



Review

Edible mushrooms: Functional foods or functional ingredients? A focus on *Pleurotus* spp.

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Abstract: The increasing consumer demands for healthier and more sustainable foods has pushed the food industry in the constant research of new foods, new functional ingredients and bioactive compounds, whose production can be considered as far as sustainable. In this sense, application of the edible mushrooms has attracted the attention of industries because of their good nutritional quality, simple and economically affordable growth, taste, flavor, and textural properties, as well as the presence of bioactive compounds with positive effects on human health. Among edible mushrooms, *Pleurotus* spp. are considered among the most popular all over the world. Their cultivation is very simple and sustainable, because *Pleurotus* spp. efficiently grow on several substrates and can degrade various lignocellulosic waste materials. This means that *Pleurotus* mushrooms can be cultivable all over the world. From the inclusion in food products as extracts to the incorporation as fresh or into powder form, several works have been published in the literature concerning the use of mushrooms as functional ingredients. However, mushroom addition can modify functional and physicochemical properties of the supplemented foods, hence the main challenge to overcome is to not negatively affect the sensory properties. Although many scientific works have been published on the matter, further research is needed to better understand the role of mushrooms as functional ingredients, due to the different results reported. This review aims for providing the more recent information about *Pleurotus* incorporation into foods, with a critical vision looking forward to the future, without forgetting an overview of the more recent literature about *Pleurotus* spp. nutritional value and their healthy promoting compounds.

Keywords: *Pleurotus*; primary metabolites; functional compounds; dietary fiber; β -glucans

1. Introduction

Cultivation of mushrooms has significantly increased in recent years thanks to their use for both therapeutic and medicinal purposes, but they also represent an excellent food resource. According to the Food and Agricultural Organization (FAO) of the United Nations, in the three-years period 2019-2021 Asia was the first world producer (contributing to 95.54% of the world production), followed by Europe (3.02%), America (1.25%), Oceania (0.12%), and finally by Africa (0.07%) [1].

Out of 14,000 mushroom species documented, 3000 are classified as edible, and among them, 270 species are reported to have therapeutic potential on human health [2], such as antioxidant, anticarcinogenic, antimicrobial, immunomodulating, anti-inflammatory, hepato- and neuro-protective activities (3).

Cultivation of edible mushrooms also represents an eco-friendly solution to convert lignocellulosic organic wastes into food proteins, thus also reducing their environmental impact [4]. However, the inedible part of the mushrooms is generally discarded as processing waste [5]; instead, it could be used as waste with high added value in several applications [6]. The mycelia of some edible mushrooms, for example, still contain umami ingredients, so they could be used in application for umami taste products [7]. Similarly, the fruiting body and mycelium of edible mushrooms are sources of several natural compounds with antioxidant activity, such as ascorbic acid, tocopherols, carotenoids, and polysaccharides [8,9]. Furthermore, there are lots of unmarketable mushrooms, such as those smaller in size, which are usually sold for cheap prices [10] or used as feed additives [11], which instead could be valorized and provide added value. Thanks to their chemical, nutritional, sensory, and technological properties, edible mushrooms have attracted the attention of the food industry in last few years. In the food industry mushrooms can be directly added to products as an ingredient, to increase food functionality, or used indirectly as a source of fermentation [12]. They also can be added in different forms: as mycelia [13], fruiting body, powder [14] or extract.

The presence of various nutritional and bioactive compounds in edible mushrooms makes them healthy foods for consumption [3]. The principal nutritional component of mushrooms dry matter is carbohydrates, both in digestible and non-digestible form. Besides providing dietary fiber, mushrooms are also a good source of protein, containing all essential amino acids; they are low in fat, salt, and calories and are cholesterol-free. Furthermore, mushrooms represent a source of vitamin D, but also possess a good amount of B vitamins and minerals, as well as many other bioactive compounds. All these characteristics make mushrooms “perfect” functional foods, but also a functional ingredient because, when added to a portion of conventional food, mushrooms increased food functionality as a result of their beneficial compounds.

Due to the presence of different compounds, such as sodium salts of glutamic and aspartic acids and 5'-nucleotides, mushrooms have umami taste, so they could be used as flavor enhancers (umami taste-based products) [7] or to produce natural seasonings [15]. Furthermore, the enzymatic activities of some mushrooms could be exploited to produce particular types of foods: the presence of both alcohol dehydrogenase and amylase in *Agaricus blazei*, for example, has allowed the production of sake, a typical fermented alcoholic beverage in Japan [16], while lactate dehydrogenase and milk clotting activity of *Shizophyllum commune* have been used to produce a cheese-like food [17].

Pleurotus genus is universally known as oyster mushrooms, and more than 200 different species are dispersed all over the world [18,19]. *P. ostreatus*, *P. sajor-caju*, *P. cornucopiae*, *P. pulmonarius*, *P. tuber-regium*, *P. citrinopileatus*, and *P. flabellatus* all belong to this big family. Oyster mushroom

is recognized as one of the most popular mushrooms worldwide, and it is the third in the production of edible mushrooms after the genus *Agaricus* and *Lentinula* [20]. *Pleurotus* cultivation is very simple, and their great ability to degrade lignocellulosic materials makes them cultivable all over the world, depending on the local availability of the various substrates on which *Pleurotus* easily grow [4]. Thanks to their pleasant taste and pharmacological properties, *Pleurotus* can be used for both culinary and medicinal purposes [21].

From a nutritional point of view, the genus *Pleurotus* is characterized by high protein, ash, total dietary fiber, and β -glucan contents, but low levels of fat, sugars, and calorific value [22]. Hence, it represents a potentially rich ingredient in food formulations.

Controversial results are instead reported in the literature about *Pleurotus* addition to foods and its effect on the sensory acceptability: this can probably depend on the level of mushroom supplementation, since low amount of *Pleurotus* addition generally doesn't seem to negatively affect sensory acceptability of the enriched foods [23], but also on the attitude of the consumers to the mushroom taste [24].

The nutritional value, antioxidant activity and the presence of several health-promoting compounds in *Pleurotus*, which means that these mushrooms can be considered functional foods, had already been the focus of the scientific research in the past (mainly late 90's and early 2000's). However, from 2010 onwards, there has been a surge in the published works concerning the role of mushrooms as food ingredient in different sectors of the food industry. This is confirmed by a simple search throughout the Scopus online database, carried out by means of the string "Article title, Abstract, Keywords": (mushroom AND ingredient) AND *Pleurotus*, which returned 345 publications covering the time range from 1991 to 2023 (Figure 1).

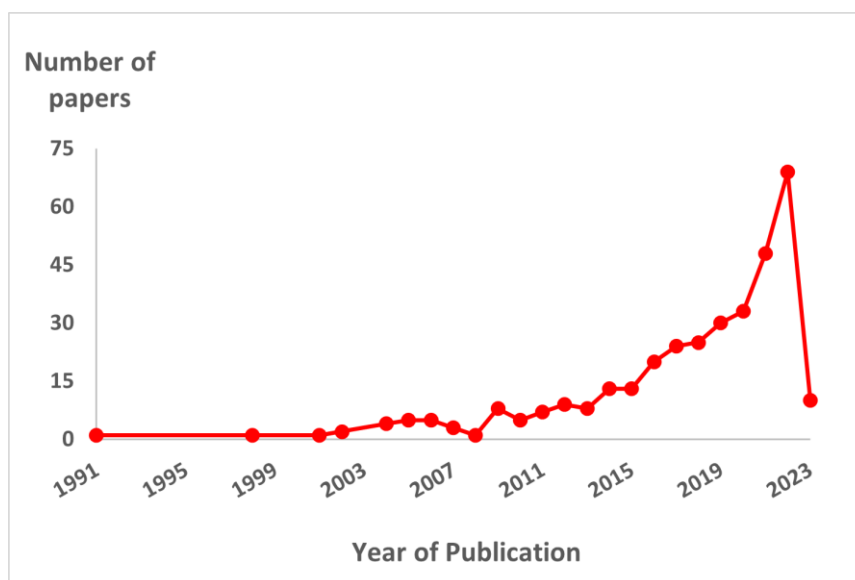


Figure 1. Trend of scientific works concerning the use of *Pleurotus* as food ingredient (research by Scopus online database, carried out by means of the string "Article title, Abstract, Keywords" (mushroom AND ingredient) AND *Pleurotus*).

However, even more food formulations can be developed. Hence, the aim of this review is to provide a critical overview of the scientific literature, from 2010 onwards, concerning chemical and

nutritional properties, as well as medicinal value, of *Pleurotus*, with a focus on its use as food ingredient and its effect on the quality of the supplemented foods, to demonstrate that *Pleurotus* should no longer be considered niche foods but could be promising foods of fundamental importance. Also, a brief overview concerning the effects of the processing and storage conditions on the quality of *Pleurotus* is provided.

2. *Pleurotus* nutritional and functional characteristics

Functional foods can be defined as foods that have satisfactorily demonstrated to affect beneficially one or more target functions in the body, beyond adequate nutritional effects, in a way that is relevant to either improved state of health and well-being and/or reduction of risk of disease [25]. Oyster mushrooms, generally consumed for their organoleptic characteristics and culinary qualities, have very interesting chemical and nutritional characteristics, but they can also be considered a functional food: hence, their consumption should be encouraged especially in the context of a correct and healthy diet.

2.1. Protein

Among nutrients, proteins represent a remarkable amount in oyster mushrooms and this characteristic can be useful for vegans. There are a lot of papers concerning the protein contents of the oyster mushroom. Manzi et al. [26] reported the protein content (Nx4.38) of different strains of *Pleurotus*: eight commercial strains of *P. ostreatus* showed a wide protein range, between 19.9 and 31.2 g/100g dry weight (dw); three strains of *P. eryngii* had more similar protein levels (ranging from 22.9 to 23.2 g/100g dry weight), while *P. pulmonarius* had a protein content of 30.5 g/100g dry weight. These data agreed with the work of Valencia del Toro et al. [27], where different strains of *Pleurotus*, grown on a commercial substrate, showed protein contents ranging from 26.7 to 28.2 g/100g dry weight, and with the work of Chirinang and Intarapichet [28], where the protein content of commercial *P. ostreatus* was 20.8 g/100g dry weight.

However, it is well known that the growing substrate may affect the protein content of oyster mushrooms [4]. For example, *P. pulmonarius*, grown on different substrates (*Medicago sativa* L. straw, *Prangos pabularia* Lindl. wastes with *Medicago sativa* L. straw (1:1), and *Poplar* sawdust residues), showed statistically significant differences in the protein contents (38.6 g/100g dw, 30.9 g/100g dw and 27.3 g/100g dw of protein, respectively) [29]. Recently, Ivarsson et al. [30] used faba bean hulls as growing substrate for *P. ostreatus*: the fruiting bodies produced were comparable to commercial mushrooms. Moreover, after mushroom harvesting, there was an increase in protein content (from 20.8 to 34.7 g/100g dry weight). Different nitrogen sources (wheat bran, cauliflower leaves, and ammonium sulphate), added to sugarcane bagasse and saw dust as growing substrate for *Pleurotus* spp. cultivation, were instead evaluated in the study of Maheswari et al. [31]. According to the authors' results, the best appropriate substrate for oyster mushrooms was made by sugarcane bagasse + sawdust + wheat bran (75:25:1, w/w), which showed the highest protein content and the highest yield production. Indeed, nitrogen-rich substrates may enhance mushrooms' protein contents, as observed in the work of Naraian et al. [32], where corn cob was used as lignocellulosic substrate supplemented with various additives. In addition to nutritional benefits, such as increased protein content, the cultivation of *Pleurotus* spp. on agro-industrial wastes can be considered a sustainable process for the conversion of

various agricultural waste into human food. However, when it comes to proteins, from a nutritional point of view it's worthwhile knowing not only the total content but also the different amino acid composition: according to a recent work by Lin et al. [33], who tested 132 samples for genetic and chemical diversity, the amino acid content varied within the different species of the genus *Pleurotus*.

Pleurotus spp. include all essential amino acids; however, there is a difference in the identification of the limiting amino acid (such as methionine, tryptophan, lysine, and leucine) among the authors [26,28,34–36]. This difference is probably due to the strain of the fungus studied and/or to the composition of the growing substrate.

More recently, new analytical techniques have been applied to mushrooms research: in the work of Pellegrino et al. [37] the metabolomic investigation by liquid chromatography/mass spectrometry combined with quadrupole time-of-flight (LC/MS Q-TOF) of *P. ostreatus*, grown on black poplar wood logs and on lignocellulosic byproducts, allowed the identification of essential and nonessential amino acids together with the remarkable presence of dipeptides that could be fruitfully exploited in the production of mushrooms as a base of functional ingredients.

2.2. Lipid compounds

Mushrooms are generally low in lipid contents (usually less than 5% dw) [38–40]. Lavelli et al. [41] reported a data set of different fat contents in various *Pleurotus* spp. cultivated on different substrates, ranging from 0.9 g/100 g dry weight in *P. sajor-caju* to 7.5 g/100g dry weight in *P. eryngii*. The main fatty acids in *Pleurotus* are linoleic (C18:2), linolenic (C18:3) and oleic (C18:1) acids, as reported in the work of Sande et al. [42]. From a nutritional point of view, this lipidic composition is very interesting: linoleic and linolenic acids are the main essential polyunsaturated fatty acids (PUFA), that have shown a positive role in preventing human diseases, while oleic acid is the principal monounsaturated acid with a beneficial effect on cancer, autoimmune and inflammatory diseases, and on protective action on health risk parameters [43].

Other fatty acids present in good amounts are palmitic (C16:0) and stearic (C18:0) among the saturated fatty acids; however, a great variability can be observed among species and growing substrates [33,34,44–46].

An interesting work [47] analyzed the effect of the growth temperature on the lipid composition in *P. ostreatus* and *P. citrinopileatus* Singer. In the experimental design 90% of relative humidity and different temperatures (12, 17, 21, and 27 °C for *P. ostreatus*, and 17, 21, and 27 °C for *P. citrinopileatus* Singer, respectively) were evaluated for the cultivation of mushrooms. It is recognized that fatty acid unsaturation increases as temperature decreases, but the authors also observed a modification in the fatty acid profile both in *P. ostreatus* and in *P. citrinopileatus* Singer, hence suggesting that variations in the growth temperatures may be a useful tool to positively affect the nutritional value of mushrooms. However, many other variables, such as nutritional factors and oxygen, may affect the lipid composition of mushrooms [47].

Among lipidic compounds, also sterols were identified in mushrooms, ergosterol being the most abundant in *Pleurotus* spp., followed by minor ergosterol isomers [45,46]. Cultivated mushrooms are found to be rich sources of ergosterol, a component of fungi membranes, which is the precursor of vitamin D₂. Vitamin D plays a vital role for humans due to its importance in calcium metabolism and bone mineralization. There are two forms of Vitamin D: cholecalciferol, or vitamin D₃, and ergocalciferol, or vitamin D₂. Vitamin D₃ originates from animal sources, while vitamin D₂ comes

mainly from vegetable sources. Fungi can be considered a good source of Vitamin D₂, where it originates from UV irradiation of ergosterol [48,49]. Due to the low abundance of vitamin D in plant foods, vegetarians are generally at risk of vitamin D deficiency [50], unless they resort to food supplements; hence, mushrooms could be a valid alternative of vitamin D source for meat-free diets. The regular consumption of mushroom products can help to achieve the recommended dietary allowance (RDA) of vitamin D₂ (15 mg/day).

Several studies have been carried out concerning the effects of different irradiation types on the production of vitamin D₂ in mushrooms. Jasinghe and Perera [51] demonstrated how the conversion of ergosterol to vitamin D₂, by exposure to UV irradiation, was significantly affected by orientation of mushroom tissues to UV radiation. Furthermore, the conversion was about four times higher when exposing gills to UV-A irradiation rather than the outer caps. Finding the best kind of irradiation was the focus of further work by the same authors [52]. According to their results, the conversions of ergosterol to vitamin D₂ under UV-A, UV-B, and UV-C were shown to be significantly different, with the highest vitamin D₂ content in oyster mushrooms irradiated with UV-B at 35°C and around 80% moisture. Similarly, Kortei et al. [53] evaluated the content of vitamin D₂ in dried *P. ostreatus* after exposure to different doses of gamma radiation prior to storage (0 months) and after storage (12 months) at room temperature (28°C). Unfortunately, the authors [53] observed that gamma radiation and storage time did not significantly affect the contents of vitamin D₂ in mushrooms. These results disagreed with what was reported by Jiang et al. [49], according to which exposure to ultraviolet radiation is a useful tool to increase the content of vitamin D₂ in mushrooms. It's worth noticing that the processing methods reported in the literature to which mushrooms are subjected differ from each other. At the same time, many other factors (mushroom species, growing substrate, harvesting time, mushrooms in the fresh or dried form, positioning of the mushrooms to the light source) may deeply affect the results, thus making it rather difficult to compare the results from the different studies.

2.3. Carbohydrate

Mushrooms usually contain 40–60% of carbohydrates on dry weight basis [54,55]. They include low-molecular-weight carbohydrates, such as mono and disaccharides, and sugar alcohols (such as glucose, trehalose, mannitol, and arabitol) [56], while, polysaccharides, such as glycogen, chitin, α - and β - glucans and other hemicelluloses, are the main high-molecular weight carbohydrates [57]. Carbohydrates are distributed in different amounts in the fruiting bodies of mushrooms (stipe, base, and pileus region) and their content vary during the various growth phases [56].

Among low-molecular weight carbohydrates, trehalose plays important physiological roles: it acts not only as a reserve but also as a thermoprotectant under different stress conditions [58]. In *P. pulmonarius* strains, the possible role of trehalose in the heat resistance was studied by Liu et al. [58]. The authors [58] studied two different strains of *P. pulmonarius* and they found that heat stress inhibited the mycelial growth of *Pleurotus* and accelerated lipid peroxidation, but trehalose played protective roles on heat resistance with an accumulation in the more sensitive strain. Analogously, trehalose alleviates high-temperature stress in *P. ostreatus* due to the inhibition of glycolysis and lactate accumulation, stimulating the pentose phosphate pathway, as demonstrated by Yan et al. [59].

Among high-molecular-weight polysaccharides, such as glycogen, chitin, α - and β - glucans and other hemicelluloses, are the most abundant compounds in mushrooms [57], being mainly located in the fungal cell walls. These compounds are non-digestible carbohydrates, which are not hydrolyzed

by human digestive enzymes, so they can be considered a part of the dietary fiber. Among these, water insoluble fiber represents the main contribution (about 80-90%) to total dietary fiber of mushrooms, while water soluble fiber is present to a lesser extent (about 10%); as usual, their contents vary according to the mushroom species [35,60–62].

One of the principal compounds of water-insoluble fiber of mushrooms is chitin, a long-chain polymer of N-acetylglucosamine: it represents the principal component of cell walls in fungi. In commercial *P. ostreatus* chitin is about 8% of the total dietary fiber [61] and about 11% in *P. eryngii* [62]. Nitschke et al. [63] described a new method for quantifying chitin in different fruiting bodies of mushrooms provided by a local producer: among the studied mushrooms, the content of chitin ranged from 0.4 g/100g dm (dry matter) in *Hypsizygus tessulatus* (or Shimeji Mushroom) to 9.8 g/100g dm in *Flammulina velutipes* (or *Enokitake*), while the chitin content of *P. ostreatus* was 3.2 g/100g dm. According to Vetter [64], the chitin content of the cultivated mushrooms is a characteristic of the species, and it does not seem to depend on the cultivars; moreover, chitin levels are higher in the pileus (cap) than in the stipes.

The partial deacetylation of chitin produces chitosan: its antibacterial, antifungal and antioxidant properties make chitosan a molecule with large applications in several sectors, such as foods, pharmaceuticals, medicine, cosmetics, agriculture, and environmental chemistry. Chitosan is often used as an active preservative in packaging, for its hypolipidemic and hypocholesterolemic activity, for the encapsulation capacity of nutraceuticals etc. [65].

In the cell walls of fungi, there are other high-molecular-weight polysaccharides, such as β -glucans. They refer to any polymers built of glucose units linked by 1→3 β linkages and a small number of branches bound by 1→6 β linkages [66]. However, mushrooms also contain 1,4/1,4-6 α -glucans. Both in chitin and β -glucan compounds, individual chains are connected via hydrogen bridges, resulting in covalent connections between the two polymers. As a result, a strong cell wall is produced, in which chitin fibers connect to create a network within a glucan matrix. In addition to these essential building components, mushrooms may also contain lower quantities of various additional saccharides [67].

The content of β -glucans is strictly dependent on the genus: *Pleurotus* and *Lentinula* are the most valuable sources, even if substrate, growing condition, and maturity of the fruiting body may affect the amount of β -glucans in mushrooms, as reported by Rop [67]. β -glucans may have several molecular masses and tertiary structures, different solubility, viscosity, and ability to gel formation, [68]. For example, β -glucans have been determined both as insoluble (54–82%) and soluble dietary fiber (16–46%) [35,69]. The importance of β -glucans is that they are not digested by humans. Therefore, they arrived intact in the human colon, where bacteria can hydrolyze and metabolize sugars with distinct linkage patterns and configurations, including glucans [70]. Hence, β -glucans can be considered a potential source of prebiotics.

A unique β -glucan of *Pleurotus* mushroom is pleuran, identified for the first time in 1994 [71]. Many studies have been performed about the health benefits of pleuran for humans: the first works were about its antidiabetic and lipid-lowering effects, with particular reference to cholesterol decrease in the blood, but recently various studies also focused on pleuran immunomodulatory, antitumour and antioxidative properties, as well as on its therapeutic effects in respiratory infections [66,72]. Recently, a study by Urbancikova et al. [73] used a supplement containing β -glucan from *P. ostreatus* for the management of Herpes simplex Virus Type 1 infection, in children aged over 6 years. The results showed that, compared to a control group who received a placebo, the use of the supplement caused a significantly shorter duration of herpes symptoms, leading to an earlier improvement compared to the

patients treated with placebo. A recent study of Rennerova et al. [74] also confirmed the beneficial effect of pleuran supplementation in preventing respiratory infections in the pediatric population.

Oral supplementation of β -glucan probably has an immune system-enhancing effect, although confirmation is required to establish the optimal dose and molecular mechanisms [75].

2.4. Other bioactive compounds

Not only β -glucans but also bioactive compounds are involved in the antimicrobial and antioxidant activities of *Pleurotus* spp. A recent study by Mkhize et al. [76] evaluated the effect of supplementing mushroom-growing substrates (sugar cane with increasing levels of wheat bran from 0% to 20%) on the content of bioactive compounds and antimicrobial and antioxidant activities of *P. ostreatus*. Several bioactive compounds such as vitamin E, phenols, fatty acids, and terpenoids were detected. Mushrooms grown on sugar cane supplemented with wheat bran showed compounds such as beta, gamma, and alpha tocopherols. However, methanolic extracts of *P. ostreatus* grown either on unsupplemented or on supplemented substrates showed similar properties. This was probably due to the presence of phenolic compounds in the methanolic extracts of mushrooms, which are also known to show antioxidant activity.

Phenolic compounds, that showed a wide range of physiological properties among which antioxidant activity, are detected in several mushrooms' studies [77, 78]. In the work of Gąsecka et al. [79] phenolic compounds (mainly ferulic and p-coumaric acids) and ascorbic acid were detected both in *P. ostreatus* and *P. eryngii* grown on a substrate enriched with selenium and zinc (1.5mM each). The enrichment of the substrate with Se and Zn resulted in increased amounts of the two elements in *Pleurotus*, due to the ability of the mushrooms to accumulate minerals, and in increased levels of total phenolic content (TPC) and ascorbic acid content. According to the authors [79], the increase in antioxidant content could be due both to the oxidative stress induced by zinc, resulting in the stimulation of antioxidant compounds synthesis, and to the inhibition of enzymatic polyphenol oxidation by selenium.

In the research of Bruno et al. [80] five different strains of *P. eryngii* were characterized for their antioxidant properties: ascorbic acid contents were less than 6 mg/g dry weight, comparable, according to the authors, to the ascorbic acid levels in foods of plant origin, while total phenolic content ranged from 22.6 to 72.4 mg/g dry weight.

The content of bioactive compounds and their properties can be affected by heat treatments and cooking methods [78], to which mushrooms are generally subjected to increase their shelf life and to be consumed. As an example, in the work of Mutukwa et al. [81] tray-dried and freeze-dried mushrooms showed significant differences in total phenolic content and antioxidant activities (measured with DPPH and ABTS), with tray drying having higher TPC [82].

A microwave system could be a good alternative to heat treatments to retain mushrooms antioxidant molecules. It can be easily developed both for industry and home cooking purposes, and even though home microwave is not as effective as industrial systems, it can be useful for retaining the mushroom's antioxidant molecules [78].

3. Processing and storage conditions affecting *Pleurotus* quality

The optimal temperature for the growth of *Pleurotus* mycelium is around 25–28 °C with pH value

of 5.5–6.5, while for fruiting body formation, key environmental factors are represented by temperature, sufficient ventilation and high relative humidity, luminosity and air composition of the surrounding substrate, concentration of oxygen and carbon dioxide [39,83,84].

The relatively high water content of fresh *Pleurotus* makes this mushroom a highly perishable commodity featuring a short shelf life: 24–48 h at ambient conditions [85], and from seven to ten days in cold storage [86]. Its high water content (about 94–79 g/100g) generally depends upon the substrate on which the mushroom was grown [26,29,54,61,87–89]. During post-harvest different changes may appear, such as microbial decay, stipe elongation, surface discoloration, and cap expansion [90]. Therefore, processing of oyster mushrooms is highly recommended to extend their shelf life.

Processing of mushrooms generally starts with washing, to remove adherent soil and other soil impurities, or with blanching, to inactivate mushroom enzymes [3]. Also soaking in antibrowning solutions, such as citric acid, sodium metabisulfite, etc. can be employed to preserve mushroom freshness [91].

Drying and further dehydration, however, represent the most widely used processes to produce *Pleurotus* powder, which in this way can be stored and used for further industrial exploitations [92] or can be consumed in the off-seasons. Times and temperatures of drying must be carefully set to avoid pigments degradation due to oxidation, with consequent variation in the color of dried mushrooms. Demiray [86], for example, observed that drying temperature at 45 °C was the most preserving color in mushroom slices compared to 55 and 65 °C: increasing hot air temperature resulted in decreased L* value and increased b* and chroma values. The vacuum drying process, which has the advantages of an oxygen-free environment and low drying temperatures, could be a feasible technique for oxidizable and temperature-sensitive compounds. A work by Demiray & Çalışkan Koç [93] showed that combination of higher vacuum temperature (65 °C) with lower absolute pressure (0.04 MPa) led to the lowest drying time (240 min) for mushroom slices compared to other vacuum drying conditions. However, these settings were not effective in preventing the degradation of lovastatin, a compound belonging to the group of statins, which has been revealed to be contained in the fruiting bodies of edible mushrooms: lovastatin, in fact, is heat, oxygen, and light sensitive [94]. Also, mushroom variety and chemical composition, as well as sample thickness and drying area are variables affecting the time of drying [93]. When evaluating different drying techniques on the nutritional quality of the oyster mushroom, Aishah and Wan Rosli [95] concluded that low-heat air blow was the most effective method in reducing water activity and retaining the highest levels of fat and carbohydrate contents compared with the other two methods, sun drying and gas laboratory oven, which instead revealed most efficient in preserving β -glucan and dietary fiber contents, respectively. Sun drying also had the lightest color (highest L* and b* values) compared to the others, while low heat air blow has the lowest color measurement for brightness (L* value). The work of Çelebi Sezer et al. [96], instead, showed that total phenolic compounds and antioxidant activities of *P. ostreatus* were not affected by microwave power and conventional drying temperature.

Many other processing techniques are reported in the literature concerning mushrooms storage, such as the use of modified atmospheric packaging, preservation by chemical treatments, lactic acid fermentation or irradiation [22]. The use of modified atmosphere packaging (MAP) is encouraged to lower the rate of respiration and, as a result, the rate of substrate depletion. Villaescusa and Gil [97] studied different storage temperatures as MAP conditions and different gas permeabilities films to maintain *Pleurotus* quality and prolong its shelf life. The main result of this study was that low temperature and proper internal humidity are the main parameters to consider for extending the shelf

life of oyster mushroom. The best MAP was 12–15 kPa O₂ + 5kPa CO₂ and this condition was found beneficial for maintaining quality and extending the shelf life of *Pleurotus*. Furthermore, the authors highlighted the issue on the use of some films normally employed in mushrooms packaging (such as the stretch PVC) and their negative impacts on the environment [97]. More recently some authors [98] studied the effect of combining modified atmosphere packaging (MOP, medium oxygen packaging 50% O₂ and 50% N₂) with bilayer active packaging (made of gelatin with pomegranate peel powder coated on the polyethylene film) on the shelf life of oyster mushrooms. This kind of combinations (MOP with active layer) was useful to increase the shelf life of *P. ostreatus* up to 11 days compared to the control (3 days), showing the lowest weight loss (namely only 0.60 % decrease in weight), throughout the storage time.

The storage conditions have also been revealed to affect nutritional and functional characteristics of mushroom: Ng et al. [99] reported that 4 °C was the best storage temperature for *P. sajor-caju* powder, compared to other storage temperatures (–20, 4, 25, 35 °C). Increasing storage temperature resulted in higher water activity, lower moisture, and L* value during storage, while no significant differences were detected for pH and microbial counts [99]. Lowering the storage temperature generally slows down the respiration and transpiration of mushrooms, postponing senescence, avoiding wilting, and extending shelf life.

4. *Pleurotus* as functional food ingredient

Pleurotus mushrooms show several functional properties, such as Water Holding Capacity (WHC), oil holding capacity (OHC), swelling capacity (SC) and emulsifying activity [99]. These are very important for food industry applications: high WHC values, for example, help to prevent food structure deterioration, while high OHC values are essential to prevent oil losses during cooking [99]. Total dietary fiber (TDF) and insoluble dietary fiber (IDF) may account for mushroom high WHC [100,101], while insoluble dietary fiber may be responsible of mushroom high OHC [102]. Drying and grinding mushrooms into powder increase the superficial area, thus resulting in improving absorption, and consequent increase of WHC, OHC, and SC [101]. The emulsifying ability of mushrooms, which could be helpful in food formulations requiring emulsion formation and extended shelf life, maybe due to their proteins since these are recognized as strong emulsifying agents [103].

Hereafter, several applications of *Pleurotus* as food ingredients in the different sectors of the food industry are reported.

4.1. Meat-based foods

The ever-increasing growth of the world population along with industrial development is causing an expansion of food production and an increased demand for animal protein [104]. However, this increasing requirement must deal with the low efficiency of animal protein production as well as with its poor sustainability. Among animal proteins, meat and meat products provide high-protein quality, together with fundamental micronutrients such as iron, zinc and selenium, and important vitamins, such as B₁₂ and D [105]. For this reason, there are current tendencies in the food companies in formulating new ingredients or new alternatives for meat products, with similar nutritional, technological and sensory attributes. Plant-based ingredients (pulses, cereals, tubers, and fruits) have been largely used as meat replacers for up to 50%, and recently also insects and by-products from the

food industry represent novel approaches developing more sustainable meat alternatives [106].

Among meat replacers, oyster mushrooms are worth exploring because they are a good source of protein, fiber, vitamins, and minerals, and are low in calories, fat, and sodium [26]. However, their nutrient composition depends on growing conditions and mushroom species, as well as on the strain employed [4].

A typical trait of meat-based foods is their fibrous structure. Plant-derived proteins, such as soy protein, which are generally used to prepare meat alternative foods, do not achieve acceptable sensory characteristics, due to their poor texture and presence of off-flavor [107]. The use of edible mushrooms, instead, could gain better sensory scores: they have, in fact, a peculiar umami taste, generally described as savory, broth-like or meat-taste [108], which makes mushrooms palatable. Among edible mushrooms, *P. ostreatus* has been revealed with high umami amino acid content [7]. Hence, the umami taste of mushrooms could represent a potential strategy to reduce salt in meat-based products, contemporarily providing high fiber content to the supplemented meat-based products. Furthermore, the bitter taste and the metallic notes generally obtained when partially replacing salt with potassium chloride or calcium chloride [109] could also be avoided by using edible mushrooms as salt replacer. Optimal results in salt reduction, for example, were achieved when *P. ostreatus* flour or powder were added to partially replace salt content in meat products such as beef patties [24], frankfurter sausages [110], and liver pâté [111]. However, maturity stages, mushroom parts and species, as well as storage conditions can affect the contents of umami ingredient in mushrooms, *i.e.*, umami amino acids (aspartic and glutamic acids) and 5'-nucleotides [112–116]. Finally, technological food processes, such as drying or thermal treatments, can also affect the umami compounds modifying and even intensifying the umami taste [117]. Another aspect to consider is the consumers' attitude to umami taste, which generally depends on how accustomed the consumer is to this intense flavor [24]. Therefore, different results regarding consumers' acceptability of foods supplemented with mushrooms can be obtained according to the consumers' origin: in the work of Wan Rosli et al. [118], for example, where chicken meat was substituted up to 50% with fresh *P. sajor-caju* in patties, no difference in the overall acceptability was found by the panellists who were Asiatic and therefore usually familiar with umami taste. In the work by Gaglio et al. [119], instead, where bread supplemented with *P. eryngii* powder was evaluated by an Italian panel, odor was worse evaluated compared to control bread. A recent work by Lang [120] also highlighted that consumers' acceptance towards meat-mushroom blend products heavily depends on their combined assessment of healthfulness, cost, taste, novelty, and environmental sustainability (listed in order of benefit ranking). However, results also reported that differences in cooking habits were not found to influence consumers' acceptance, probably due to the exposure and trial of blended products occurring with equal frequency in the home and restaurants.

Regarding mushroom influence on the flavor of meat supplemented with *Pleurotus*, Qing et al. [121] found that edible mushrooms not only supply flavor components but also promote the formation of flavor substances in the meat products: both free amino acids and volatile compounds were affected by mushroom addition, due to moderate oxidation of proteins and lipids.

When incorporating fresh mushrooms into meat, generally softer products are expected, due to the high moisture content of mushrooms. Furthermore, *Pleurotus* may compete with the myofibrillar proteins from meat for water adsorption, thus lowering hardness. This was observed, for example, in the works of Chung et al. [10], Wu et al. [122], and Wan Rosli et al. [123] (see Table 1). Chung et al. [10] exploited the ability of raw *P. eryngii* to form a viscous paste, following the interaction of mushroom

polysaccharides with water, to develop a surimi gel by replacing fish meat with cuttlefish and mushroom. With the increasing amounts of king oyster mushrooms (20, 30, 40 and 50%) the authors [10] observed significant decrease in hardness, cohesiveness, and gumminess, while springiness of the cuttlefish pastes increased with the addition of mushroom paste, probably due to its viscoelastic properties. The supplemented samples also received a good sensory evaluation, but when mushroom addition rose up to 50% flavor, softness, chewiness, and overall quality tended to decrease. Similar results were reported by Wu et al. [122], who incorporated fresh *P. ostreatus* puree into a pork sausage: increasing mushrooms levels (0, 10, 20, 30, and 40%) resulted in decreased hardness, gumminess, and chewiness, and increased springiness, cooking loss and water holding capacity. Indeed, *Pleurotus* has a higher moisture content compared to meat: hence, during cooking processes water evaporate, thereby increasing cooking loss. Furthermore, the high water content of the mushroom prevented the gel production of the pork myofibril protein, thus causing lower cohesiveness of sausage fillings and the appearances of more inside gaps [122]. At the same time, the high dietary fiber of *Pleurotus*, with a high water holding capacity, could be related to the decreasing of hardness, gumminess, and chewiness in the meat formulations. These attributes were also responsible for the lowering of sausages texture with increasing mushroom levels, which were disliked by the sensory panel. Mushroom supplementation level at 20% had the best aroma and flavor compared to the experimental sausages.

A decrease in textural properties with increasing mushroom addition was also observed by Süfer et al. [124] when adding *P. ostreatus* powder into beef meatball: however, compared to the control, patties with 5% supplementation level resulted in higher texture and no statistically significant difference in sensory scores. Wan Rosli et al. [123] instead partially replace chicken meat with *P. sajor-caju* up to 50% in chicken patty: although mushroom supplementation did not affect sensory attributes, supplementation at level of 25% was recommended by the authors because it resulted in lower loss of nutrients (protein, fat, and carbohydrate), while moisture and ash increased (see Table 1). In a similar work [118] increasing levels of *P. sajor-caju* as a partial replacement of chicken meat in patties also resulted in decreased lightness and yellowness, increased total dietary fiber (particularly the insoluble fraction) and β -glucan content, and reduced textural properties (except springiness) and water retention. By partially replacing 25% of chicken meat in the patty with *P. sajor-caju* was the most suitable formulation for commercial chicken patties. Similar results were obtained by Wan Rosli et al. [125], who replaced beef meat in patties with *P. sajor-caju* (see Table 1). A further study by the same authors [126] reported that mushroom addition into these patty formulations (chicken or beef) did not affect their apparent digestibility as well. Also replacing chicken frankfurters with *P. sajor-caju* resulted in increased nutritional value of the final products (see Table 1), although a reduction in the protein content was observed due to meat replacement with mushroom powder [127]. Similar results were reported by Özünlü and Ergezer [128] when replacing meat in salami with dried oyster mushroom: increasing levels (0, 1, 3 and 5%) of *P. ostreatus* resulted in the reduction of moisture, fat and protein compared to the control, together with increases in ash and carbohydrate contents. However, dried oyster mushroom significantly decreased lipid and protein oxidations during storage compared to the control, especially the 5% level of supplementation [128], probably due to the antioxidant activity of phenolic compounds present in mushroom [129]. Although a significant decrease of color (L^* , a^* and b^* values) was observed for salami supplemented with mushrooms, at a sensory analysis addition of dried *P. ostreatus* improved flavor, color, and odor of the products, but higher supplementation levels (>3%) resulted in texture losses (lower firmness) compared to the control [128].

In the work of Pachekrepapol et al. [130], fish burgers supplemented with mushrooms (both at 10

and 15% level) showed a significant decrease in thawing loss and a significant increase in cooking yield compared to the control, due to the improved water holding capacity of the burgers caused by mushroom addition. As usual, decreasing in hardness and chewiness together with increased cohesiveness were observed in fish burgers supplemented with mushrooms, even if sensorial attributes were not affected by mushroom addition. As already observed by Özünlü and Ergezer [128], *Pleurotus* incorporation into the meat matrix slowed down lipid oxidation during storage compared to the control.

As it can be seen from the results reported in Table 1, generally lower percentages of meat substitution have been tested when mushroom addition was made in the dried form. This is because dried mushrooms have higher nutrient contents and more intense taste, which could negatively affect chemical, nutritional, sensory, and technological characteristics of the supplemented meat-based products. When meat is replaced with mushrooms a general decrease in hardness is observed (see Table 1), probably due to the reduction of protein content with meat replacement, which can affect meat emulsion [131].

When completely replacing meat with mushroom in meat-based products, the quality of the mushroom-based foods may be affected by the presence of other ingredients. Lu et al. [132], for example, employed *P. eryngii* pulp as the main ingredient to make sausage-like gel food, and observed that the addition of a certain amount of edible gum (0.6% carrageenan) in a particular form (powder rather than reversible gel form), as well as synergistic effects between gums (0.4% carrageenan with 0.2% konjac gum), can improve the textural and sensory properties, reduce cooking loss, and increase water holding capacity of the final product. A further work of Arora et al. [133] confirmed that carrageenan (0.8%) was the most effective in reducing purge and cooking loss and increasing emulsion stability in the mushroom-based sausage analogue compared to other binding agents (soy protein concentrate, casein, and xanthan gum). Lu et al. [134] also added soybean to increase the protein content of mushroom-based sausage: increased water holding capacity, emulsibility and chewiness of sausage were observed, while the addition of corn starch contributed to making the sausage tissue fine and smooth.

Another problem to be faced in meat-based products is discoloration during storage or after thawing. This process is due to the oxidation of deoxymyoglobin and oxymyoglobin to metmyoglobin (metMb), which could be prevented by using compounds with antioxidant activity. Among mushroom bioactive compounds, ergothioneine (ESH) and phenolic compounds have been shown to exert antioxidant activity [135]. Furthermore, ESH has been proven to have the highest antioxidant activity *in vitro* compared to traditional antioxidant agents, such as glutathione, uric acid and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) [136]. For this purpose, mushrooms aqueous extracts (containing both ESH and phenolic compounds) have been tested as color stabilizer in processed fish meats [5]. The authors also reported that the antioxidant activity against lipid oxidation and metMb formation by ESH depended on the purity of the mushroom extracts, because phenolic compounds contained in the extract could have secondary negative effect on the meat color, thus decreasing the antioxidant activity of native ESH [5]. It is probably due to this mechanism that Cerón-Guevara et al. [24] observed the discoloration phenomenon (increase in L* and decrease in a* values) during cold storage of beef patties despite the addition of *P. ostreatus* flour to meat products.

Akinwande and Abegunde [137] tried to replace turkey meat and beef with oyster mushroom (*P. sajor-caju*) in the production of Nigerian pepper soup: mushroom pepper soup had higher protein content and higher preference for taste compared to beef pepper soup, as well as the highest crude fiber and the lowest fat content.

Table 1. Effects of *Pleurotus* spp. on quality attributes of meat-based foods.

Mushroom variety and Levels used	Types of foods	Mushroom pretreatment	Effects	References
Crude mushroom ergothioneine extract (<i>P. cornucopiae</i> , <i>P. eryngii</i>) 1 mL (from 10 g wet material) added to 100 g of meats	Processed meats	fish	Extraction of the grounded fruiting body with H ₂ O (95 °C × 1h), centrifugation and evaporation. Further extraction with aqueous EtOH (70%, v/v), centrifugation, evaporation and redissolution in H ₂ O	Anti-discoloration effect (delayed lipid oxidation and metmyoglobin formation) during ice storage [5]
Fresh mushrooms (<i>P. ostreatus</i>) at 5%	Beef paste		Washed with tap water, drying to constant weight at 45 °C in a heat pump drying machine, grinding into powder, and sieving (20 mesh). Addition of the other ingredients and further processing	Increased cooking loss (+83.9%), a* (+12.8%) and b* (+10.4%) values. Decreased hardness (-13.7%), gumminess (-23.3%), chewiness (-30.8%), springing (-11.7%), cohesiveness (-13.2%), and L* (-8.7%) value. Increased moderate oxidation of proteins and lipids, with consequent increase of free amino acids (197.4% of total free amino acids) and increased number of volatile compounds (21 vs 14) compared to the control. [121]
Fresh mushrooms (<i>P. eryngii</i>) at 40%	Cuttlefish gel	surimi	Washing, cutting into small cubes (3 x 3 x 3 cm), addition of the other ingredients and grinding. Further processing	Decreased hardness (-59.0%), cohesiveness (-40.7%), and gumminess (-52.3%). Increased springiness (+11.7%), flavor (+9.4%), taste (+4.5%), and softness (+17.9%). [10]

Continued on the next page

Mushroom variety and Levels used	Types of foods	Mushroom pretreatment	Effects	References
Fresh mushrooms (<i>P. ostreatus</i>) at 20%	Pork sausages	Cleaning with water, cutting into small pieces, and mashing with a chopping machine. Addition of the other ingredients and further processing	Increased moisture (+13.0%), amino acids (+24.8% essential amino acids, +21.2% non-essential amino acids), L* value (+7.8%), cooking loss (+320.0%), WHC (+9.5%), and springiness (+47.4%). Decreased protein (-1.9%), fat (-6.1%), ash (-8.7%), a* value (-11.2%), hardness (-28.1%), gumminess (-45.2%), chewiness (-15.1%). Reduced lipid oxidation during storage (TBARs: -9.8% at time 0 and -7.6% after 20 days of storage)	[122]
Fresh fruiting body (<i>P. nebrodensis</i>) at ≈ 73%	Sausage (cooked)	Cleaning, slicing, blanching (in boiling water × 3 min) and crushing into pulp. Addition of the other ingredients and further processing	Same taste, color, texture, and acceptance of common sausage (used as control)	[134]
Fully-grown mushrooms (<i>P. sajor-caju</i>) at 13.5%	Chicken patty (cooked)	Rinsing with clean water, blanching, and chopped coarse chopping (sizes 2-5 mm), draining of excess water. Addition of the other ingredients and further processing	Decreased L* (-11.8%) and b* (-10.7%) values, increased TDF (+78.9%) and β-glucan (+89.2%); reduced hardness (-42.5%), cohesiveness (-19.6%), gumminess (-41.1%), and chewiness (-40.1%). Reduced fat (-17.4%) and carbohydrate (-15.7%) contents, increased moisture (+14.1%); same aroma, color, springiness, juiciness, flavor, and overall acceptance of chicken patty (used as control). Similar apparent digestibility	[118,123,126]

Continued on the next page

Mushroom variety and Levels used	Types of foods	Mushroom pretreatment	Effects	References
Fully-grown mushrooms (<i>P. sajor-caju</i>) at 13.5%	Beef patty (cooked)	Rinsing with clean water, blanched, and ground for 30 sec. Addition of the other ingredients and further processing	Reduced protein (−14.8%) and fat (−9.8%) contents, decreased cooking yield (−4.1%) and moisture retention (−8.3%), increased TDF (+79.5%); same color, juiciness, elasticity, flavor, and overall acceptance. Similar apparent digestibility	[125,126]
Fully-grown mushrooms (<i>P. sajor-caju</i>) at 3.1%	Chicken frankfurter	Rinsing with clean water, chopped coarsely (sizes 2–5 mm), draining of excess water, drying at 55 °C and grinding with a food grinder for 60 sec. Addition of the other ingredients and further processing	Reduced protein (−22.4%) and fat (−7.4%) contents, increased carbohydrate (+46.9%), TDF (+7650%) and β -glucan (from 0 to 1.43 g/100g) levels; decreased hardness (−53.8%) and increased cohesiveness (+13.2%) and springiness (+2.2%)	[127]
Mushroom powder (<i>P. ostreatus</i>) at 3%	Beef salami	Washing with tap water, chopping into small pieces (0.5–0.7 cm thickness), drying at 50 °C in a cabinet laboratory type dryer (until reaching 10% moisture content). Addition of the other ingredients and further processing	Reduced moisture (−1.4%), protein (−4.0%), and fat (4.4%) contents, increased ash (+29.2%) and carbohydrate (+183.3%) levels. During storage at day 0 and 90, respectively, changes in: L* (−7.5% and −3.6%), a* (−8.8% and +10.2%), and b* (−18.0% and +28.0%) values; TBARS (−25.0% and −60.0%) and carbonyl content (−51.5% and −55.1%); sensory color (+36.4%, only at 90 days), flavor (+26.7% and +53.5%), odor (+13.3% and +13.6%), overall acceptability (+17.2% and +27.0%), and reduced firmness (−12.5% and −13.0%)	[128]

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Mushroom variety and Levels used	Types of foods	Mushroom pretreatment	Effects	References
Fresh mushroom powder (<i>P. ostreatus</i>) at 2.5%	Low-fat and low-salt beef patty	Washing, draining, and cutting into 5mm thick slices, oven drying (60 °C for 18 h) and milling (0.5 mm mesh). Addition of the other ingredients and further processing	Increased protein content (+8.1%), better flavor, and taste, and decreased ash (-20.7%) and Na (-50.2%) levels compared to the control. During cold storage: higher increase in L* and higher decrease in a* compared to the control; slower increase in hardness and no change in springiness compared to the control. (Control contained the same fat amount, twice the salt content and no mushroom flour)	[24]
Mushrooms flour (<i>P. ostreatus</i>) at 7.5% [hydrated with water in a ratio 50/50 (w/w)]	Low-fat and low-salt liver pâté	Washing, draining, and cutting into 5mm thick slices, oven drying (60 °C for 18 h) and milling (0.5 mm mesh). Addition of the other ingredients and further processing	Increased moisture (+8.7%), protein (+5.1%), carbohydrate (+304.1%), dietary fiber (from 0 to 3.31 g/100g), and a _w (+0.9%) values compared to the control. Decreased fat (-44.3%), ash (-28.9%), and Na (-40.3%) levels compared to the control. No variation in the amino acid composition compared to the control. During storage: decreased L* value (-14.8% and 16.9%, at 0 and 90 days of storage, respectively), higher gumminess and chewiness only at 0 time storage (+17.2% and +18,3%, respectively). (Control contained twice contents of salt, fat, phosphates and nitrates, and no mushroom flour)	[111]

Abbreviations: WHC: water holding capacity; TBARS: Thiobarbituric acid reactive substances (byproducts of lipid peroxidation); TDF:total dietary fiber.

P. ostreatus powder has also revealed a feasible strategy to reduce the caloric value and improve the nutritional content of liver pâté [111]. The authors reduced 50% of fat, salt, phosphate, and nitrite contents, and increased the dietary fiber in pâté by adding mushroom powders. The addition of dietary fiber and protein from mushroom contributed to increasing the pâté moisture content. Despite this, an increase in softness, generally observed when substituting fat by other vegetable sources or vegetable oils due to the increase of water content or the presence of more unsaturated fatty acids [138,139], was not observed in this case: probably, addition of protein content from mushroom affected the protein/fat/water ratio, thus modifying the protein–water and protein-protein gel network, and, therefore, the gel consistency [139]. Increasing *P. ostreatus* powder up to 10% resulted in the worst sensory scores, while 7.5% of mushroom addition showed acceptable levels and was comparable to the control. A similar work was carried out by Cerón-Guevara et al. [110], who partially replaced 30 and 50% of pork backfat, 50% of salt, and 50% of phosphates in frankfurters sausages with mushroom flours. All the experimented formulations (sausages containing 2.5% or 5% of *P. ostreatus* flour, and sausage containing 2.5% of *P. ostreatus* plus 2.5% of *A. bisporus*) resulted in increased moisture and dietary fiber contents, keeping the amino acid profile. Although color, flavor and taste of the sausages supplemented with mushrooms had lower scores compared to the control, they ranked in the acceptable level. However, it must be highlighted that when replacing, and thus reducing, fat in meat products, this can negatively affect the sensory attributes [140], since fat provides foods with yellowness and palatability. *P. ostreatus* flour addition also resulted in softer and less cohesive sausages, probably because the reduction of fat was not compensated by increasing protein, thus resulting in losing stability and negatively affecting the texture of the low-fat systems [110].

4.2. Dairy products

The high fiber content of *Pleurotus* species has been exploited to produce functional dairy products [141] because both powder and extracts of *Pleurotus* have revealed a good source of prebiotics [142,143]. However, few works (see Table 2) are present in the literature concerning the use of *Pleurotus* spp. as ingredient in dairy products.

Pelaes Vital et al. [144] incorporated *P. ostreatus* aqueous extract (POE) to produce a low-fat yogurt with improved functional and rheological properties. Total phenolic compounds and antioxidant activity were higher in all supplemented yogurts (from 0.25 to 1% of the final concentration of POE), as well as the viable counts of *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. Furthermore, the high content of phenolic compounds in the extract of *P. ostreatus* provided more stability to the casein networks, due to the interaction of protein-polyphenol and their strong internal bonds: this resulted in the reduction of protein arrangement during storage, with consequent maintenance of water in the network and reduced syneresis. Increased water in the gel system also increased cohesiveness, adhesive, and springiness, and decreased firmness of the supplemented yogurts compared to the control. However, the authors [144] did not evaluate the taste of the new products, which could be negatively affected by their high contents of phenolic compounds.

Table 2. Effects of *Pleurotus* spp. on quality attributes of dairy products.

Mushroom variety and Levels used	Types of foods	Mushroom pretreatment	Effects	References
Mushroom aqueous extracts (<i>P. ostreatus</i>) at 0.50, 0.75, 1%	Low-fat yogurt	Drying at 55°C, crushing into powder, stirring in Millipore water for 30 min at 70°C, and filtering (250 mm)	Darker color, increase in phenolic compounds, antioxidant activity, and viable count of <i>S. thermophilus</i> and <i>L. bulgaricus</i> . Lower syneresis and firmness, more adhesiveness, springiness, and cohesiveness than control	[144]
β -glucans extract from mushroom body (<i>P. ostreatus</i>) at 0.4% (w/w)	Fat white-brined cheese	Defatting of the freeze-dried powder with EtOH (Soxhlet \times 8h), residue soaking with 0.9% NaCl solution (70°C \times 24h), centrifuge (5700rpm \times 10 min), residue extraction with NaOH 1M (40°C \times 8h), neutralization with CH ₃ COOH 1M, washing of the precipitate (β -glucans) with distilled water until blenching	During storage no statistical differences in composition, color parameters, proteolysis and organoleptic evaluation compared to the control. During storage decreased hardness (-24.4% after 120 days) and brittleness (-33.2% after 120 days)	[145]
	Ovine soft spreadable cheese		During storage no statistical differences in composition, color parameters, viscosity, antioxidant activity, appearance, texture, and overall acceptability. Increased flavor from the 14 th day of storage onwards (+10.7% and +8.0% after 14 and 21 days of storage, respectively)	[146]
Tocopherol-rich extracts from mushroom mycelia (<i>P. ostreatus</i>)§ (<i>P. eryngii</i>)§	Yogurt	Extraction with MeOH and hexane (1:8:8) by vertexing, addition of saturated NaCl aqueous solution and homogenization, centrifugation (5 min, 4000g), transfer of the hexane fraction to a 25 mL amber vial, re-extraction (twice) with hexane, evaporation to dryness (under N ₂) of the combined extracts	Increased carbohydrates content (+5.3%, both <i>P. ostreatus</i> and <i>eryngii</i>) and antioxidant activity (DPPH and reducing power) for both <i>P. ostreatus</i> and <i>eryngii</i> , compared to the control (<i>P. ostreatus</i> higher than <i>P. eryngii</i>). Decreased ash levels (-5.7% <i>P. ostreatus</i> ; -4.5% <i>P. eryngii</i>). No significant differences in moisture, protein, fat, lactose, and fatty acids profile	[148]

Abbreviations: DPPH: 2,2-diphenyl-1-picrylhydrazyl (antioxidant activity assay)

§ each one incorporated at a concentration corresponding to the EC50 obtained in the reducing power assay.

Low-fat cheeses generally result in harder and more rubbery texture as well as inferior taste compared to their full-fat counterparts [145]; for this reason, β -glucan extracts from *Pleurotus* were employed to improve textural properties in a feta-type cheese with reduced fat content [145]. Hardness and brittleness of the cheeses supplemented with β -glucans were better compared to the control until 120 days of storage, while no significant difference was observed at the end of storage (180 days). The same β -glucan extract from *Pleurotus* (0.4% w/w) was also used to fortify ovine soft spreadable cheese [146], where did not significantly affect the composition, color, viscosity, proteolysis, and lipolysis of the supplemented cheese, while resulted in higher moisture at 1st (+2.6%) and 21st (+4.3%) days of storage, and in higher flavor scores after 14 days of storage. Higher moisture values were due to the high water binding ability of β -glucans, while better flavor evaluation agreed with that reported by Khorshidian et al. [147], according to which supplementation of dairy products with β -glucans higher than 1% has negative effects on their sensory characteristics.

Finally, Bouzgarrou et al. [148] enriched yogurt, which is generally poor in vitamin E (except when mixed with nuts or seeds) with tocopherol-rich extracts from mushroom mycelia: the new yogurt formulations resulted in higher antioxidant activity compared to the control, which remained unchanged even during storage. Interestingly, *P. ostreatus* extract showed higher antioxidant activity (both DPPH and reducing powder) compared to *P. eryngii*, probably due to their different composition in tocopherol profiles: this also resulted in higher antioxidant activity of the yogurt supplemented with tocopherol-rich extract from *P. ostreatus* compared to that obtained from *P. eryngii*.

4.3. Cereal-based products

Cereal-based products, widely consumed all over the world [149], are not recognized as reach protein foods and are deficient in the essential amino acid lysine [150]. Furthermore, some technological processes often result in the removal of important nutrients from the grains, notably iron, zinc, B-vitamins (thiamine, riboflavin, niacin, folate), as well as several phytochemicals [151]. Different attempts have been made to fortify cereal-based products with proteins, vitamins, minerals, phenolic compounds, and fiber [149] coming from different dietary sources, such as legumes or other vegetable plants, to enhance their nutritional quality [92].

In the last years also the possibility of using mushrooms as fortifying agents has been exploited [152–154] thanks to their good nutritional characteristics. Siyame et al. [152], for example, observed increasing levels of protein, ash, crude fiber, and decrease in carbohydrate content with increasing mushroom supplementations (30, 40 or 50%) into maize flour. Similar trends were also reported by Bamidele and Fasogbon [153]. However, when the same flours were used to prepare porridge, significant decrease of sensory attributes were observed with increasing mushroom addition.

Dietary fiber (DF) is defined as “the edible constituent of analogous carbohydrates or plants which is resistant to digestion and absorption in the human small intestine, with partial or complete fermentation in the large intestine” [155]. It is recognized as a fundamental constituent of a health-promoting diet because DF has been positively associated with a decreased risk of development of chronic diseases [156]. Furthermore, high fiber content has been correlated with high satiety value [157]. Traditionally, cereals such as oat, wheat and corn were used to improve the DF content of foods [158], but nowadays also edible mushrooms are recognized as a good source of dietary fiber [159]. Since dietary fiber might absorb a large amount of water, its presence in bakery products could also affect the water holding capacity, thus reflecting in different purposes of the raw materials: flours with high

water absorption are required for bread production, whereas flours with low water holding capacity are generally used for cookies [160].

4.3.1. Bread

Gaglio et al. [119] functionalized bread with mushroom powder, increasing the bread content of biotin, cobalamin, and cholecalciferol, which are not generally present in white bread, thanks to the addition of 5% or 10% (w/w) of *P. eryngii* powder. The overall assessment of bread functionalized with mushroom powder (at both levels) was comparable to the control, even if significant differences were observed for several aspects. Some bread attributes (height and softness) decreased with mushroom addition; on the contrary, the redness of crust and void fraction and cell density of crumb increased. A similar work was carried out by Ndungu et al. [161], who supplemented bread with 0, 5, and 10% of dried *P. ostreatus* powder: as usual, an increase in protein content of the supplemented bread, together with increased moisture and ash levels, were observed. It is worth noting that supplementation with 15% of *P. ostreatus* powder resulted in no formation of a visco-elastic dough, with no consequent bread production, despite the authors [161] blanched the mushroom before milling to inactivate proteases, which could decrease dough strength with negative effects on bread volume and height. Contrary to what Gaglio et al. [119] reported, supplementation of bread with *P. ostreatus* increased niacin, and decreased thiamine, while cobalamin was not present; also, other B vitamins and all amino acids significantly increased with increasing mushroom content (see Table 3). Regarding sensory evaluation, bread supplemented with 5% of *P. ostreatus* powder gave comparable results to the control (white bread), while supplementation with 10% was judged by the semi-trained panellists with lower scores (see Table 3). A further work of the same authors [162] also reported a significant increase of phenolic compounds in bread supplemented with *P. ostreatus* powder (see Table 3). The positive effect of *Pleurotus eryngii* powder addition in bread made with tender wheat on the vitamin contents (B₁, B₂, B₃ and D), as well as total polyphenols and β -glucans levels, were observed also in the work of Cirlincione et al. [163].

Okafor et al. [150] evaluated the effect of different supplementations (5, 10, 15, 20 and 25%) of *P. pulmonarius* powder on bread quality. The authors observed a significant increase in the content of crude protein (from 7.96 to 14.62%), ash (0.90–2.64%) and crude fiber (from 0.51 to 2.48%). Regarding some main bread attributes, water absorption increased with increasing mushroom supplementation, while other parameters decreased (see Table 3). From an organoleptic point of view, bread was acceptable up to 10% of *P. pulmonarius* supplementation, after which its sensory score significantly decreased.

4.3.2. Biscuits and snacks

Different studies have evaluated the inclusion of different *Pleurotus* powders in biscuits to improve the nutritional qualities or technological characteristics.

Kim et al. [164] evaluated the quality characteristics and antioxidant activity of cookies added with different percentages (10, 20 and 30%) of *P. eryngii* powder. The authors observed no significant differences in bulk density and water content among the doughs. Increasing the content of *P. eryngii* powder in cookies resulted in decreased spread factors, leavening rates, and L* value of cookies, while a* value, hardness and antioxidant activity were gradually increased. Taste and overall acceptability

resulted highest in cookies with 10% of *P. eryngii* powder.

Some studies have been performed on *P. sajor caju*. Ng et al. [156], for example, developed cinnamon biscuits formulated with different levels of *P. sajor-caju* powder: gradually replacing wheat flour with mushroom powder reflected in increasing levels of total dietary fiber, protein, ash, a* and b* values and firmness, whereas L* value and sensory scores decreased. Although the biscuit with the highest (12%) mushroom powder still had high nutrient contents, it resulted in the lowest sensory scores (even if still acceptable), probably due to the high degree of firmness, stronger mushroom aroma, and flavor, as well as dark color of the biscuit surface. Incorporation of *P. sajor-caju* powder up to 8%, instead, was a good compromise to develop a biscuit with high nutrient contents, together with acceptable physical and sensory characteristics. A similar work was reported by Prodhan et al. [165], who incorporated increasing levels (0, 5, 10 or 15%) of *P. sajor-caju* powder into biscuits: total dietary fiber was significantly higher than control in biscuits supplemented with 10 and 15% of *P. sajor-caju* powder. Although the sample supplemented with 15% mushroom powder significantly increased the protein content compared to the control (+10.7%), it received the lowest sensory evaluation. No significant differences ($p>0.05$) were observed for ash, moisture, protein, and fat contents, even if an increasing trend could be seen; the same was observed for the decrease in carbohydrates ($p>0.05$). At the same time, mushroom powder supplementation in biscuits did not affect the bulk density, the spread ratio, and the spread factor. Biscuits incorporated with 10% of *P. sajor-caju* powder resulted in the best compromise for both improved nutritional quality and sensory evaluation. The incorporation of *P. sajor-caju* in biscuits was also performed by Wan Rosli et al. [92] and Bello et al. [166], whose results showed an improvement in the nutritional qualities of the biscuits. However, Bello et al. [166] showed that biscuits supplemented with 20 and 30% *P. sajor-caju* powder had the lowest overall acceptability, probably due to their darker color and unpleasant taste. All these studies on *P. sajor-caju* confirm that the addition of more than 10% of this mushroom in the powdered form into biscuits, although it improves some nutritional and/or technological characteristics, is not acceptable for consumers.

Recently, Ng et al. [21] also reported that the high dietary fiber content of *P. sajor-caju* might affect the glycemic index of foods. When incorporating mushroom powder into biscuits, in fact, the authors observed a decrease in the sizes of the starch granules as well as irregular spherical shapes. At the same time, a lower starch digestion rate index was observed, together with high protein, insoluble dietary fiber, and β -glucan contents derived from mushroom powder: all these phenomena resulted in markedly reduced glycemic index of the biscuits [21].

Variations in the pasting and thermomechanical properties of wheat flours supplemented with *P. eryngii* powder were also observed by Biao et al. [167], with decreasing viscosity attributes and dough stability time with increasing mushroom levels. This was due to the dilution effect on the content of wheat gluten by high levels of mushroom powder, with a consequent decrease in dough stability, and to the greater water absorption capacity of the mushroom fiber, which therefore reduced the amount of water available to exhibit plasticizing effects [168]. When the blended flours were used to produce cookies, sensory scores improved with increasing mushroom levels up to 15%, after which they decreased due to the adverse effect of mushroom powder on color, flavor, and texture of the cookies [167].

Recently, a whole grain cereal product (breadsticks) enriched with vitamin D₂ from *P. ostreatus* was developed by Proserpio et al. [169] and tested for children's acceptance: although control samples were significantly preferred, samples with mushroom powder fortification at 2% and 4% were well accepted and obtained comparable liking scores to each other, while increasing mushroom powder up to 6% received the lowest liking scores. It is worth noting how breadsticks enriched with 2% and 4%

respectively provided 32% and 54% of the recommended daily amount of vitamin D₂ [170] per single dose (50g), much more than other fortified products with vitamin D usually present at the market [169].

4.3.3. Pasta

Kim et al. [171] prepared pasta by replacing common wheat flour at 2%, 4%, and 6% with β -glucan-rich fractions (BGRFs) from *P. eryngii* mushroom powder. Increasing BGRFs level resulted in decreased peak viscosity, holding strength, and final and setback viscosity, due to the lower amylose content probably caused by the high fiber content of BGRFs. Generally, high setback and final viscosity values are related to a high rate of soluble amylose retrogradation in starch [172,173] and are often associated with the firm texture of pasta and noodle products [174]. Hence, the addition of BGRFs may represent a potential way to increase dough stability and reduce pasta quality deterioration [171]. High supplementation levels of β -glucans also resulted in the increased cooking loss, hardness, and adhesiveness, probably due to the more regular network and porous structure of the supplemented cooked pasta. At the same time, high supplementation of BGRFs, which are considered a soluble fiber, decreased swelling index, water absorption, and color.

P. ostreatus has also successfully replaced wheat flour in noodles [175–177]. In the work of Arora et al. [175] increasing levels (0, 2, 4, 6, 8, 10%) of mushroom addition to instant noodles resulted in increased cooking time, water absorption, and tensile strength as well as solid loss during the cooking of noodles, although the increase was significant only after 4% addition of mushroom powder. It is interesting to note how peak viscosity first increased with increasing mushroom levels and then sharply declined (from 6% to 8%). This is because mushroom fiber is known to absorb water, therefore at a certain mushroom concentration, the reduction of available water in the system would reduce initial starch granule swelling, thus explaining the lower peak viscosity of the pastes [178]. The same trend was observed for breakdown, which is a measure of cooked starch granules' susceptibility to disintegration [179]. Breakdown strength first increased with mushroom powder addition (up to 6%) and then decreased, probably due to starch brittleness because of reduced water availability due to the high content of mushroom proteins [178]. Supplemented noodles with mushrooms had lower sensory attributes compared to the control, but their nutritional profile was certainly improved [175]. Similar results were obtained by Parvin et al. [176] where increasing levels (0, 5, 8, and 10%) of *P. ostreatus* were used to fortify noodles: the only exception was the cooking time, contrary to what was reported by Arora et al. [175], which decreased with increasing mushroom supplementation. Also, Wahyono et al. [177] reported an optimal supplementation level with *P. ostreatus* (5%) in noodles similar to those reported by Arora et al. [175] and Parvin et al. [176]. Even though increasing levels of mushrooms resulted in increasing trends of ash, protein, crude fiber, cooking loss and cooking yield, the differences were not statistically significant ($p > 0.05$), but high standard deviations were reported by the authors [177]; on the contrary, water activity significantly increased with mushroom supplementation. Enrichment of noodles with *P. osteratus* powder at the level of 5% allowed not to compromise color, aroma, and the texture of the noodle, despite higher supplementation levels.

Table 3. Effects of *Pleurotus* spp. on quality attributes of cereal products.

Mushroom variety and Levels used	Types of foods	Mushroom pretreatment	Effects	References
Fresh mushroom (<i>P. ostreatus</i>) at 30%	Maize flour	Washing with tap water, slicing, solar-drying to a moisture content below 10%, milling, and sieving (1mm)	Increased protein (+110.9%), ash (+472.3%), crude fiber (+102.8%), Fe (+169.5%), Ca (+234.5%), K (+206.2%) and decreased carbohydrate (-33.4%). In porridge prepared with mushroom flour at 30%, decreased color (-10.6%), aroma (-4.6%), flavor (-11.2%), texture (-10.2%), overall acceptability (-9.0%)	[152]
Mushroom powder (<i>P. pulmonarius</i>) at 10%	Bread	Drying and milling (60 mesh). Addition of the other ingredients and further processing	Increased water absorption (+17.8%), protein (+39.1%) and ash (+73.3%) contents. Decrease in carbohydrate (-6.7%), loaf volume (-25.9%), specific volume (-23.6%), crumb grain (-17.1%) and loaf quality (-25.0%).	[150]
Mushroom powder (<i>P. eryngii</i>) at 10%	Bread	Chopping into small pieces, blanching in boiling tap at 100 °C for 3 min, drying in a cabinet drier at 40 °C for 6 h, and milling into flour (sieve size 500 mm). Addition of the other ingredients and further processing	Increased levels of moisture (+16.6%), total ash (+32.1%), protein (+109.8%), P (+44.5%), K (+44.3%), Fe (+99.0%), Zn (+107.8%), Cu (+200.0%), Glu (+47.2%), Asp (+237.9%), Leu (+86.9%), Phe (+44.9%), Arg (+78.5%), Ile (+62.7%), Thr (+104.3%), Trp (+540.0%), Val (+14.3%), Lys (50.9%), Met (+118.2%), riboflavin (+219.4%), niacin (+206.4%), pantothenic acid (+222.1%), pyridoxine (+328.6%), biotin (+85.7%), folate (+61.3%), caffeic acid (+131.0%), ferulic acid (+27.6%), acetylated dimethyl flavone (+68.1%), apigenin hexoside (+82.1%), procyanidin (+637.5%), isovitexin (+57.1%), C-xyloside (+700.0%), vicenin (+933.3%), luteolin (+933.3%), apigenin glucoside (+69.0%), orientin (+875.0%), lucenin (+1050%), chlorogenic acid (+7.1%), genistein (+2.6%), daidzein (+3.6%). Decrease in carbohydrate (-62.0%), thiamin (-14.1%), malonylglycitin (-3.1%), malonyldaidzin (-20.7%), acetylgenistin (-26.7%), glycitein (-43.2%), genistin (-16.7%), and sensory scores [color (-42.4%), texture (-46.9%), aroma (-56.0%), taste (-59.0%), overall acceptability (-61.4%)]	[161,162]

Continued on the next page

Mushroom variety and Levels used	Types of foods	Mushroom pretreatment	Effects	References
Mushroom powder (<i>P. eryngii</i>) at 10%	Cookies	Slicing into 3 mm tick, drying at 50 °C × 5 hours, and then pulverization. Addition of the other ingredients and further processing	Decreased spread ratio (-11.1%) and loss rate (-15.3%), increased leavening rate (+9.7%) and hardness (+11.6%). Increased total phenolic content (+142.9%), antioxidant activity (FRAP +71.4%, DPPH +200.2%), and overall acceptability (+21.4%). Increased a* value (from 0 to 1.23) and decreased L* value (-5.4%)	[164]
Polysaccharide extract of flour of fruiting body base (<i>P. sapidus</i>) at 10%	Cookies	Cleaning, drying (food dehydrator at 40 °C), grinding and sieving. Flour washing with 96% EtOH (v/v) for 24 h (continuous stirring at room T), filtering, drying (food dehydrator: 60 min, 40 °C), extraction with distilled H ₂ O, autoclaving (45 min, 121 °C), cooling down, centrifuge (10 min at 6000 rpm), volume concentration (1:10 by rotary evaporator at low temperature), washing with cold EtOH 98.8% (1:3), centrifuging (4000 rpm for 10 min) and freeze-drying. Addition of the other ingredients and further processing	Decreased appearance (-13.8%) and color (-14.9%). No significative difference in taste, aroma, flavor, overall acceptability compared to the control	[6]

Continued on the next page

Mushroom variety and Levels used	Types of foods	Mushroom pretreatment	Effects	References
Polysaccharide extract of fruiting body base (<i>P. sapidus</i>) at 20%	Steamed buns		Decreased appearance (-16.7%) and color (-18.2%). No significant difference in taste, aroma, flavor, overall acceptability compared to the control	
Mushroom powder (<i>P. sajor-caju</i>) at 10%	Biscuits	Cleaning with water, chopping into small pieces, blanching in hot water (3% salt and 0.01% citric acid) at 100 °C for 3 minutes, draining off the water, drying in sunlight at 33 °C for 48 hours; cooling at room temperature, grinding into powder, and sieving (65 mesh). Addition of the other ingredients and further processing	Increased crude fiber (+10.1%), reduced texture (-17.1%), taste (-28.2%), aroma (-12.7%), overall acceptability (-23.0%), color and acceptance (-14.0%) compared to the control	[165]
Mushroom powder (<i>P. sajor-caju</i>) at 8%	Biscuits	Low-heat air-drying at 50–55 °C until yield 10% (w/w), milling and sieving (125 mm). Addition of the other ingredients and further processing	Increase in protein (+14.3%), ash (+10.5%), TDF (+155.8%), IDF (+148.3%), SDF (+183.8%), β -glucan (+975%), a* (+5.3%) and b* (+4.7%) values, aroma (+13.3%), color (+4.7%), pasting and gelatinization temperatures. Decreased L* value (-4.2%) and moisture (-8.8%). Decrease in all the pasting viscosity and gelatinization enthalpy	[21,156]

Continued on the next page

Mushroom variety and Levels used	Types of foods	Mushroom pretreatment	Effects	References
Mushroom powder (<i>P. sajor-caju</i>) at 4%	Biscuits	Rinsing with clean water, blanching, and coarse chopping (sizes 2–5 mm), draining of excess water (50 °C until constant weight), grinding into powder and sieving (125 mm). Addition of the other ingredients and further processing	Increase in ash (+21.4%), TDF (+10.3%), β -glucan (+ 9.1%), crispiness (+15.0%). Decreased moisture (–30.9%) and color (–18.7%)	[92]
Mushroom powder (<i>P. sajor-caju</i>) at 10%	Biscuits	Cleaning, cutting into slices, drying at 60 °C for 8 h, grinding and sieving (80 mesh). Addition of the other ingredients and further processing	Increased moisture (+40.1%), protein (+3.2%), crude fiber (+8.1%), ash (+84.9%), Ca (+123.6%), K (+55.2%), Mg (+43.0%), P (+266.6%), Fe (+41.7%), Cu (+25%), Zn (+675%). Decreased fat (–5.5%), carbohydrate (–1.1%), Na (–34.3%), crispness (–22.0%), taste (–23.3%), aroma (–20.4%), color (–20.8%), overall acceptability (–25.3%)	[166]
β -glucan-rich fraction of mushroom powder (<i>P. eryngii</i>) at 4%	Pasta (common wheat flour)	Oven drying at 55 °C for 24 h, grinding and sieving (50 mesh); mixing of mushroom powder with distilled H ₂ O (5%, w/v), stirring for 5 min, filtering (Miracloth), resuspending in distilled H ₂ O, autoclaving (120 °C, 10 min), filtering (Miracloth), and freeze-drying. Addition of the other ingredients and further processing	Decreased holding strength (–8.0%), final viscosity (–7.1%), setback viscosity (–5.9%), swelling index (–9.0%), water adsorption (–14.1%), L* value (–25.2%, on cooked pasta). Increased a* (+299.4%) and b* (+6.3%) values	[171]

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Mushroom variety and Levels used	Types of foods	Mushroom pretreatment	Effects	References
Fresh mushroom powder (<i>P. ostreatus</i>) at 4%	Instant noodles	Sun-drying for 5 h, drying in a cabinet drier for 2 h at 50 °C (to a final moisture content of 5%) Grinding by powder-making machine. Addition of the other ingredients and further processing	Increased antioxidant activity (DPPH +77.0%; total phenols +16.3%), protein (+17.3%), ash (+1.1%), dietary fiber (+8.9%), holding strength (+307.7%), and breakdown strength (+76.3%). Decreased fat (-3.5%), moisture (-8.6%), final viscosity (-14.7%), color (-19.2%), aroma (-6.8%), and overall acceptability (-8.6%)	[175]
Fresh mushroom powder (<i>P. ostreatus</i>) at 5%	Noodles	Slicing into 3 mm thick, blanching in boiling brine (2% NaCl for 3 min), cooling down, spreading on trays, and drying in a thermostatically controlled oven (50 °C for 6 h), grinding and sieving (80 mesh). Addition of the other ingredients and further processing	Increased protein (+12.9%), ash (+2.9%), fat (+10.2%), dietary fiber (+441.7%), Ca (+37.9%), Fe (+146.9%), K (+322.6%), Na (+80.3%), water absorption (+10.2%), and solid loss (+6.5%). Decreased moisture (-6.8%), carbohydrate (-2.4%), texture (-7.1%), and cooking time (-18.5%)	[176]
Fresh mushroom powder (<i>P. ostreatus</i>) at 5%	Noodles	N.R.	Increased water activity (+1.7%) and redness (+100.0%); decreased whiteness index (-6.9%)	[177]

Abbreviations: FRAP: Ferric Reducing Antioxidant Power (antioxidant activity assay); DPPH: 2,2-diphenyl-1-picrylhydrazyl (antioxidant activity assay); TDF: total dietary fiber; IDF: insoluble dietary fiber; SDF: soluble dietary fiber; N.R.: not reported.

4.4. Other plant-based foods

The characteristics of herbal seasoning enriched with *P. sajor-caju* powder were evaluated in the works of Bahri and Wan Rosli [180,181]. The authors [181] observed that replacing more than 40% of coconut milk in the herbal seasoning with *P. sajor-caju* powder resulted in better functionality of the paste because viscosity and all textural attributes resulted increased, due to the high fiber content of mushroom. Moreover, herbal seasoning with *P. sajor-caju* powder resulted in improved nutritional quality (increase in protein and total dietary fiber, together with decreased fat and carbohydrate levels) and no significant variation in sensory attributes, except for lower aroma and overall acceptability compared to the control, which, however, were not statistically significant in 100% replacement of coconut milk with *P. sajor-caju* powder [180]. That's because increasing levels of mushroom powder reduced the aroma of coconut milk powder, even if for the 100% substitution the sensory panels accept the replacement of ingredient, probably due to the heat treatments influence on the acceptance of the product [180].

Powder of *P. sajor-caju* was also employed to develop a novel snack food based on Papad, a typical food adjunct consumed in India [182,183], resulting in increased mineral, crude protein, and fiber contents, as well as sensory score. Also, Balan et al. [184] developed a novel snack, a mushroom protein crisp, which was healthier compared to commercially popular snacks sold in the USA markets: it was lower in calories and salt, with no saturated fatty acids, and with increased content of protein, dietary fiber and some minerals, as well as with an accepted flavor profile by consumers.

Verma and Singh [185] fortified a typical Indian food (potato pudding) by replacing potato with increasing levels (5%, 10%, 15% and 20%) of *P. ostreatus* powder: replacement at 5% level resulted in improved nutritional content (mainly protein and crude fiber) and better sensor characteristics compared to the control.

Proserpio et al. [186] developed a vegetable-based product (pumpkin and carrot soup) with increasing concentration of *P. ostreatus* (from 0% to 6% of mushroom powder), observing that 2% of *P. ostreatus* supplementation resulted in comparable liking scores to the control. Even if vegetable soup is already reached in fiber, the addition of *P. ostreatus* improved soup functionality thanks to the β -glucans from mushroom, providing 2.2 g of β -glucans per serving (300g). Increasing mushroom levels, however, decreased the sensory scores of the supplemented vegetable soups evaluated by adolescents.

Table 4. Effects of *Pleurotus* spp. on quality attributes of other plant-based foods.

Mushroom variety and Levels used	Types of foods	Mushroom pretreatment	Effects	References
Mushroom powder (<i>P. sajor-caju</i>) at 20%	Papad (Indian snack food)	Treatment with 1% KMS × 30 min, dipping in whey × 30 min, drying at 50 °C, powder passing through 350 mm Addition of the other ingredients and further processing	Increased amount of protein (+15.8%), mineral (+38.9%), fiber (+218.2%), and acceptability. Reduction in energy value	[182]
Mushroom powder (<i>P. sajor-caju</i>) at 20% (on the total)	Herbal seasoning	Drying under Biodehydration™ system, and grinding into fine powder (1mm mesh size) Addition of the other ingredients and further processing	Decreased fat (−69.4%), carbohydrates (−4.5%), and total solid (−9.9%) contents. Increased moisture (+5.7%), protein (+63.9%), TDF (+41.8%), viscosity (+108.9%) and textural attributes [firmness (+93.9%), consistency (+75.7%), adhesiveness (+105.2%), and index of viscosity (+ 68.3%)] compared to the control (containing 20% of coconut milk and 0% of mushroom powder)	[180,181]
Mushroom powder (<i>P. ostreatus</i>) at 1.2% (on the total)	Potato pudding	Washing, trimming, blanching for 3 min, draining, oven drying (110 °C for 10 hours)/sun drying, grinding, and sieving Addition of the other ingredients and further processing	Increased protein (+20.0%), fiber (+766.7%), carbohydrate (+1.2%), fat (+0.7%), and ash (+1.6%) levels. Decreased taste and flavor (−2.2%), increased texture (+7.5%), color and appearance (+2.5%), overall acceptability (+2.4%).	[185]

KMS: potassium metabisulphite.

5. Fermentation of foods with *Pleurotus mycelia*

Fermentation is a process generally used in the food industry to stimulate the formation of biologically active substances derived from raw materials and/or fermentation products [187]. It is also one of the solutions employed, along with boiling, milling or sprouting, to reduce or prevent the negative effects of some antinutritional factors on the bioavailability of some nutrients [188]. The presence of phytic acid and phenolic compounds in some vegetables which can bind to minerals and proteins, thereby reducing mineral bioavailability and protein digestibility, is only an example.

Among fermentation processes, solid-state fermentation is carried out by microorganisms grown on solid particles in the absence of water but with enough moisture to enable their growth [189]. Compared to the liquid-state type (submerged fermentation), solid-state fermentation has the advantages of being faster, less expensive, and most approaches to the natural environment of several microorganisms [190]. While solid-state fermentation by mushrooms is a well-known process for degrading lignocellulosic materials, including agri-food by-products [4], it is still poorly explored in the food industry, unlike fermentation in solid-state carried out by bacteria and yeasts [188].

To enhance the nutritional value and digestibility of lentil flour, Asensio-Grau et al. [188] fermented lentils with *P. ostreatus*: fermented flours resulted in lower carbohydrate level (−6%) and increased content of protein (+18.5%) and phenolic compounds (+53%). Carbohydrates represent the main carbon source for mushroom growth, thus explaining their decrease in fermented products [191]. The increase in protein content could be due to different phenomena: i) bioconversion of some carbohydrates into protein [192]; ii) prevention of nitrogen losses for ammoniac volatilization, due to the acidophil nature of *Pleurotus* and their ability to reduce pH by releasing organic acids [193]; iii) increase of unicellular protein biomass due to *Pleurotus* growth [57]; iv) production of extracellular fungal enzymes during growing and bioconversion [194]. According to Espinosa-Páez et al. [195], increased phenolic compounds in fermented foods by mushrooms were due to different mechanisms: i) synthesis of phenols in the mycelium or hydrolysis of conjugated phenolics; ii) deamination of aromatic amino acids phenylalanine and tyrosine, which are precursor of phenolic acids [196]; iii) phenol oxidases secreted by mushrooms, which are employed to degrade lignocellulosic materials and obtain nutrients as substrates. Furthermore, Hur et al. [197] reported that some microbial enzymes secreted during fermentation can hydrolyze glucosides and break the cell matrix of seeds and starch, thus enabling the release of phenolic compounds. Finally, Asensio-Grau et al. [188] observed an increase of hydrolyzed protein fraction in fermented flours, because proteins were already partially hydrolyzed by mushroom lytic mechanisms during fermentation.

Espinosa-Páez et al. [195], instead, evaluated the effect of fermentation by *P. ostreatus* on flours of kidney beans, black beans, and oats: antioxidant activity and total phenolic compounds significantly increased in fermented products compared to the controls, even after simulated digestion, together with significant increase in protein digestibility and essential amino acids. Increased amino acids in fermented products confirmed the effect of *P. ostreatus* in the synthesis of essential amino acids [198]. A decrease of the antinutrient tannins was also observed in fermented products, thanks to tannase activity present in *Pleurotus* [199], which also contributed to improving protein digestibility. In a further work by Espinosa-Páez et al. [195] the same flours of kidney beans, black beans, and oats fermented by *P. ostreatus* were employed in the development of functional cookies with different flours formulations: antioxidant activity and bioavailable proteins remained higher in cookies made with fermented flours, which also showed higher fiber content and lower sugar levels compared to

commercial cookies. Also, the sensory acceptability of the cookies with fermented flours was positively evaluated.

Sánchez-García et al. [200] obtained similar results in increased protein content after fermentation of quinoa and lentil seeds, and quinoa flour, while a decrease in total phenolic content and antioxidant activities were observed during fermentation. A previous work of Xu et al. [201] had already observed that total phenol contents (TPCs) of fermented cereals varied with fermentation time and the starter organisms. Hence, with equal fermentation time and *Pleurotus* species employed (2 weeks and *P. ostreatus*, respectively), differences in the results observed by Sánchez-García et al. [200] compared to those reported by Espinosa-Páez et al. [195] and Asensio-Grau et al. [188] regarding TPCs evolution during fermentation were probably due to the different strains employed and to differences in the enzyme production by mushroom, whose optimal activity is obtained on a specific time in a given culture [200]. Fermentation by *P. ostreatus* of quinoa and lentil seeds and flour also resulted in decrease of the antinutrient phytic acid [200].

Oh et al. [187], instead, fermented tofu with mushroom mycelia to increase tofu functionality. The authors observed an increase of isoflavone aglycones in the fermented product compared to the unfermented one, probably due to the action of β -glucosidase produced by the mushroom mycelia, which catalyzes the release of isoflavone aglycones [202]. The increase in isoflavone aglycone contents also resulted in improved physiological activities, such as the inhibition of angiotensin I-converting enzyme (ACE) and α -glucosidase, as well as anti-cancer and anti-inflammatory activities. In particular, tofu fermented with *P. ostreatus* mycelia showed higher percentage of isoflavones in the aglycone form compared to tofu fermented with *L. edodes* (89.1% vs 51.3%). This is of particular importance because isoflavones, which are considered phytoestrogens and may have a preventive effect on the development of some cancers [187], are less absorbed by the small intestine in the glycosidic form compared to isoflavone aglycones, due to the higher hydrophilicity and greater molecular mass of isoflavone glycosides [203].

6. Discussion and future aspects

Like most of edible mushrooms, *P. ostreatus* have a good nutritional profile, which results from the low content of calories, fat and sodium, and high levels of dietary fiber, protein, and minerals, as well as the presence of many secondary metabolites (bioactive compounds) showing several health benefits. However, mushrooms are generally consumed after cooking, so further studies should be carried out on cooked mushrooms. Furthermore, mushrooms are taken into the human body after ingestion, hence further studies should be carried out, both *in vivo* and *in vitro*, to study in deep the metabolic pathways of mushroom bioactive compounds and their actual actions on human health. On the other hand, mushrooms also contain chitin, lectins, phytates, oxalates, tannins, and saponins, all of which belong to the class of antinutrients, which can interfere with the uptake/absorption of nutrients by humans and limit the bioavailability of essential nutrients [204]. However, very few works have been published on *Pleurotus* antinutrients [205,206]. Even if many antinutrients are well-known to possess many beneficial effects and therapeutic potential on several human diseases [207], further studies should be carried out to better understand the role of antinutrients on mushroom intake and absorption, since it has already been reported, for example, that chitin decreases mushroom digestibility [64]. Toxicological aspects or side effects of mushrooms consumption also need to be addressed to guarantee safe delivery of mushroom products to the market.

Edible mushrooms in general, and therefore also the *Pleurotus* genus, possess all three functionalities of food: nutrition, taste, and physiological functionality [208]. All these characteristics make them suitable for many applications as dietary supplements. However, mushrooms are still an under-exploited source of food ingredients and potentially bioactive compounds.

Although many scientific works have been published about the most sustainable preservation methods to retain mushrooms' nutritional value and bioactive properties, the results cannot be compared because the preservation methods were applied to different mushrooms species. Even when different preservation methods were used on the same mushrooms species, it can be incorrect to compare the results from different studies because mushrooms' chemical composition varies significantly within the same species [91], and according to the growing substrate, as well as postharvest methods. Therefore, further research is needed to be focused on the development of mushrooms post-harvest preservation methods, and the valorization of the waste generated during these processes.

The use of mushroom powder facilitates its incorporation into new formulations and its use as food ingredient also represents a tool to increase mushrooms' shelf life, which is generally very short. The production of mushroom powders or flours, generally obtained through drying and grinding, results in water removal with consequently increased mushroom shelf life, concentrates mushroom nutrients and facilitates the handling and storage of mushrooms.

The addition of *Pleurotus* to foods generally results in darker products, due to the dark color of this mushroom [208]. On the other hand, polyphenol oxidase present in mushrooms could promote oxidation, thus affecting the color of the new products [209], but also Millard reaction and lipid peroxidation following technological processes could lead to nonenzymatic browning [210]. When using mushrooms as flour replacer, generally a decrease in pasting properties is observed, together with darker and firmer products [140]. It had already been reported in the literature that addition of fibers to bread resulted in decreased volume and increased firmness, but the extent of modification depended on the type of fiber [211]. Therefore, variations in volume and firmness observed in bakery products following mushroom addition would mainly be due to the contribution of fiber derived from oyster mushroom. Decreased specific and loaf volumes of bread could be come from the dilution of gluten protein in dough system obtained following mushroom powder supplementation [212]. Furthermore, mushroom powder could cleave gluten strands during mixing, impair gas retention and change crumb texture [213].

The high content of dietary fiber (DF) in mushrooms could be a useful tool to claim a portion of food as "source of DF" or "high in DF", depending on the amount of mushrooms added to food to reach at least 3 g or 6 g of DF per 100 g of serving, respectively [214]. In this way, enriching foods with mushrooms into staple food could offer the promising potential to increase the daily dietary fiber intake of the population. The same approach could be used by fortifying foods with *Pleurotus* to meet the recommended daily intake of vitamin D: these foods could be a valid alternative for subjects who generally consume a low amount of fish, such as children, or for people who are intolerant to lactose, since most of the fortified products with vitamin D available on the market are dairy-based products. Furthermore, vitamin D from mushrooms certainly represents a more sustainable production than vitamin D from animal source.

Supplementation of mushroom powder in pasta has some negative effects on cooking and textural properties, i.e., increased cooking loss and firmness. However, improved nutritional value is gained, thanks to the enrichment with dietary fiber, protein, and other bioactive ingredients.

Partially meat replacing with mushrooms was shown to affect the nutritional composition of the

final products, with a general decrease in protein and fat contents and increasing total dietary fiber and β -glucan levels. Textural and sensory properties of meat-based products with mushrooms incorporation could be affected by mushroom addition, but they also depend on cooking processes and the presence of other ingredients. Generally, when adding or supplementing *Pleurotus* to meat, polysaccharides and protein of mushroom form a three-dimensional matrix able to bind water and fat in the meat system, thus increasing cooking yield and decreasing thawing loss [215], especially during storage of the frozen products [130]. Although *Pleurotus* can be considered an excellent source of protein, before completely replacing meat proteins with mushroom proteins further research is needed, especially regarding the technological functionality of mushroom. Excessive reduction of meat proteins, in fact, can usually result in weakening of the protein-protein gel emulsion matrix, thereby reducing the ability to hold water, and increasing cooking loss and shrinkage. However, the several endogenous enzymes of mushrooms with different activities can exert opposite effects on the meat structure, such as improving meat tenderness by protease [216] or promoting lipid oxidation by lipoxygenase with consequent formations of protein cross-linking structure [217]. This could be partly associated with the different results reported in the literature regarding the influence of mushroom addition on the sensory and textural properties of the supplemented meat products. Therefore, further research is needed to deepen these mechanisms. Indeed, proteins from mushroom sources could be considered more sustainable and with lower cost compared to proteins from meat origin. Other plant proteins, although lower production cost and environmental impact compared to meat [218], cannot be defined as complete: for example, cereals have low lysine contents, while legumes are deficient in sulfur amino acids (e.g., methionine and cysteine) [219]. Mushrooms are considered to be richer in essential amino acids when compared to vegetables [35], and their proteins show high thermal and pH stability [220], hence potential applications of mushrooms in the food industry seem to be wider compared to vegetable proteins. It should be also noted that the addition of edible mushrooms to meat products represents a potential alternative to the use of salt and phosphate [221] as well as nitrite [122], thus improving the nutritional quality of the supplemented meat products.

Moreover, current alternatives to meat products are mainly represented by soy-based foods, but soy is one of the major allergens, which could limit consumers' choices of foods [130]. Therefore, the substitution of soy proteins with mushrooms could be a safe alternative to consider, but it needs further research.

The intense umami attribute of mushrooms could be exploited as a feasible strategy to reduce the salt content of foods, especially in meat-based products, since the umami taste of mushrooms is often referred to as a meaty taste. However, acceptability and consumption of supplemented foods with mushrooms could be limited for those consumers which are unfamiliar with umami flavor, unlike Asian people.

According to Qing et al. [121] the effect of *Pleurotus* on the overall flavor of the new supplemented foods could be due to the flavorful substances of mushrooms or to endogenous enzymes of mushrooms able to promote the oxidation of proteins and lipids, hence strengthen the formation of flavor substances. However, excessive oxidation could lead to bad flavor and taste; hence, the optimal levels of mushroom supplementation need to be further investigated.

Antioxidant and antimicrobial activities of some compounds naturally present in *P. ostreatus* are well known, and several works focused on the antioxidant activity of mushroom powders or extracts in foods, especially in products with high fat levels. However, the use of *P. ostreatus* as an alternative to synthetic antioxidants and antimicrobials is still poorly explored. Further research could be carried

out also in the sector of emulsified meat products, such as frankfurters or burgers, whose generally high fat contents may be the cause of lipid oxidation and microbiological spoilage, with negative effects also on the product shelf life.

Finally, solid-state fermentation by *Pleurotus* spp. has revealed a powerful tool to improve the nutritional value of the fermented foods. This process, in fact, results in reduced contents of antinutrients (such as phytic acid and tannins) with consequent increase of nutrients bioavailability (especially minerals and proteins), and increased levels of some compounds, such as proteins and phenolic compounds.

7. Conclusions

Nutritional value, antioxidant activity and the presence of several health-promoting compounds in *Pleurotus* make these mushrooms really functional foods. All these properties, together with *Pleurotus* flavor and texture properties, have recently attracted the food industry's attention to use oyster mushrooms as a valuable food ingredient or replacer in several food formulations, also to meet the increasing consumers' demand for more healthy and sustainable foods.

According to the several results reported in this review, *Pleurotus* are particularly suitable to be defined as functional ingredients. Addition of 4–10% of *Pleurotus* mushroom (in the dried form) generally results in improved nutritional attributes and acceptable sensory characteristics of the supplemented foods. Hence, mushroom fortification could represent a potential way to develop functional foods well accepted by consumers. Fortification with *Pleurotus* of such foods that represent the main source of nutrients in low- and middle-income countries or that are largely consumed all over the world could have important health impacts as well.

Finally, considering that *Pleurotus* spp. can grow efficiently on low-cost substrates, they play a fundamental role in the development of “sustainable nutritious foods”. They also well fit with the concept of the circular economy since mushroom by-products and surplus materials can be easily converted into new valuable resources.

What results from this review supports the idea that *Pleurotus* have no longer to be considered niche foods but could be promising foods and ingredients.

Conflict of interest

All authors declare no conflicts of interest in this paper.

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