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Use of Medicinal mushrooms in the preparation of "*Superfoods*" for sustainable nutrition and human health

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Every great advance in science has issued from a new audacity of imagination.

John Dewey, 1929

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Chapter 1. General introduction

In the last few years, the interest of research and food industries is focusing on the development of foods that can prevent several diet-related diseases and disorder. Foods that satisfy these requirements are commonly called *"Superfoods*". This is the commercial term, popularized by the media, to refer to foods that may have health properties; it is the commonly used and best-known term for "*Functional foods*" [1].

The term "*Functional food*" was coined in Japan in the early 1980s with the definition of: "*processed foods that have disease prevention and/or health promotion benefits in addition to their basic nutritional value*" [2]. These foods can lower the cost of healthcare of the aging population and, at the same time, give a commercial potential for the food industry [3]. A food (natural or processed) is therefore defined as superfood if, in addition to its nutritional properties, it is scientifically proven that have a positive influence on one or more physiological functions, reducing the risk of diseases related to improper diet and, in general, improve the health of the consumer [4].

According to Robertfroid [5] the objectives of functional foods are different:

- Gastrointestinal functions: microflora balancing, immune activity, nutrient bioavailability, increase intestinal motility, probiotics and prebiotics effects.
- Oxidative stress reduction: stabilize free radicals resulting from oxidative processes (polyphenols, carotenoids, flavonoids, vitamins) to balance oxidant activity and defense systems.
- Metabolism regulation: regulate the assimilation of macronutrients by the modulation of relative's hormone (e.g. carbohydrates and insulin) and prevent risk of pathogenic effects (insulin resistance).
- Improves fetal and early life development: for example the relevance of folic acid in pregnant women's diets, as well as the involvement of long-chain polyunsaturated fatty acids in the early stages of brain development.
- Protective: role of phytonutrients in protecting the body from toxicity and carcinogenicity caused by xenobiotics.
- Regulation of physical and cognitive functions: improves memory, attenuates neuronal damage and prevent cognitive dysfunction.

There are between 2.2 and 3.8 million known species of fungi worldwide, of which 150,000 have been named, 2000 are thought to be edible, and more than 200 species of wild mushrooms are thought to have therapeutic properties [6].

For millennia, mushrooms have been considered by Eastern civilizations as a nutritional and pharmaceutical resource because they are able to influence human health. People appreciated mushrooms for their culinary properties (rich in volatile organic compounds) and nutritional value (high proteins and vitamins content), following the discovery of their medicinal properties, used as dietary supplements and as drugs in "mycotherapy" [7].

Medicinal mushrooms are macroscopic fungus, mainly Basidiomycetes, that are used as extracts or powder to treat, prevent, or cure illnesses and/or for nutritional purposes.

Although they are mostly utilized as functional foods or dietary supplements, several studies have indicated that they may also be used as genuine medications [8].

These properties were imputable to different molecules with biological activities included in mycelia and fruiting bodies. Many of these biomolecules, processed and accumulated by fungi during their biological cycle, play a fundamental role in nutrition and human health and belong to different classes of chemical compounds and these are vitamins, polysaccharides, proteins, peptides, lectins, fatty acids, lipids, phenolic compounds, terpenoids, triterpenoids and sterols [9].

Over years, it has been studied that Macromycetes (Basidiomycetes and Ascomycetes) possess various pharmacological properties such as anticancer, antibacterial, antifungal, antiviral, cytotoxic, immunomodulatory, anti-inflammatory, antioxidant, antiallergic, antidepressant, antihyperlipidemic, antidiabetic, digestive, hepatoprotective, neuroprotective, nephroprotective, osteoprotective and hypotensive [10].

The worldwide mushroom market was 14.35 million tonnes in 2020, and it is expected to increase to 24.05 million tonnes by 2028. Consumers most frequently purchase Reishi (*Ganoderma lucidum*), Lion's Mane (*Hericium erinaceus*), Chaga (*Inonotus obliquus)*, Turkey Tail (*Trametes versicolor*), Shiitake (*Lentinula edodes*), and Cordyceps (*Ophiocordyceps sinensis*) [11].

There are several papers in the literature (Table 1) in which the addition of fungal mycelium or fruiting bodies in various foods has been shown to increase the nutritional value and total availability of vitamins, minerals, fiber, β-glucans and antioxidants, making these foods true "Superfoods."

Table 1. Foods and beverages fortified with mushrooms.

Mushroom species	Functional food/beverages	References
Agrocybe aegerita	Snack food	Brennan *et al*., 2012; 2013
Auricularia auricula	Bread	Fan *et al*., 2006; Yuan *et al*., 2017
Agaricus bisporus	Snack food Bread Sponge cake Meat balls	Singla *et al*., 2009 Ahmad & Singh, 2016 Salehi *et al*., 2016; Arora *et al*., 2017

		Süfer *et al*., 2016
Agaricus blazei	Yoghurt Milk	Stojkovic *et al*., 2014 Vital *et al*., 2017
Agaricus blazei, Antrodia camphorata, Hericium erinaceus and *Phellinus linteus*	Bread	Ulziijargal *et al*., 2013
Agaricus bohusii	Cream cheese	Reis *et al*., 2012
Boletus aereus	Pork meat product	Stojkovic *et al*., 2015
Boletus edulis	Beef burger Wheat Bread	Barros *et al*., 2011 Vlaic *et al*., 2019
Boletus edulis, Lentinua edodes and Agaricus bisporus	Extruded product	Xikun *et al*., 2020
Calocybe indica	Cookies Bread	Rathore *et al*., 2019 Oyetayo & Oyedeji, 2017
Cordyceps militaris	Extruded product	Zhong *et al*., 2017
Flammulina velutipes and *Tricholoma matsutake*	Beer-like drink	Okamura *et al*., 2001
Ganoderma amboinense, *Agaricus* spp., *Fomes yucatensis* or mixed mushrooms	Soup and sauce	Laroche & Michaud, 2007
Ganoderma lucidum	Beer Wine Emulsion type sausage	Leskosek-Cukalovic *et al*., 2010 Nguyen *et al*., 2020 Ghobadi *et al*., 2018
Laetiporus sulphureus	Chicken pate	Petrovic *et al*., 2014
Lentinula edodes	Baked food Noodle Frying batter Bread	Kim *et al*., 2011 Kim *et al*., 2008; 2009 Kim *et al*., 2010 Li Yun *et al*., 2008
Lentinula edodes, Boletus edulis and Agaricus bisporus	Bread	Lu *et al*., 2021
Pleurotus eryngii	Bread	Gaglio *et al*., 2019
Pleurotus ostreatus	Bread Meat balls Potato pudding Whole-Grain Cereal Product Noodles Vegetable soup	Ndung'u *et al*., 2015 Süfer *et al*., 2016 Verma & Singh, 2017 Proserpio *et al*., 2019 Parvin *et al*., 2020 Proserpio *et al*., 2019
Pleurotus plumonarius	Bread	Okafor *et al*., 2012
Pleurotus sajor-caju	Papad (An Indian snack food) Biscuits Wheat and rice based products	Parab *et al*., 2012 Bello *et al*., 2017 Aishah & Wan Rosli, 2013
Schizophyllum commune	Cheese-like food	Okamura-Matsui *et al*., 2001
Suillus luteus and *Coprinopsis atramentaria*	Cottage cheese	Ribeiro *et al*., 2015
Tirmania pinoyi	Soup	Stojkovic *et al*., 2013

1.1 Objective of PhD research

Several works have focused on the use of mushroom species native to Eastern countries, and only a few authors have considered the use of native strains, generally known only for their culinary value, for the development of Functional foods. For these reasons, the research project of this thesis focused on the use of wild-collected *Pleurotus eryngii* var. *eryngii* mushrooms, isolated and grown in the laboratory, to produce different types of functional foods and beverages.

Specifically, in Chapter 2, knowledge about the bioactive compounds possessed by the best-known medicinal mushroom species, their use as mycotherapeutics and the clinical studies

conducted were explored, while in Chapter 3, "myco-chemical" compounds in wild and cultivated mushrooms were studied with a special focus related to nutrition and health. Chapters 4 and 5 reported the experimental activities conducted for the development of two products, a food (bread) and a fermented beverage (beer), respectively, into which *P. eryngii* var. *eryngii* was added in powder form to improve their technological, sensory, and functional characteristics. In conclusion, Chapter 6 provides final remarks on the work done and future perspectives.

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Chapter 2. Medicinal Mushrooms: Bioactive Compounds, Use, and Clinical Trials

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2.1 Abstract

Medicinal mushrooms have important health benefits and exhibit a broad spectrum of pharmacological activities, including antiallergic, antibacterial, antifungal, anti-inflammatory, antioxidative, antiviral, cytotoxic, immunomodulating, antidepressive, antihyperlipidemic, antidiabetic, digestive, hepatoprotective, neuroprotective, nephroprotective, osteoprotective, and hypotensive activities. The growing interest in mycotherapy requires a strong commitment from the scientific community to expand clinical trials and to propose supplements of safe origin and genetic purity. Bioactive compounds of selected medicinal mushrooms and their effects and mechanisms in *in vitro* and *in vivo* clinical studies are reported in this review. Besides, we analyzed the therapeutic use and pharmacological activities of mushrooms.

2.2 Introduction

Mushrooms, which have always been appreciated for their culinary and nutritional value, are now increasingly valued for their many important medicinal properties, so much so that they are used not only as dietary food (functional foods) but also in the form of dietary supplements, nutraceuticals, and mycotherapy products [1,2]. Their use for promoting and maintaining a good state of health and the treatment of diseases has been around since ancient times in Asian regions, while in the West, this approach is considerably more recent. Medicinal mushrooms (MMs) are reported to have numerous pharmacological actions such as antimicrobial, anti-inflammatory, immunomodulatory, antidiabetic, cytotoxic, antioxidant, hepatoprotective, anticancer, antioxidant, antiallergic, antihyperlipidemic, and prebiotic properties, among others [2–5]. These activities are attributable to many bioactive metabolites present in the mycelium but above all in the fruiting body, whose biological effect varies according to the chemical nature and whose distribution varies according to the fungal species. A great deal of research has been carried out and is increasingly being undertaken to identify and characterize mycochemicals and to define their actions and mechanisms, due to the growing interest in the use of natural products, including as adjuvants in traditional therapies. The bulk of research carried out has focused on a few genera or species, for example, those of the oldest and most traditional use among Asian populations, while for those remaining, current scientific support is still lacking. Several studies have so far investigated the various activities of MMs, highlighting their enormous

potential for use in the medical sector, but a particular effort has been put into studying their antitumor and immunomodulatory properties, as cancer remains one of the most difficult challenges to date. The pharmacological activities of a medicinal fungus are detected primarily by *in vitro* assays, generally accompanied or followed by *in vivo* studies in animal models, which together reveal the great potential of a mushroom, fungal extract, or chemical compound. On the other hand, a small number of clinical studies carried out on humans and published in the peer-reviewed literature are available. Clinical studies are necessary to assess the efficacy of medicinal mushrooms within the complex human body system but also to assess their safety [2,5,6]. This review aims to provide a discussion of some of the best-known and most studied bioactive metabolites of medicinal mushrooms. In particular, we highlight the mechanisms of action by *in vitro* and preclinical scientific studies.

2.3 Bioactive Compounds in Medicinal Mushrooms: Effects and Mechanisms of in vitro and in vivo Preclinical Studies

As mentioned above, fungal compounds with bioactivity, and which are potentially useful for the prevention and treatment of various diseases, are very diverse. The most important are polysaccharides, structural components of the fungal cell wall. The polysaccharides have a strong ability to carry biological information. More specifically, they have antitumor, immunomodulatory, antioxidant, anti-inflammatory, antimicrobial, and antidiabetic activity. In reality, the type and modulation of these biological activities are influenced by the specific structural features of the molecule, such as the weighted degree of branching, backbone linkage, side-chain units, and the type of constituent monosaccharides. The best known and most abundant are α- and β-glucans.

Heteroglycans, peptidoglycans, and polysaccharide–protein complexes also contribute to biological activity [7–9]. They are primarily responsible for immunomodulatory effects due to their ability to bind to specific cell wall receptors and stimulate specific immune responses. Medicinal mushrooms are usually used in cancer treatments as biological response modifiers (BRMs), useful for treating cancer, reducing the side effects of therapies, and improving the quality of the patient's life [1,3].

Another class of compounds that are very important for their bioactivity are the terpenes, characterized by units of five-carbon isoprene atoms and whose addition of functional groups produces the terpenoids. They modulate the immune system by stimulating the expression of genes coding for proteins involved in the immune response, but also have anti-inflammatory, antioxidant, and antitumor properties. High terpenoid contents are found in mushrooms belonging to the genus *Ganoderma* P. Karst. [2,7,9].

Mushrooms are rich in proteins, which have cytotoxic and anticancer properties. Some of them are known for their characteristic and marked immunomodulatory effect. These proteins are indicated as fungal immunomodulatory proteins (FIPs) whose mechanisms of action can be diverse [2,9]. Proteins also include lectins, which bind reversibly to mono- and oligosaccharides with high specificity, recognizing and interacting with various carbohydrates and proteoglycans on the cell surface. They are involved in many biological activities, such as innate immunity and cell-to-cell interaction, and their immunomodulatory mechanism varies depending on the origin of the compound. They also have immunomodulatory, antitumor, and antiproliferative properties [7,9]. Other fungal metabolites with bioactivity are phenolic compounds, antioxidants with different mechanisms of action (oxygen scavenging, metal inactivation, free radical inhibition, peroxidase decomposition), laccases (copper-containing oxidases), and fatty acids [2].

2.3.1 *Coriolus versicolor* (L.) Quél.

Coriolus versicolor, commonly known as tunzhi in China or turkey tail, has always been used as a "magic herb" in Asian regions and particularly in China, where ancient formulations based on this mushroom have been and are still widely used to promote good health, strength, and longevity [10,11]. Thousands of years ago in China, its medicinal properties were reported in the "Compendium of Materia Medica" and "Shen Non-Compendium Medica", and currently in China, since 1987, and Japan, since 1977, *C. versicolor* extracts have been approved in routine clinical practice, especially in integrated cancer therapy in conjunction with chemotherapy or radiotherapy [10–12]. In China, there are currently at least 12 *C. versicolor*-based drugs approved by the State Administration of Food and Drugs (SAFD) for clinical use. The immunomodulatory properties of this mushroom are due to two protein-bound polysaccharides present in the fungal extract: the polysaccharide peptide (PSP), extracted from the deep layer cultivated mycelia of the COV-1 fungal strain and used most extensively in China, and the glycoprotein PSK (krestin), derived from the strain CM101 and used most widely in Japan. They are mainly composed of β-glucans and are among the most studied mushroom bio-compounds.

PSP extraction is done by boiling mycelia or even basidioma in water and further precipitation in ethanol. This protein-bound polysaccharide has a molecular weight of approximately 100 kDa, has a polysaccharide-to-peptide balance of 90–10%, is highly water soluble, and its carbohydrates are mannose, xylose, galactose, and fructose. [11].

PSP possesses immunomodulating, antitumor, anti-inflammatory, and antiviral effects, as reported by several *in vitro* and *in vivo* studies and some clinical trials; it has also shown other physiological effects, such as liver-protecting, system-balancing, antiulcer, antiaging

and learning, and memory-enhancing properties, as well as reducing adverse events related to chemotherapy and radiotherapy treatments [10,12]. The immunomodulatory activity is due to the ability to a) act on cytokine release, b) increase the expression of cytokines and chemokines such as tumor necrosis factor-α (TNF-α), interleukins (IL-1β and IL-6), histamine, and prostaglandin E, c) activate natural killer (NK) cells and enhance dendritic and T cell infiltration into tumors. These actions are attributable to the β-glucan component of the polysaccharide, as these compounds are known to activate various immune cells expressing the corresponding receptors (such as dectin-1, toll-like receptors TLR-2, TLR-4, and TLR-6, and CR3 complement receptors) [11,12]. PSP induces apoptosis in human promyelocytic leukemia HL-60 cells by lowering the Bcl-2/Bax ratio and the mitochondrial transmembrane potential, releasing cytochrome c and activating caspase-3, -8, and -9 [13].

Studies *in vitro* or in mice models have shown an increase in lymphocyte proliferation and immunoglobulin IgG levels, suggesting effects on humoral immunity by PSP. Other results suggest a role for PSP in the activation of various pattern recognition receptors (PRRs), fundamental, therefore, in the innate immune response upon an encounter with a pathogen-associated molecular pattern (PAMP) [11]. One type of PRR whose specific level of action is a function of the infecting pathogen is toll-like receptors, which represent the body's first line of defense and on which the PSP can act positively. Antiviral action of this kind was demonstrated *in vitro* by Rodríguez-Valentín *et al*. [14], showing that PSP not only downregulated viral replication and promoted the upregulation of specific antiviral chemokines, such as RANTES, MIP-1α/β, and SDF-1α, with blocking action on HIV-1 coreceptors in THP1 cells and human peripheral blood mononuclear cells (PBMCs), but upregulated TLR4 expression. A study lead by Wang *et al*. [10] in mice carrying a defective or normal TLR4 gene recorded how PSP stimulates the expression of both cytokines and TLR4 and its downstream signalling molecule TRAF6 and increases the phosphorylation of the transcription factor NF-κB p65 and the activator protein AP-1 transcription factor component γ-Jun in peritoneal macrophages from TLR4+/+ mice, but not from TLR4-/- mice. This shows that PSP-mediated immunomodulatory action occurs via the TLR4 signalling pathway. Besides, a reduction in tumors was also found compared to normal saline treatment.

Many *in vitro* studies in different models (human PBMCs from healthy or cancer subjects, murine splenic lymphocytes, primary mouse peritoneal macrophages, etc.) have shown that PSP treatment induces higher levels of TNF-α and the cytokines associated with it and IL-1β (the proinflammatory signal that enhances lymphocyte proliferation), but also IL-12 (enhancer of NK and CD8+ T cell activity and inducer of interferon IFN-γ), IL-6, and Il-1α,

affecting the expression of many other pleiotropic cytokines, e.g., transforming growth factor (TGF)-β (proinflammatory effects on monocytes and Th17 cells; anti-inflammatory effects on B cells and regulatory T cells (T(regs)) and the activation of macrophages, and it also induces superoxide dismutase (SOD) and increases the sensitivity of immune cells to other stimuli [11,12].

PSK is a proteoglycan of about 100 kDa, with a polysaccharide-to-peptide balance of 40–60%. The carbohydrates are mannose, xylose, galactose, arabinose, and rhamnose [11]. Since it is also composed mainly of β-glucans, its activities and mechanisms are similar to PSP, again demonstrated *in vitro* or in mice models. Krestin showed both direct and indirect cytotoxic effects on cancer cells *in vitro*. As shown by Lu *et al*. [15] in their *in vitro* study on splenocytes from *neu* transgenic mice and in mice carrying a defective TLR2 gene, the antitumor action of this compound is mediated by TLR2 in mice. It increased dendritic cells (DC)s and CD4+ and CD8+ T cells, decreased B cells, induced the secretion of Th1 cytokines and IL-2, increased IFN-γ levels, and promoted DC maturation (CD86+ MHCII; higher levels of IL-12p40 and IL-12p70 were recorded, as was the inhibition of tumor growth. Other research has reported similar results, such as the activation of cytotoxic T cells (TC cells), improved DC maturation, the increased production of IL-8 and other cytokines (TNF-α, IL-1, IL-4, IL-6, IFN-γ) through the activation of T cell receptors (TCRs), enhanced major histocompatibility complex (MHC) class I expression by tumor cells, the inhibition of tumor growth, and the *in vitro* reduction of tumor growth factor-β (TGF-β) [16–18]. Thus, PSK can strengthen the body's natural immune response.

In a study on mice [19], this molecule was also shown to improve insulin resistance and hyperlipidemia by regulating the expression of inflammatory cytokines; a reduction in plasma triglycerides (TGs) and free fatty acids, the downregulation of the proinflammatory factors IFN-γ, IL-6, and IL-1β, and the upregulation of the expression of the anti-inflammatory factor IL-10 were observed. Therefore, PSK could be an important adjuvant in the management of cardiovascular risk related to hyperlipidemia. Despite the many results obtained to date, the mechanisms of action of these two protein-bound polysaccharides are still not fully understood. The peptide component may also play a role in the bioactivity of PSK and PSP.

2.3.2 *Ganoderma lucidum* (Curtis) P. Karst.

Long known as the "mushroom of immortality", *Ganoderma lucidum*, also known as ling zhi or reishi, is one of the most widely used medicinal mushrooms in the world today. It has been used to promote well-being and longevity since ancient times in traditional Chinese medicine, as it was included in Shen Nong's Materia Medica (206 BC-8 AD), and it is now

listed in the American Herbal Pharmacopoeia, Chinese Pharmacopoeia, and Therapeutic Compendium, and is also widely used as an adjuvant in the treatment of various types of cancer [20]. More than 100 reishi-based products are currently marketed, such as the nutraceuticals Ganopoly and Immunlink MBG, containing aqueous polysaccharide fractions, as well as a wide range of supplements often also containing other mushroom species extracts, functional foods, mycopharmaceuticals, and cosmeceuticals, prepared from carpophores, mycelia, or spore powder. *G. lucidum* is recognized for its numerous pharmacological properties, such as anticancer, hypoglycemic, immunomodulatory, antihypertensive, cytotoxic, anti-diabetic, antioxidant, antihyperlipidaemic, antimutagenic, antiaging, antimicrobial, and hepatoprotective properties, and many others. These properties are mainly due to two major groups of metabolites present in *G. lucidum*: triterpenes/triterpenoids and polysaccharides. Triterpene compounds are derivatives from lanosterol, including ganoderic acids, ganodermic acid, ganodermic alcohols, lucidones, and lucinedic acids, and they possess marked antitumor, antimetastatic, cytotoxic, and enzyme inhibitory properties. The main polysaccharides are α-1,3, β-1,3 and β-1,6-D-glucans and ganoderan with glucose as a major sugar component, characterized by a strong antiangiogenic and immune system-strengthening properties [20,21]. These two categories of molecules are primarily responsible for the anticancer properties of reishi, both by suppressing cell proliferation, metastasis, and invasion and by promoting apoptosis, combined with its immunomodulating, immunostimulating, antioxidant, and anti-inflammatory activities. In particular, concerning immunomodulatory action, it has been observed that this occurs through multiple mechanisms, such as the activation of cytotoxic T cells, B lymphocytes, dendritic cells, macrophages, NK cells, the TLR-4 pathway, and other immune cells, as well as their by-products TNF-α, interleukins IL-1, IL-2, IL-3, and IL-6, and active nitrogen and oxygen intermediates [20]. Lee *et al*. [22] found that the *G. lucidum* triterpenes butyl ganoderate A and B and butyl lucidenate A and N exert an inhibitory effect on adipogenesis in 3T3-L1 cells. The first and last of these compounds exerted this action by suppressing the mRNA expression levels of fatty acid synthase (FAS) and acetyl-CoA carboxylase (ACC) genes.

The researchers concluded that the inhibitory action of these triterpenes on 3T3-L1 cells is at least partly due to the downregulation of the adipogenic transcription factor sterol regulatory element-binding protein 1 (SREBP-1c) and its target Fanconi anemia (FA) group C gene (FAC) and ACC. Lucialdheydes A and C, ganodermanonol, and ganodermanondiol showed cytotoxic activity *in vitro* in Lewis lung carcinoma, sarcoma 180, T-47D, and Meth-A tumor cell lines [21]. An *in vivo* study on mice injected with inflammatory breast cancer

(IBC) and treated with the commercial extract ReishiMax GLpTM (carpophore and cracked spores) highlighted a selective action on gene and protein expression, with smaller tumor size and weight and reduced expression of E-cadherin, mammalian target of rapamycin (mTOR), human eukaryotic translation initiation factor 4G (eIF4G), and p70 ribosomal protein S6 kinase (p70S6K) and the activity of extracellular regulated kinase (ERK 1/2) [8]. It has been observed *in vitro* that the antiproliferative and proapoptotic action of reishi terpenes, polysaccharides, and proteins occurs by stimulating the proliferation of undifferentiated spleen cells and the production of cytokines and antibodies.

They also have an antimetastatic action, which they exert by activating the NF-κB and MAP kinase pathways, promoting cytokine release [20]. A great deal of research has been carried out on ganoderic acids; ganoderic acid T, for instance, has been shown to induce apoptosis in metastatic lung cancer cells by acting on the pathway linked to mitochondrial dysfunction and the expression of the tumor protein p53. Moreover, ganoderic acid D inhibited the proliferation of HeLa human carcinoma cells and induced G2/M cell cycle arrest and apoptosis [21]. Ganoderic acid DM has been shown *in vitro* to arrest osteoclastogenesis in bone marrow cells and RAW 264 cell D-clone (RAWD) by suppressing the expression of γ-Fos and nuclear factor of activated T-cells c1 (NFATc1), with the consequent inhibition of dendritic cell-specific transmembrane protein (DC-STAMP) expression and reduced osteoclast fusion [23].

In addition to anticancer activity, cardioprotective activity was also reported by [24] in their preliminary study using transverse aortic constriction (TAC) in mice to model pressure overload-induced cardiomyopathy and treatment with spore oil; the treatment resulted in a normalized ejection fraction, corrected the fractional shortening generated by TAC and reduced left ventricular hypertrophy; analysis of total RNA expression revealed the reduced expression of genes associated with cardiac failure, as well as reduced levels of RNA circ-Foxo3.

Ganodermanondiol has also been reported by Kim *et al*. [25] for its inhibitory effect on melanogenesis by assaying its inhibitor effects on tyrosinase activity and melanin biosynthesis in B16F10 melanoma cells. The researchers pointed out the inhibition of activity and the expression of cellular tyrosinase, as well as of the expression of tyrosinase-related protein-1 (TRP-1), TRP-2, and microphthalmia-associated transcription factor (MITF), leading to reduced melanin production. The mitogen-activated protein kinase (MAPK) cascade and the cyclic adenosine monophosphate (cAMP)-dependent signaling pathway were also affected. These results make reishi the perfect candidate for the preparation of skincare products. An antiaging action of *G. lucidum* was shown in a recent

study [26] by assaying the effects of ganoderic acid D on oxidative stress-induced stem cell senescence, using an H2O2-induced stem cell senescent model using human amniotic mesenchymal cells (hAMSCs) with a high expression of β-galactosidase, a senescence-associated marker. Ganoderic acid D inhibited the generation of reactive oxygen species (ROS) and senescence-associated markers, such as β-galactosidase, p21, and p16INK4a, and enhanced telomerase activity through the activation of the PERK/NRF2 signaling pathway. *G. lucidum* possesses a plethora of other bioactive metabolites with numerous effects, more than 400 of which can be found in the literature (Bulam *et al*. 2019). In addition to those already seen, there are also peptides (GLP from the water-soluble extract, perhaps the main agent responsible for the fungus's alternative oxidase (AOX) activity), peptidoglycans (GLPP, capable of neutralizing ROS damage in rat macrophages; ganoderan C with hypoglycemic activity), polyphenols, ergostan sterols and ergosterol, alkaloids, fatty acids with tumor proliferation-inhibiting action, nucleotides and nucleosides with platelet aggregation effects, the α-glucosidase inhibitor SKG-3, and laccase isoenzymes with antiviral properties [20,21].

2.3.3 *Lentinula edodes* (Berk.) Pegler

Lentinan, a β-1,3-D-glucan extracted from *Lentinula edodes* or shiitake, is another compound perdmitted in and widely used in Japan for the treatment of cancers, especially gastric cancer, due to its immunomodulatory action. Lentinan is a BRM capable of promoting Th1 response and improving Th1/Th2 balance. In several *in vitro* studies and in *in vivo* mice models, the polysaccharide activates dendritic cell function by increasing levels of tumor-infiltrating CD86+ cells, stimulates T and NK cell production, restores the killer/survival cell ratio, increases FcR receptor expression and thus promotes NK cell-mediated tumor cell killing, and increases IL-2 levels. The metabolite also appears to enhance complement-dependent cytotoxicity (CDC) and complement-dependent cell-mediated cytotoxicity via the CR3 receptor, known to be triggered by iC3b in fungal cell walls [27]. It can activate downstream signaling pathways, such as MAPK-NFκB and Syk-PKC, through binding to pattern recognition receptors such as TLR2/4/6/9 and Dectin- 1, the complement receptor CD11b, and other membrane receptors, with the consequent activation of T cells, NK cells, and macrophages [28]. In both *in vitro* and *in vivo* studies, it has also been shown to increase the cytotoxic activity of primary macrophages and RAW264.7 cell lines, as well as cytotoxic activity and TNF secretion in macrophages, and cytotoxicity against sarcoma S180 cells by upregulating the proapoptotic protein Bax and downregulating the antiapoptotic protein Bc1–2, thus inducing apoptosis [28,29].

The action of lentinan is also expressed by interfering with cell cycles, as demonstrated in an *in vitro* study on rat C6 glioma cells [30]. The C6 cells' activity was strongly inhibited in a dose- and time-dependent manner and the induction of apoptosis, cell cycle blockage, an increase in the proportion of cells in G0/G1 phase, and a decrease in those in S-phase were observed. Lentinan is also able to act on the activation of inflammasomes, components of the innate immune system responsible for triggering inflammatory responses. This action is inferred from the results of a study conducted by Ahn *et al*. [29] on myeloid cells, involving mouse bone marrow-derived macrophages treated with lentinan with/without inflammasome triggers. Lentinan was found to selectively inhibit absent in melanoma 2 (AIM2) inflammasome activation, upregulate proinflammatory cytokines, and induce the expression of inflammasome-related genes through TLR4 signaling. Moreover, assessing the effect of lentinan on mice treated with *Listeria monocytogenes* or lipopolysaccharide as an AIM2 or non-canonical inflammasome-mediated model, the researchers found that the polysaccharide reduced IL-1β secretion due to activation of the *Listeria*-mediated AIM2 inflammasome, and also reduced endotoxin lethality by inhibiting the activation of the non-canonical inflammasome. An inhibitory effect of lentinan on tumor angiogenesis mediated by increased IFN-γ production was demonstrated in a study on the lung carcinoma cell line LAP0927 and colorectal carcinoma cell line CT26 [31]. The polysaccharide resulted in the upregulated expression of angiostatic factors and especially IFN-γ, as well as increased tumor infiltration of IFN-γ-expressing T cells and myeloid cells.

Underpinning the current use of lentinan as an adjuvant in oncological therapies are also several preclinical studies in which the polysaccharide was tested alongside substances used in chemotherapy treatment. For example, lentinan has been shown to alleviate the ROS-mediated nephrotoxicity of cisplatin (a key drug in lung cancer treatment) by activating the Nrf2-ARE signaling pathway. Similarly, a synergistic action with the tumor drug paclitaxel on A549 cells was exerted by activation of the ASK1/p38 MAPK signaling pathway and, consequently, of the thioredoxin-interacting protein (TXNIP)-associated NLRP3 inflammasome (TXNIP-NLRP3) [28].

An aqueous extract, particularly rich in polyphenols, of shiitake, was tested on human tumor cell lines of laryngeal carcinoma (Hep-2) and cervical adenocarcinoma (HeLa) for assessing its antiproliferative activity [32]. The extract displayed high free radical scavenging and catalase-like and cytotoxic activities, as well as the inhibition of cell proliferation and the induction of apoptosis as well as the *Pleurotus sajor-caju* (Fr.) Singer extract also tested in this study, albeit to a lesser extent.

2.3.4 *Pleurotus* spp.

Although not the best-known medicinal mushrooms, the *Pleurotus* species also have proven biological and effects. Several studies have been carried out to assess their antioxidative, antimicrobial, antidiabetic, anticancer, anti-inflammatory, immunomodulatory, antihypercholesterolemic, antihypertensive, antimicrobial, hepatoprotective, and antiaging properties, although the mechanisms underlying these effects have often not been elucidated, nor have the metabolites responsible been identified or characterized. The immunomodulatory and antitumor activities of *Pleurotus ostreatus* (Jacq.) P. Kumm. were reported by Sarangi *et al*. [33] by assaying, *in vitro* and *in vivo*, the effect of water- soluble proteoglycan fractions extracted from *P. ostreatus*, or oyster mushroom, on a sarcoma-180-bearing mouse model. Treatment with the mushroom resulted in a quantitative reduction of tumor cells and their arrest in the pre-G0/G1 phase of the cell cycle, increased cytotoxicity of NK cells, and the stimulation of macrophages to produce nitric oxide. In a study by Jedinak and Sliva [34] comparing the impact of different medicinal mushrooms on the growth of breast and colon cancer cells, *P. ostreatus* (PO) proved to be the most effective, suppressing cell proliferation via the p53-dependent and p53-independent pathways. More specifically, the methanolic extract of the mushroom induced the suppression of the proliferation of the human breast cancer cell lines MDA-MB-231 and MCF-7 and colon cancer cell lines HCT-116 and HT-29, and caused cell cycle arrest in the G0/G1 phase in MCF-7 and HT-29 cells. Furthermore, in MCF-7 cells, it induced the expression of the tumor suppressor p53 and the cyclin-dependent kinase inhibitor p21(Cip1/Waf1) and inhibited phosphorylation of the retinoblastoma protein Rb; the upregulation of p21 expression and inhibition of Rb phosphorylation was also observed in HT-29. A few years later, Jedinak *et al*. [35] demonstrated the anti-inflammatory properties of PO by testing a mushroom concentrate *in vitro* on the RAW264.7 murine macrophage cell line and murine splenocytes, in the absence or presence of lipopolysaccharide (LPS) or concanavalin A (ConA), and *in vivo* on Balb/c mice with LPS-induced inflammation. PO suppressed the LPS-induced secretion of TNF-α, IL-6, and IL-12 from macrophages and inhibited the LPS-induced production of prostaglandin E2 (PGE2) and nitric oxide (NO) through, respectively, the downregulation of the expression of COX-2 and iNOS, the suppression of the LPS-dependent activation of AP-1 and NF-κB, the suppression of the secretion of TNF-α and IL-6 in mice challenged with LPS *in vivo*, and the inhibition of ConA-induced splenocyte proliferation and the production of IFN-γ, IL-2, and IL-6. A polypeptide (PEMP) extracted from *Pleurotus eryngii* (DC.) Quél. Mycelium demonstrated significant free radical scavenging and antitumor activity in breast, cervical, and stomach cancer cells, whose

growth it inhibited, and an activating effect on the macrophage-mediated immune response [36]. In a dose-dependent manner, it inhibited tumor cell proliferation, promoted macrophage proliferation and the expression of TNFand IL-6 secretion, TLR2 and TLR4, and stimulated macrophage phagocytosis through the release of NO and H2O2. Cold-water extracts of *P. eryngii* var. *ferulae* (Lanzi) Sacc. And *P. nebrodensis* (Inzenga) Quél. showed marked *in vitro* anticancer activity on human HCT116 colon cancer cell lines [37]. Both treatments showed a marked inhibition of cancer cell viability and apoptosis induction, and a significant increase in the Bax/Bcl2 mRNA ratio; they also inhibited cell migration and affected homotypic and heterotypic cell–cell adhesion by inducing an increase in E-cadherin expression and negatively modulated the phosphorylation of both protein tyrosine and extracellular signal-regulated kinase ERK1/2.

A hot-water extract of *Pleurotus pulmonarius* (Fr.) Quél. has been shown to reduce *in vitro* and *in vivo* liver cancer cell proliferation and invasion by inhibiting the autocrine vascular endothelial growth factor (VEGF)-induced PI3K/AKT signaling pathway [8].

2.3.5 *Grifola frondosa* (Dicks.) Gray

Grifola frondosa or maitake is another major medical mushroom with numerous medicinal properties and whose main bioactive metabolite is the so-called D-fraction or GFP, a β-glucan proteoglycan compound. Several studies have demonstrated its antitumor effect, such as the one conducted by Alonso *et al*. [38] on MCF-7 human breast cancer cells. Not only did it activate macrophages, T cells, and NK cells, but it also triggered the expression of BCL2-antagonist/killer 1 (BAK-1) and several other genes (RASSF-2, FADD, IGFBP-7, ITGA2, ICAM3, SOD2, CAV-1, Cul-3, NRF2, Cycline E, ST7, and SPARC) involved in apoptotic stimulation, the inhibition of cell growth and proliferation and cell cycle arrest, the suppression of tumor cell migration and metastasis, and the downregulation of the PI3K-AKT signaling pathway. In a subsequent study in rodent LM3 mammary adenocarcinoma cells, it was observed that D-fraction, in addition to inducing apoptosis and reducing cell motility and invasiveness, increased cell adhesion by upregulating E-cadherin protein levels and inhibiting matrix metalloproteinase-2 (MMP-2) activity [39], while in triple-negative breast cancer (TNBC) cells, in addition to the effects just described, it also suppressed MMP-9 and reduced cell–cell adhesion by also increasing the membrane localization of the β-catenin protein [40].

Two polysaccharide fractions obtained from GFPs and named as F2 and F3 showed promising hypoglycemic effects *in vitro*, most likely due to enhanced insulin resistance stimulated by the reactivation of insulin receptor (IR) and insulin receptor substrate-1 (IRS-1); both fractions reduced levels of fasting serum glucose (FSG), fasting serum insulin (FSI),

and the homeostasis model assessment of insulin resistance (HOMA-IR), and also enhanced IR and IRS-1 activities and the protein level of IR, reducing, on the contrary, that of IRS-1, and consequently acted on the PI3K/Akt pathways, as seen from the increased mRNA levels of PI3K and Akt [41].

Many other biologically active compounds have, however, been extracted from maitake and investigated for their effects. This is the case, for example, with a new glycoprotein extracted from the fermented mycelium of *G. frondosa*, GFG-3a. It can induce apoptosis in human SGC-7901 gastric cancer cells by acting on the stress response, p53-dependent mitochondrial-mediated, caspase-8/-3-dependent, and PI3k/Akt pathways [42]; apoptosis, cell cycle arrest at S phase, and the modulation of the expression of 21 proteins were observed and, in particular, the upregulation of 10 proteins, including RBBP4, associated with cell cycle arrest and the downregulation of 11 proteins, including RUVBL1, NPM, HSP90AB1, and GRP78, involved in apoptosis and stress response.

2.3.6 *Hericium erinaceus* (Bull.) Pers.

Very important and well-studied bioactive metabolites also include the erinacines (A-I), a group of cyathin diterpenoids extracted from the mycelium of *Hericium erinaceus* or lion's mane or yamabushitake, and hericenones (C-H), benzyl alcohol derivatives extracted from the fruiting body. Both groups of compounds can easily pass through the blood–brain barrier and have demonstrated neurotropic and neuroprotective effects. They are reported to induce nerve growth factor (NGF) synthesis, both *in vitro* and *in vivo*. However, this medicinal mushroom also has antioxidative, anti-inflammatory, anticancer, immunostimulant, antidiabetic, antimicrobial, hypolipidemic, and antihyperglycemic properties, although its most frequent use is for the treatment of neurodegenerative diseases and cognitive impairment [4,43,44]. Erinacin A, the main representative of the erinacine group, has been proven to have an effective protective effect against Parkinson's disease. In a 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse model of Parkinson's disease, erinacin A produced a reduction of MPTP-induced dopaminergic cell loss, apoptotic cell death induced by oxidative stress, and the levels of glutathione, nitrotyrosine, and 4-hydroxy-2-nonenal (4-HNE); it also reversed MPTP-associated motor deficits, and reduced the impairment of 1-methyl-4-phenylpyridinium (MPP)-induced neuronal cell cytotoxicity and apoptosis, through an endoplasmic reticulum (ER) stress-sustained activation of the IRE1α/TRAF2, JNK1/2, and p38 MAPK pathways, the expression of C/EBP homologous protein (CHOP), IKB-β, and NF-κB, as well as Fas and Bax [45]. This metabolite was also found to be effective against ischemic stroke, as reported in a study on rats in which the reduction of neuronal apoptosis, as well as the size of the stroke cavity in the brain by targeting

iNOS/reactive nitrogen species (RNS) and p38 mitogen-activated protein kinase (MAPK)/CCAAT enhancer-binding protein homologous protein (CHOP) pathways, was observed [46].

Erinacin A was also reported to have significant antitumor activity in human gastric cancer TSGH 9201 cells, in which it induced significant apoptosis associated with increased phosphorylation of focal adhesion kinase/protein kinase FAK/Akt/p70S6K and serine/threonine kinase PAK-1 pathways. It also resulted in increased cytotoxicity and ROS generation, the reduced invasiveness and activation of caspases, and the expression of tumor necrosis receptor TRAIL [47]. The strong antitumor action of this metabolite was subsequently confirmed by a recent study both *in vitro* in two human colon cancer cell lines (DLD-1 and HCT-116) and *in vivo* in a mouse model [48] that further clarified its mechanisms. Treatment effects included stimulation of the extrinsic apoptosis activation pathways (TNFR, Fas, FasL, caspases), suppression of the expression of the antiapoptotic molecules Bcl-2 and Bcl-XL, and phosphorylation of Jun N-terminal kinase JNK1/2, responsive to stress stimuli, NF-κB p50 and p330. It was also demonstrated that the upregulation of death receptor molecules through the JNK MAPK/p300/NF-κB pathway is mediated by the modification of histone H3K9K14ac; the results of the *in vivo* assay revealed, in fact, increased levels of histone H3K9K14ac, as well as histone acetylation on Fas, FasL, and TNFR promoters.

Another erinacin, erinacin C, is known for its antineuroinflammatory and neuroprotective actions, which could be achieved through a mechanism of inhibition of IκB, p-IκBα (involved in the upstream NF-κB signal transduction cascade), and inducible nitric oxide synthase (iNOS) protein expression, and the activation of the Nrf2/HO-1 stress-protective pathway [49]. The treatment of human BV2 microglial cells with LPS-induced inflammation resulted in reduced levels of nitric oxide (NO), IL-6, TNF-α, and iNOS, the inhibition of NF-κB expression, and the phosphorylation of IκBα (p-IκBα) proteins, as well as the inhibition of Kelch-like ECH-associated protein 1 (Keap1), and increased nuclear transcription factor erythroid 2-related factor (Nrf2) and the expression of the heme oxygenase-1 (HO-1) protein.

2.3.7 *Antrodia cinnamomea* T.T. Chang and W.N. Chou

Anctin-A (ATA) is a bioactive steroid-like compound isolated from *Antrodia cinnamomea* or the AC mushroom, a little-known medicinal mushroom in the West, but very popular in Taiwan, where this endemic species is traditionally used to treat liver disorders resulting from alcohol intake and has many recognized therapeutic properties in addition to its hepatoprotective one. This mycochemical was reported to have antitumor effects on human

MCF-7 and MDA-MB-231 breast cancer cells, in which it arrested epithelial-to-mesenchymal transition (EMT) processes by upregulating E-cadherin and occluding proteins and downregulating N-cadherin and vimentin proteins through suppressing their transcriptional repressor ZEB-1; it also caused induction of the ZEB-1 repressor miR-200c, associated with the transcriptional activation of p53, and the inhibition of cell motility and invasiveness [50].

The AC ethanolic extract also showed marked antiproliferative activity in human T47D breast cancer cells *in vitro* and *in vivo*. AC caused cell cycle arrest at the G1 phase, thus inhibiting proliferation, and induced autophagy. The reduced expression of cell cycle-related proteins and the increased expression of the transcription factor FOXO1, the autophagy marker LC3 II, and the protein p62 were recorded. The extract also mediated endoplasmic reticulum stress by upregulating the expression of inositol-requiring enzyme 1-α (IRE1), glucose-regulating protein 78 GRP78/Bip, and C/EBP homologous protein CHOP. Tumor growth was inhibited *in vivo* [51].

2.3.8 *Agaricus bisporus* (J.E. Lange) Imbach

Agaricus bisporus contain beta-glucans, ergosterol, ergothioneine, vitamin D, and flavonoids, with varying concentrations depending on the cooking method and duration, and UVB exposure [52]. Besides, essential amino acids, peptides, glycoproteins, nucleosides, triterpenoids, lectins, fatty acids, and their derivatives make this mushroom of considerable importance for its potential application as an antimicrobial, anticancer, antidiabetic, antihypercholesterolemic, antihypertensive, hepatoprotective, and antioxidant agent [53]. The consumption of *A. bisporus* in the diet is recommended to prevent prostate cancer due to the action of conjugated linoleic acid (CLA). Specifically, CLA inhibits proliferation in prostate cancer cell lines *in vivo*. The treatment showed an antiproliferative and proapoptotic action of mushroom extracts by inhibiting the growth of prostate cancer in athymic mice [54].

The nephroprotective effects of *P. ostreatus* and *A. bisporus* aqueous extracts on hyperoxaluria-induced urolithiasis induced in Wistar rats through the addition of 0.75% (v/v) ethylene glycol in drinking water for nine weeks have been investigated. The mushroom extracts inhibited the progression of nephrolithiasis and showed nephroprotective effects against ethylene glycol-induced kidney dysfunction [55].

The anti-inflammatory and antioxidant properties of *A. bisporus* biomass extracts from *in vitro* cultures were highlighted by Muszynska *et al*. [56]. The incubation of Caco-2 cells with *A. bisporus* extracts produced a decreased expression of cyclooxygenase-2 and prostaglandin F2α receptor compared with the LPS- and/or TNF-α-activated cells.

2.3.9 *Agaricus blazei* Murrill

Agaricus blazei contains several bioactive components which activate the immune system for a multitude of defensive functions [57]. Several animal studies and clinical experience have demonstrated that *A. blazei* possesses antitumor and immunological enhancement activity, and the fungus is also effective for the treatment of diabetes, HIV/AIDS, hypotension, and hepatitis [58]. β-glucans in *A. blazei* are the main constituents that stimulate the immune system and also act as antitumorals against myeloma and hepatic cancer in *in vivo* and *in vitro* studies [59,60].

Although further investigation of the efficacy of *A. blazei* extracts is required, some *in vitro* and *in vivo* preclinical studies have shown activity against Gram-positive and Gram-negative bacteria. In particular, the *A. blazei* mushroom extract promotes an antimicrobial effect against peritonitis, as well as deadly oral infections [61].

Another study highlights the clinical effect of the oral administration of *A. blazei* on the antibody response to β-glucan (anti-BG) titer [62]. The oral administration of *A. blazei* induced a β-glucan-specific response. The resulting anti-BG antibody production could be used as an index of the immune response to β-glucan in humans.

The influence of the β-glucans of *A. blazei* on the immune system can result in antiallergic effects. Such activities have been shown *in vitro* and in animal assays with an impact on the balance between Th1/Th2 cells in the immune system [63].

2.4 Mushroom Therapeutic Use: An Overview of Dietary Supplement Affairs

Mushrooms have been traditionally used for the maintenance of physical well-being and the treatment of numerous diseases since ancient times, especially in Asian regions. Since fairly recent times, they have become part of the sphere of dietary supplements widely employed for their health benefits, the use of which has largely entered into complementary alternative medicine (CAM) and complementary integrated medicine (CIM). Today, they are among the most commonly used of all integrative, complementary, and alternative therapies, especially in the field of oncology. This is especially the case in Asian countries, where mycotherapy has ancient and deep-rooted origins, while in Western areas, the application of mushrooms in medical therapies is still rather limited, especially in conventional medical institutions [5,20]. However, this is still a complex field in many respects. First of all, although a great deal of research has been carried out to highlight their various beneficial properties and thus their potential use in therapeutics, many mushrooms have only been tested *in vitro* or *in vivo* in animal models, mainly mice and rats, with little or no scientific support *in vivo* in humans [5,6]. Thus, although supplement companies often specify research to support their product claims, these are preclinical or even *in vitro* studies.

Another aspect to take into account is undoubtedly the fact that there are innumerable mushroom supplements on the market today, but for the same species, doses, preparations, manufacturing practices, and claims vary considerably between manufacturers. In the absence of standardization, significant differences can be found even in different batches from the same manufacturer. This leads to considerable difficulties in the scientific practice of clinical trials, both because they are difficult to compare, but also and above all because there is no standardization of parameters such as dose, active ingredient/s, composition, adverse effects, and interactions, which ends up compromising the validity and repeatability of any results obtained and, consequently, their use in medical practice according to the criteria it requires.

It is precisely the heterogeneity, reduced quality, and lack of standardization of these mixtures that make it difficult for supplements to be considered by the Western medical community and integrated into conventional therapeutic practices [20]. However, there is a slow increase in the number of doctors using them in their daily practice. Another problem is the autonomous choices of people to take supplements, convinced of the efficacy of a "natural" substance.

Regarding claims about and labelling of dietary supplements, the American Food and Drug Administration (FDA) does not require manufacturers to prove safety and efficacy, although products must have a history of safety [64]. The European Food Safety Authority (EFSA) sets the rules for the use of nutritional health and disease risk reduction claims, requiring toxicological data; since 2011, a new "botanical" can only be registered as a food supplement and not as an actual drug, falling under EU Regulation No. 1924/2006 [65]. Therefore, for most mushroom-based supplements, safety and efficacy are generally supported by traditional use, *in vitro* studies, animal model studies, and some case reports. It has to be said, however, that the increasing focus on these two attributes is resulting in more and more clinical investigative studies being carried out to prove them, albeit currently with the limitations mentioned above. The lack of regulation and monitoring by many governments means that supplements are often not monitored to ensure that they contain the ingredients or the amount of active ingredient declared by the manufacturer.

Indeed, unlisted components may be present, which may be either harmful or inert. Furthermore, the very fact that a fungal extract contains a multitude of demonstrably or potentially bioactive compounds often makes it difficult to link the effect to its true responsible agent, which also requires knowledge of the real concentration of the bioactive compounds contained in a supplement. Moreover, there is a risk that the consumer will not ingest the correct dose of an active substance, which may be higher, lower, or even non-

existent [64]. In addition to all this, the presence of several compounds in the same supplement makes it difficult to carry out rigorous clinical studies. This is due to the complexity of identifying both the "optimal dose" of the preparation needed to guarantee the desired effect and the cause–effect relationship, since the different substances may act on several parameters at the same time and, moreover, in a synergistic or antagonistic manner.
In addition to all this, numerous other factors often limit the validity of the clinical studies conducted to date, even in the case of promising results. They often involve too small a sample (among other things, the enormous variability in sample size makes it difficult to compare the various studies), or lack a control or placebo group, or the two groups compared are numerically very different, there is a very frequent lack of replication, adverse events are poorly reported or investigated, the statistical methods are deficient, and the results are poorly described in various respects [5,6,9,66]. However, many clinical investigations have shown very encouraging or promising results, thus underlining the great potential of mushrooms in therapeutic applications.

2.5 Pharmacological Activities of Mushrooms: Medical Evidence through Clinical Trials

The effects of some fungal metabolites or extracts found to be effective in *in vitro* tests or preclinical research, and therefore of potential medical interest, have also been evaluated *in vivo* in human subjects in clinical trials.

2.5.1 Medicinal Mushrooms and Cancer Clinical Studies

An extract of *Agaricus blazei* Murrill Kyowa, known to have antimutagenic and antitumor properties, was used in a randomized clinical trial (RCT) of 100 patients suffering from various gynecological cancers (cervical, ovarian, and endometrial) and receiving chemotherapy [67]. The treated group showed increased NK cell activity, while no significant differences were found in lymphokine-activated killer and monocyte activities.
Furthermore, administration of the fungal extract resulted in a clear reduction in chemotherapy-related side effects, such as loss of appetite, alopecia, emotional instability, and general weakness, as well as a marked improvement in mood-related parameters (anxiety, depression, mental stability).
Ineffectiveness, on the other hand, was the result of an open-label trial conducted in 2010 by Yoshimura *et al*. [68]. They compared two different supplements, Rokkaku Reishi containing *G. lucidum* and Senseiro containing *A. blazei* Murrill, and administered them orally for six months to a total of 51 prostate cancer patients following radical prostatectomy. The parameters assessed before and after treatment were serum prostate-specific antigen

(PSA) level and PSA doubling time, with a partial response rate evaluated as the primary outcome; hormonal status (represented by serum testosterone levels) and toxicity were also evaluated. Both medicinal mushrooms had no significant cancer effect, showing no partial response in terms of PSA, and no correlation between PSA doubling time and serum testosterone levels. Ascertaining the safety of an *A. blazei* Murrill treatment for cancer patients was the subject of a phase I clinical trial conducted by Ohno *et al*. [69]. Seventy-eight cancer survivors (30/24/24) were given one, two, or three packs of Senseiro (1800 mg/pack) daily for 6 months. Adverse events were defined by subjective/objective symptoms and laboratory data according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 3.0 (NCI-CTCAE v3.0). Consumption of the mushroom proved safe in almost all patients. Only in nine cases were adverse events recorded, mainly digestive, such as nausea and diarrhea. Only one patient complained of a liver dysfunction-related food allergy, a drug-ymphocyte product. None of these adverse events occurred in a dose-dependent manner. No immune outcome was implemented. An interesting case is AndosanTM (ACE Co. Ltd. produced for Immunopharma, Gifu-ken, Japan), a product made from the mycelium of *A. blazei*, as well as smaller amounts of *Grifola frondosa* (3%) and *Hericium erinaceus* (15%), three mushrooms traditionally used for therapeutic purposes in Asia. This product has been tested in various clinical trials, demonstrating antitumor, anti-inflammatory, and antiallergic action, presumably mainly due to β-glucans and isoflavonoids [70]. Although to date it remains unknown what the main constituent of this product is, as only small amounts of β-glucans have been detected in it, it seems that the results obtained in humans are due to the β-glucan stimulation of Peyer's patches in the gut-associated lymphoid tissue (GALT), together with other less defined absorbable low-molecular-weight (LMW) substances, such as flavonoids [70]. In 2015, Tangen *et al*. [71] reported the results of a clinical trial in which AndosanTM extract was orally given (60 mL/d) for seven weeks to patients with multiple myeloma undergoing high-dose chemotherapy with autologous stem cell transplantation (ASCT). In patients receiving AndosanTM, increased percentages of Treg cells (CD4+, CD127d+, and CD25+) and plasmacytoid dendritic cells (CD303+) were observed, as well as a significant increase in serum levels of interleukins IL-1ra (receptor antagonist), IL-5, and IL-7. Furthermore, gene expression studies were also carried out, showing increased expression of immunoglobulin genes, killer immunoglobulin receptor (KIR) genes, and Human Leukocyte Antigens (HLA) genes in the bone marrow in the *Agaricus* group; in more detail, an upregulation of endosomatic HLA genes and the plasma membrane CD86 gene was observed, and a downregulation of IL-7 and the proinflammatory chemokine CCL2 (MCP-1) genes, whereas

the expression of the IL-5 gene was unaltered. In addition, the overall survival increased notably.

When the D-fraction (β-glucan) of *G. frondosa* was tested in patients with cancer (lung, lingual, breast, gastric, or liver cancer), inhibition of the progression of metastasis was observed and a reduced expression of tumor markers (carcinoembryonic antigen (CEA) and cancer antigen 15–3 (CA15–3) and CA19–9) [70]. The mechanisms underlying these effects are an increase in NK cell activity and Th1 response, besides a reduction in Th2 activity.

AndosanTM has also shown anti-inflammatory effects *in vivo* in human subjects. In two different trials with healthy individuals, the ingestion of this drug provoked a reduction in proinflammatory cytokines, and an increased shedding expression of the adhesion molecule CD62L (L-selectin) and decreased intracellular reactive oxygen species (iROS), both in monocytes and granulocytes [70]. An improvement of quality of life was obtained by administering 60 mL of AndosanTM for 21 days to patients with ulcerative colitis or Crohn's disease, as well as a reduction in plasma levels of IL-5 and IL-2, respectively [58]. However, this study also found additional effects (improvement of total fatigue and/or lower cytokine levels) that have not yet been fully clarified.

The drug also appears to have antiallergic properties, as demonstrated in a study on patients suffering from birch allergy and asthma, where after administration of AndosanTM before the pollen season, a clear improvement was observed, due to a reduction of specific IgE levels and mast cell sensitization, suggesting the potential use of mushroom polysaccharides as a substitute for corticosteroids [70].

Maitake's effectiveness in fighting tumors was also highlighted in another study, in which patients with stage II-IV cancers were given mushroom powder or MD-fraction [72]. In high percentages of breast cancer patients (68.8%), lung cancer patients (62.5%), and liver cancer patients (58.3%), a cancer regression or a significant symptom improvement were observed, and the stimulation of immune-competent cell activities when the mushroom treatment was given alongside chemotherapy. Interesting considerations were made by Deng *et al*. [73] following a phase I/II study with breast cancer patients who were given different doses of *G. frondosa* extract orally. They found that the resulting immunological effects were quite complex and varied according to the cell type and specific cytokine. Maitake intake seemed to have a stimulatory effect on some parameters but a suppressive one on others. In particular, the biggest functional changes were observed in granulocyte response to phorbol myristate acetate (PMA) stimulation, IL-10 production from CD14+ cells stimulated by PMA, IL-10 production from CD3+ cells stimulated by PMA, IL-2 production from unstimulated CD56+CD3+ cell, and tumor necrosis factor- α (TNF-α) production from

CD3+ cells stimulated by LPS, all parameters for which a medium dose (5–7 mg/kg) of the fungal extract was optimal, as well as for others such as IL-10 production from CD14+ cells stimulated by PMA, monocyte oxidative burst response stimulated by N-formyl methionyl-leucyl-phenylalanine (fMLP), and TNF-α production from unstimulated CD3+ cells. In contrast, for augmented granulocytes' response to fMLP, augmented IFN-γ production from unstimulated CD45RO+ CD4+ memory T helper cells, and suppressed IFN-γ production from CD45RA+ CD4+ cells stimulated by PMA, the optimal dose was the highest one (10 mg/kg). This highlights that it is often difficult to find an "optimal dose" for botanical agents, probably because they generally contain several compounds that may act differently on a variety of target cells or with different strengths. Therefore, extreme caution should be exercised when treating individuals with major illnesses.

The dietary supplement based on maitake β-glucans, with recognized immunomodulatory properties, is also known to stimulate hematopoietic progenitor cell differentiation, granulocyte colony-stimulating factor production, and the recovery of peripheral blood leukocytes after bone marrow injury. Therefore, Wesa *et al*. [74] wanted to evaluate the effects of *G. frondosa* extract on 21 patients suffering from myelodysplastic syndrome (MDS) by giving them 3 mg/kg twice a day for 12 weeks and measuring the following parameters before and after treatment: neutrophil count and function, the latter tested as endogenous or stimulated neutrophil production of ROS by flow cytometry.

Escherichia coli, the bacterial peptide fMLP, and phorbol ester were used as ROS activators. Maitake showed beneficial effects on MDS. It caused increased endogenous neutrophil and monocyte function and, compared to before treatment, an increased monocytic response to *E. coli* and fMLP-stimulated ROS production response were detected afterward.

Asian medical research has also focused on the two bioactive protein–polysaccharides extracted from *Coriolus versicolor* (L.) Quél., PSP and PSK or krestin, known for their immunomodulating and antitumor activities and which are widely employed. In a randomized, double-blind, placebo-controlled trial, subjects with advanced non-small cell lung cancer (NSCLC) who received PSP for 28 days following chemotherapy showed a significant improvement in blood leukocyte and neutrophil counts, serum IgG and IgM, and body fat percentage compared to the control group, resulting in a slower general deterioration [75].

Based on previous studies that suggested PSK could improve the survival of cancer patients when combined with chemotherapy, presumably through immunological mechanisms such as the induction of cytokines, regulation of Th1/Th2 balance, and inhibition of immunosuppressive molecules, Akagi and Baba [76] set out to test this product on advanced

gastric cancer patients. Twenty-one subjects were randomly assigned to receive 300 mg tegafur/uracil (UFT) alone or 3g PSK together with 300 mg UFT daily for at least one year after surgery; immunological parameters were monitored and measured.

Overall survival was markedly improved in the PSK group, with a 3-year overall survival of 62.2% compared with 12.5% in the untreated group. This result is possibly related to the observed reduction in CD57+ T-cells, known to indicate a poor prognosis in patients with advanced gastric cancer. Therefore, PSK is presumed to improve the overall survival of patients partly through the inhibition of CD57+ T cells. Glycoprotein PSK was evaluated by Ito *et al*. [18] for its possible influence on the expression of major histocompatibility complex (MHC) class I expression in advanced gastric cancer patients after surgery. They compared two groups of subjects, one receiving only chemotherapy and the other immunochemotherapy with 3 g/day PSK for 19 months, in a total of 349 individuals. The two groups did not differ in their MHC class I expression. Nonetheless, within the PSK group, while MHC class I-positive patients had a relatively poorer prognosis than control patients, recurrence-free survival (RFS) was significantly higher in expression-negative subjects. Concerning the pN factor (number of lymph node metastases), expression-negative patients with pN2 or greater exhibited significantly higher RFS values. In contrast, for the factors pT (depth of tumor invasion) and venous invasion, no statistically significant differences were observed. The authors hypothesized the effectiveness of PSK adjuvant immunochemotherapy in MCH class I-negative patients and patients with advanced lymph node metastasis of pN2 or greater.

Despite the positive results of some studies and the common therapeutic use of PSK on tumors in Asian regions, several studies in this area have shown it to be ineffective. This is the case of the RCT carried out by Miyake *et al*. [77], in which they compared chemotherapy treatment based on UFT plus leucovorin (UFT/LV) with immunotherapy treatment with PSK implementation (UFT/PSK) in 357 stage IIIB or III colorectal cancer patients who had undergone Japanese D2/D3 lymph node dissection. Both the 3-year disease free-survival (DFS) and the 3-year overall survival (albeit with less marked differences) were lower in the UFT/PSK group, thus demonstrating the ineffectiveness, if not the disadvantage, of using this glycoprotein as an adjuvant to standard chemotherapy treatments in this type of patient. UFT/PSK immunochemotherapy was also ineffective or even unfavorable in the 1-year randomized phase III trial performed by Okuno *et al*. [78] compared to surgery alone in 111 phase II colorectal cancer patients. Overall survival values were fairly similar in the two groups, while DFS was even worse in the treated group, although not statistically significant. *C. versicolor* was tested by Chay *et al*. [79] in an RCT with 15 severe hepatocellular

carcinoma (HCC) patients who had poor liver function or were unsuitable for standard therapy. A dose of 2.4 g/d of the extract was used. Compared to the placebo group, the mushroom-treated group had a higher median time to progression (TTP) (2.5 months vs.4.2), median progression-free survival (PFS) (2.5 months vs. 1.1), and median overall survival (OS) (6.5 months vs. 2.2). A decrease in interleukin IL-17F and MCP-1, and an increase in prolactin and TNF-related apoptosis-inducing ligands were detected. Besides, there was an improvement, even if not statistically significant, in social, emotional, physical, and cognitive parameters, as well as a significant marked reduction in symptoms normally associated with disease progression, such as loss of appetite and pain. Therefore, this mushroom seems to be particularly promising for patients with palliation needs.

Powdered mycelium of *C. versicolor* was evaluated by Torkelson *et al*. [80] in a phase I two-center, dose-escalation clinical trial to work out the maximum tolerated dose when taken daily in divided doses for 6 weeks by women with breast cancer after standard chemotherapy and the recent completion of radiotherapy. Nine participants were assigned to three cohorts to take 3, 6, or 9 g of mushroom preparation (500 mg lyophilized mycelial powder/capsule). All three doses were well tolerated. Only nine adverse events were detected, seven mild, one moderate, and one severe (anxiety, probably unrelated to the treatment). The effects on the immunological system were also positive, with an increase in lymphocyte counts at 6 and 9 g/day, increased natural killer cell functional activity at 6 g/day, and a dose-related increase in CD8+ T cells and CD19+ B cells, unlike CD4+ T cells or CD16+56+ NK cells. Although this study showed the safety of *C. versicolor* at a 9 g daily dose, did not determine the safety and tolerability of higher doses, i.e., the maximum dose tolerated (MDT), and the sample size was very small.

Ganoderma lucidum or reishi is one of the oldest medicinal mushrooms used in Asian regions, and is consequently one of the most studied up to the present day. About 400 bioactive compounds have been isolated from the mycelium, fruiting bodies, and spores of this mushroom, including polysaccharides, triterpenes, ganoderic acids, phenols, amino acids, lignin, vitamins, nucleosides, nucleotides, sterols, steroids, proteins, un saturated fatty acids, and inorganic ions [81]. However, the most important bioactive molecules turn out to be polysaccharides, the most important of which are β 1–3 and β 1–6 D glucans, and triterpenes, especially the lanostane type. They have shown multiple important therapeutic properties both *in vitro* in numerous tumor cell lines and *in vivo* in animal models, thus explaining why Chinese medicine traditionally uses extracts of this mushroom for cancer prevention and treatment. They are also used to alleviate the side effects of radio- and chemotherapy.

A randomized, double-blind, placebo-controlled, multicenter clinical trial was performed by Gao *et al*. [82] on 68 patients suffering from advanced lung cancer to assess whether *G. lucidum* had beneficial effects on the subjects' quality of life. In individuals taking 600 mg/d of Ganopoly® (Alpha s.r.l., Lodi, Italy) (a polysaccharide product from *G. lucidum*) three times daily for 12 weeks, a marked improvement in the quality of life was observed, as demonstrated by significantly higher Karnofsky scores than in the placebo group. Quality of life in general also improved significantly, regarding disease-related symptoms such as fever, weakness, insomnia, sweating, and coughing. Ganopoly® , administered at doses of 1800 mg/d three times daily for 12 weeks in 34 advanced lung cancer patients, also had positive effects on immunological functions, producing significant increases in plasma IL-2, IL-6, and IFN-γ concentrations, Cd56+, phytohemagglutinin (PHA) responses, and NK activity, as well as significant decreases in IL-1 and TNF-α [83].

Back in 2010, Oka *et al*. [84], wishing to confirm the cancer-preventive action of the water-soluble extract of *G. lucidum* mycelia (MAK) *in vivo*, conducted a no-treatment concurrent controlled study with 123 patients with colorectal adenomas who were disposed to undergo colonoscopy. They were given 1.5 g/d MAK for 12 months, after which they underwent a colonoscopy to detect the size, location, and macroscopic type of adenomas, then the data were compared with the untreated control group. In contrast to the untreated group, there was a significant decrease in both the number and size of adenomas in the individuals taking MAK, suggesting a suppressive effect of the extract on the development of colorectal adenomas and precancerous lesions of the large bowel.

Two years later, Zhao *et al*. [66] published the results of a pilot randomized clinical trial with 48 breast cancer patients with cancer-related fatigue undergoing endocrine therapy receiving 1000 mg of *G. lucidum* spore powder three times a day for 4 weeks. The survey parameters were obtained using the Functional Assessment of Cancer Therapy: Fatigue (FACT-F), Hospital Anxiety and Depression Scale (HADS), and European Organisation for Research and Treatment of Cancer Core Quality of Life Questionnaire C30 (EORTC-QLQ-C30) questionnaires. The concentrations of TNF-α and IL-6 and liver–kidney functions were assessed both before and after treatment.

Compared to the control group, significant increases in overall quality of life and physical function were observed in treated individuals, as well as an equally significant improvement in emotional and functional well-being, state of depression and anxiety, emotional functioning, cognitive function, and symptoms like fatigue, sleep disturbance, and appetite loss. Besides, significantly lower levels of TNF-α and IL-6 were found in the serum. Thus, the spore powder exerted its anticancer and immunomodulatory effect without causing any

serious adverse effects on individuals. Additionally, no alterations in kidney and liver function were found in the corresponding tests; only moderate discomfort, such as dizziness and dry mouth, occurred in a small percentage of the treated group.

The anticancer effect of *G. lucidum* glucans is attributable to immunological mechanisms, using the activation of T lymphocytes and macrophages, resulting in cytokine release [81] and the activation of B lymphocytes and NK, dendritic, and other immune cells, together with their secretory products like tumor necrosis factor-α (TNF-α), reactive nitrogen, oxygen intermediates, and interleukins (IL-1, IL-2, IL-3, IL-6) [20].

The inhibition of angiogenesis is also thought to be a mechanism involved in antitumor effects. A reduction in the mortality rate in cancer patients following the admin istration of reishi products (4–6 g/d for 2 months), or an immunological enhancement (capsules of 1800 mg/d for 12 weeks), and a general improvement in the quality of life have been observed in various clinical studies [69]. According to recent experimental evidence, *G. lucidum* inhibits the motility of tumor cells, interfering with the signaling pathway and thus having very important consequences on the spread of metastases.

In addition, *Pleurotus cornucopiae* (Paulet) Rolland or tamogitake was associated with the upregulation of the immune system in a double-blind placebo-controlled clinical trial by Tanaka *et al*. [85], suggesting its great potential for the prevention of various diseases, such as cancer and infectious diseases. This study showed that the administration of a fungal extract for eight weeks increased interferon IFN-γ and IL-12, and a smaller increase in NK cell activity, while serum levels of Th2-type cytokines IL-10 and IL-13 and other cytokines remained unchanged. The enhancement of the immune system by tamogitake appears to occur through Th1 phenotype potentiation and the macrophage–IL-12 and IFN-g pathway, thus resulting in activation of the cell-mediated immune system, but with almost no change in Th2-type cytokines (the causes of which have yet to be clarified).

Agaricus bisporus (J.E. Lange) Imbach showed promising results against prostate cancer by reducing immunosuppressive factors. In phase I, a single-arm, unblended, single-facility trial involving 36 patients, Twardoski *et al*. [86] observed that, following the administration of tablets of fungal extract (six mushroom dose cohorts: 4, 6, 8, 10, 12, and 14 g/day), there was a decline in PSA level from the first few months of intake until it became undetectable. At the same time, there was an increase in baseline IL-15 levels, and declines in myeloid-derived suppressor cells (MDSCs). The maximum dose level was capped at 14 gm daily, which was the highest dose that was thought to be practical to ingest over a long period of time, based on the number of tablets required.

Another species, *A. sylvaticus* Schaeff., was tested by Costa Fortes *et al*. in a randomized, double-blind placebo-controlled clinical trial with 56 patients with colorectal cancer in the post-surgery phase [87,88]. A mushroom extract (tablets) in doses of 30 mg/kg/d or placebo was administered to the two subgroups for six months, monitoring their metabolic, biochemical, and enzymatic profile, as well as parameters describing quality of life. In contrast to the placebo group, several significant positive effects were found in the treated group, such as the reduction of fasting plasma glucose level, reduction of total cholesterol, reduction of serum thyroglobulin and creatinine levels, decreased aspartate aminotransferase (AST) and alanine aminotransferase (ALT) and also of IgA and IgM, and the reduction of diastolic blood pressure (DBP), whereas total protein content and globulin increased. Regarding quality of life, the treated group showed improvements in various aspects (quality of sleep, appetite, pain, mood, and gastrointestinal symptoms).

The effects of *A. sylvaticus* on the side effects of chemotherapy in cancer patients were also investigated by Valadares *et al*. [89] in a randomized, double-blind, placebo-controlled trial of 46 women with breast cancer administered 2.1 g/d of the extract for 6 months. Again, there was an improvement in the patients' condition in various respects, such as pain, appetite, and bowel function, with a very low rate of the occurrence of certain side effects compared to the placebo group. In the RTC of Tsai *et al*. [90], *Antrodia cinnamomea* T.T. Chang and W.N. Chou, a popular medicinal mushroom in Taiwan, was tested in subjects suffering from different kinds of cancer (gastric, lung, breast, liver, or colorectal) alongside chemotherapy. A total of 37 patients were randomly assigned to receive either placebo or 20 mL of an *A. cinnamomea* (AC) oral formulation (mainly containing polysaccharides, triterpenoids, and γ-aminobutyric acid) twice a day for 30 days. Although lower mortality rates and longer overall survival were found in the mushroom-treated group than in the placebo group, these differences were not statistically significant.

The EORTC-QLQ-C30 also showed no substantial differences between the two groups, except for better sleep quality in the AC group. It is further important to remark that, even if most hematologic, liver, or kidney functions did not differ significantly between the two groups, a significant reduction in platelet count was observed in the AC group at the end of treatment. The study also reports a number of adverse effects in both samples, the most recurrent of which were gastrointestinal complaints, which were even more frequent in the AC group, albeit of a lesser severity, than in the control. Although this medicinal mushroom has traditionally been widely used in Taiwan for the treatment of various cancers, the results of this trial provide no evidence of its efficacy in improving the outcome of patients with

advanced cancer. The authors speculate, however, that this may be due to the limited sample size, its short duration, and, not least, the inclusion of subjects with different cancer types.

In 2011, Ina *et al*. [91] wanted to test whether the biological response modifier (BMR) lentinan, used in combination with oral fluoropyrimidines (S-1 chemotherapy), could be effective in patients with gastric cancer. For this purpose, 78 subjects with metastatic or recurrent gastric cancer undergoing S-1 chemotherapy were selected, a proportion of whom were given 2 mg/body weight of lentinan intravenously for 30 min every 2 to 3 weeks. Immunochemotherapy with lentinan has been shown to significantly increase the overall survival of patients with advanced gastric cancer, although without consistent differences in the incidence and degree of adverse effects. Another randomized clinical trial was reported by Ma [92], comparing the effect of lentinan when combined with nanoparticle (NP) chemotherapy (cisplatin plus navelbine) on advanced non-small cell lung cancer patients. A significantly lower Parkinson disease (PD) rate and significantly higher efficiency rate and control rate were observed in treated subjects compared to the control group, as well as higher levels of CD3+, CD4+, CD8+, and CD4+/CD8+, showing that the immunochemotherapy with lentinan is able to improve the therapeutic efficacy, enhance immune functions, and reduce the adverse effects.

In addition, a more recent clinical study by Wang *et al*. [93] showed that lentinan-based chemoimmunotherapy seems to be a promising strategy for antitumor activity via enhancing the proliferation of cytotoxic T cells (CD3+ CD8+) and CD3+ CD56+ NKT cells, followed by the elevation of proinflammatory chemokines/cytokines (IFN-γ, TNF-α) and proinflammatory IL-12, which would then lead to a shift in the Th1/Th2 balance towards Th1. These were the results observed in subjects treated with 4 mg/d of lentinan for 12 weeks in relation to the total number of patients with NSCLC involved in the trial.

From the mycelium of the medicinal mushroom *Lentinula edodes* (Berk.) Pegler, or shiitake or ling zhi, active hexose correlated compound (AHCC), a nutritional supplement containing polysaccharides (including α-1,4-glucans), amino acids, and minerals, has been obtained [94]. Various studies report this product to have beneficial effects in the treatment of cancer, as it acts as an immunoenhancer able to alleviate the adverse effects of chemotherapy. In 2014, Ito *et al*. [95] performed a clinical trial with 24 cancer patients undergoing chemotherapy, and showed that the administration of AHCC significantly decreased the levels of herpes virus type HHV-6 in saliva (generally increased by chemotherapeutic treatments), improving quality of life and also chemotherapy-associated hepatotoxicity and hematotoxicity in the cancer patients. Additionally, Del Buono *et al*. [96] performed a small

clinical study to assess the effectiveness of AHCC for integrative cancer treatment, evaluating its immunomodulatory effects on the lymphocyte population.

A dose of 3 g/d of AHCC was given for 1 month to seven patients suffering pancreatic, lung, or colorectal adenocarcinoma. At the end of treatment, there were consistent increases in neutrophils and the population ratios of CD3/CD4, CD4/CD8, CD8/CD3 (suppressor/cytotoxic), and CD3+/CD16+/CD56 NK cells, while lymphocytes and monocytes decreased. Thus, α-glucans were more effective on innate immunity (CD8, CD56) than acquired immunity (CD4), and exerted a marked modulation of NK cells, which are crucial for the direct destruction of neoplastic cells. No toxicity was observed.

Positive results for AHCC were also obtained in a clinical study conducted with patients suffering from epithelial ovarian cancer or peritoneal cancer undergoing platinum-based chemotherapy [94]. An intake of 3 g/d (500mg/capsule) throughout six cycles of chemotherapeutic treatment resulted in increased levels of CD8+T cell lymphocytes, and in reduced side effects, such as nausea and vomiting. However, increased muscle pain was noted. D'Orta *et al*. [97] also showed the beneficial effects of AHCC on sarcopenia in patients suffering adenocarcinoma and malnutrition undergoing radio-chemotherapy, by giving 50 of them a food therapy and 1.5 g/d of AHCC for 6 months. In 80% of subjects, no progression of cancer malnutrition or cachexia was observed, but rather an increase in body cell mass.

2.5.2 Medicinal Mushrooms and Diabetes, Hyperglycemia, Hyperlipidemia, and Cardiovascular Disorder Clinical Studies

The efficacy of *A. blazei* Murrill in diabetes control was also demonstrated in a randomized, double-blind, placebo-controlled trial involving 72 individuals with type 2 diabetes treated with gliclazide and metformin for more than 6 months [98]. The *A. blazei* mushroom (ABM) extract was administered for 12 weeks at a dose of 1500 mg/d and then the homeostasis model assessment for insulin resistance (HOMA-IR) was evaluated.

The administration of the ABM extract was associated with a significant improvement in insulin resistance, probably due to an increase in plasma adiponectin concentration, as this parameter increased reasonably in the ABM group and decreased in the placebo group.

The beneficial effects of a diet based on *A. bisporus* have been evaluated in pregnancy-related complications, like hypertension and macrosomia, in a recent randomized, placebo-controlled clinical trial performed by Sun and Niu [99]. A sample of 1244 women planning for their first pregnancy was recruited and randomly assigned to consume at least 100 g of white button mushroom (cooked according to personal taste) daily or follow a normal diet, from the pre-pregnancy stage to the 20th week of gestation. In the end, 582 women in the

WBM group and 580 in the placebo group completed the program. The primary outcome was gestational hypertension, measured by diastolic blood pressure (DBP) and systolic blood pressure (SBP); the secondary outcome included preeclampsia, gestational body weight gain, other pregnancy complications such as gestational diabetes mellitus, and birth weight. The results showed a beneficial impact of the mushroom diet on all the parameters mentioned above, and a significantly lower risk of gestational hypertension, preeclampsia, gestational weight gain, gestational diabetes, and macrosomia was observed.

No significant positive results were obtained by Klupp *et al*. 8 [100] when they investigated the effectiveness of reishi in treating hyperglycemia and cardiovascular risk components of metabolic syndrome through a prospective, double-blind, randomized, placebo-controlled trial. For this purpose, they selected a total of 84 subjects with type 2 diabetes mellitus (DMT2) and metabolic syndrome and randomized them to receive 3 g/d of *G. lucidum* alone, *G. lucidum* combined with *Cordyceps sinensis* (Berk.) Sacc, or placebo for 16 weeks. Blood glucose was measured in terms of glycosylated hemoglobin (HbA1c) and fasting plasma glucose (FPG), but also other parameters like blood pressure, triglycerides, waist circumference, body mass index (BMI), health-related quality of life, C-reactive protein, total High-Density Lipoprotein (HDL) and Low Density Lipoproteins (LDL) cholesterol, and apolipoproteins A and B. Surprisingly, at the end of the treatment, reishi had not significantly influenced any of the aforementioned factors, thus not supporting the hypothesis of its possible use in the treatment of metabolic syndrome.

The capacity of *P. ostreatus* to reduce blood glucose, cholesterol, and triglycerides in diabetic patients was investigated by Kathun *et al*. [101], while also checking for possible hepatic and renal toxicity. A clinical trial was conducted with 89 subjects consuming 50g of cooked mushroom thrice daily for 24 days, by alternating 7 days of the mushroom diet and 7 days of no mushrooms and measuring different parameters at the start and after every 7 days. The significant effects found in patients were the reduction of systolic and diastolic blood pressure (SBP, DBP), plasma glucose, total cholesterol (TC), and triglycerides (TGs), while there was no substantial change in weight and HDL. When mushrooms were not consumed, there were significant increases in DBP, FPG and PG 2 h after breakfast (2 hPG), TC, and TGs, while SBP, HDL, and weight did not change. On resuming mushroom intake, the changes described above occurred again. Therefore, this fungal species can provide important beneficial effects in diabetic patients, without compromising liver and kidney function.

Other studies followed in subsequent years, aimed at assessing the effects of ingesting *P. ostreatus* in the form of fresh, cooked, or dried mushrooms on conditions such as DMT2,

dyslipidemia, hypertension, overweight, or obesity. Kajaba *et al*. [102], for example, showed a significant hypolipidemic effect of *P*. *ostreatus* in their uncontrolled clinical trial of 57 individuals with, primarily, combined types of dyslipidemia, following a daily administration of 10 g of freeze-dried and pulverized mushroom for 6 weeks, and found a significant decrease in TGs, TC, and TC/HDL-C (High-Density Lipoprotein Cholesterol). There was also a decrease in the concentration of conjugated dienes (CDs) in plasma, while glutathione peroxidase (GPX) activity and the concentration of glutathione (GTH) in erythrocytes increased.

The cholesterol-lowering properties of an oyster mushroom diet were investigated for the first time in humans by Schneider *et al*. [103]. In their RTC, 20 individuals with moderate untreated hyperlipidemia were randomly assigned to take either a soup containing 30 g dried oyster mushrooms or a tomato soup as a placebo daily for 3 weeks. In the mushroom-fed group, significant reductions in TG concentrations and oxidized low-density lipoprotein (oxLDL) levels were detected, as well as a significant trend towards a lowering of TC values. Choudhury *et al*. [104] performed an uncontrolled trial to assess the effects of *P*. *ostreatus* on the blood pressure and glycemic status of 27 hypertensive diabetic DMT2 drug-treated males. Capsules with sun-dried powdered mushroom (3 g/d; thrice daily amounts of 1 g) were administered for 3 months. A significant reduction of both SBP and DBP was detected, as well as of FGP and glycated hemoglobin (HbA1c), resulting in an improved glycemic status and blood pressure control. Moreover, no adverse effect on renal function was detected, as demonstrated by no significant change of plasma creatinine level.

A further uncontrolled study was carried out by Choudhury *et al*. [105] to investigate the effect of oyster mushrooms on the lipid profile of obese or overweight hypertensive non-diabetic subjects. After administrating pulverized mushroom capsules at a dose of 3 g/d for 3 months to 14 patients, significant decreases in plasma TC and low-density lipoprotein cholesterol (LDL-C) levels were observed. TGs and HDL-C also decreased, although not significantly.

These results suggest that *P*. *ostreatus* might be prescribed to improve the atherogenic lipid profile, primarily TC and LDC-L, of obese hypertensive individuals. Additionally, Sayeed *et al*. [106] performed a clinical trial to evaluate the metabolic effects of *P*. *ostreatus* in diabetic DMT2 drug-treated subjects, randomly assigning 73 women to consume 200 g/d mushrooms or placebo (equal calorie diet of vegetables) for 1 year. Significant decreases of FBG, 2-hPG, TC, TG, and LDL were observed in the treated group, whereas no differences were detected among groups for BMI, SBP, DBP, HDL, Hb, ALT, and creatinine. Finally, the hypolipidemic effects of the oyster mushroom and *Pleurotus cystidiosus* O.K. Mill. were

tested by Jayasuriya *et al*. [107] on both healthy individuals and dietetically treated DMT2 diabetics, by oral daily administration for two weeks of a suspension of one of the two freeze-dried and pulverized mushrooms at a dose of 50 mg/kg BW, followed by a glucose load. In healthy subjects, both fungal species showed a significant decrease in FPG and postprandial plasma glucose (PPG). Equally significant was the lowering of PPG in diabetic patients for both mushrooms, for whom an increase in postprandial serum insulin levels was also observed, thus indicating these mushrooms as beneficial functional foods for diabetes mellitus.

2.5.3 Medicinal Mushrooms and Neuron Health Clinical Studies

G. lucidum has been widely used in Asian regions to treat not only cancer but diabetes and neurasthenia as well. For the latter, the mushroom seems to have particularly beneficial effects, as demonstrated in a randomized, double-blind, placebo-controlled clinical trial conducted by Tang *et al*. [108] with Ganopoly® . A total of 132 neurasthenic patients were randomized to receive placebo or 1800 mg three times daily for 8 weeks. At the end of treatment, patients taking *G. lucidum* were found to have an increased sense of well-being as measured by the Visual Analogue Scale (VAS), and consistent reductions in the Clinical Global Impression (CGI) severity scale score and fatigue.

The beneficial effects of *Hericium erinaceus* have been demonstrated in numerous clinical studies. Mori *et al*. [109], for example, conducted a double-blind, parallel-group, placebo-controlled clinical trial with 30 middle cognitive impairment patients, giving them four 250 mg tablets containing 96% mushroom powder or placebo three times a day for 16 weeks, continuing the follow-up for a further 4 weeks and performing assessments using a cognitive function scale based on the Revised Hasegawa Dementia Scale (HDS-R).

Compared to the placebo group, at weeks 8, 12, and 16 of treatment, and 4 weeks of follow-up, the yamabushitake group showed significantly increased scores, although at the fourth week, after the end of ingestion, the values decreased significantly. Regardless, this fungal species has proved to be very useful in improving average cognitive impairment. Additionally, on the side of these promising results, Li *et al*. [110] wished to test the efficacy of this mushroom in the treatment of early Alzheimer's disease. In this randomized, double-blind, placebo-controlled pilot study, three capsules of *H. erinaceus* (HE) mycelium (350 mg/capsule; 5 mg/g erinacin A as active ingredient) were administered daily to patients with early-ons*et al*zheimer's disease for 49 weeks. When comparing these subjects with the placebo group, there were significant differences in the Instrumental Activities of Daily Living score, a significant improvement in the Mini-Mental State Examination score, and contrast sensitivity. Furthermore, whereas in the placebo group the mean apparent diffusion

coefficient (ADC) values from the arcuate fasciculus region in the dominant hemisphere increased significantly, in the mushroom-treated group, this was not the case, and a significant decrease in ADC values from the parahippocampal cingulum region in the dominant hemisphere was detected.

Because of the effect of *H. erinaceus* (HE) on the autonomic nervous system and brain function, the effects of this mushroom on menopause, depression, sleep quality, and undefined disorders were investigated by Nagano *et al*. [111] in a randomized, double-blind, placebo-controlled trial. Evaluations were carried out according to the Kupperman Menopausal Index (KMI), the Pittsburgh Sleep Quality Index (PSQI), the Centre for Epidemiologic Studies Depression Scale (CES-D), and the Indefinite Complaints Index (ICI). Thirty females were randomly assigned to consume either four HE cookies (0.5 g powdered carpophore per cookie) or four placebo cookies daily for 1 month. In the treated group, the CES-D and ICI scores after the HE intake were significantly lower than before; compared to the placebo group, the "insensitive" and "palpitation" terms of the ICI were significantly lower, and the terms "concentration", "irritating", and "anxious" tended to be lower. Finding that *H. erinaceus* can alleviate anxiety and depression, the authors were led to suppose a different mechanism of action from the more familiar NGF-enhancing action of the mushroom.

A case study was reported by Inanaga [112] about the treatment with HE of an 86-year-old subject with recurrent depressive disorder suffering mild cognitive impairment during antidepressant therapy with mirtazapine. After 6 months of the intake of HE extracts in the form of Amyloban®3399 (US patent pending. Mushroom Wisdom, Inc., East Rutherford, NJ, USA), both cognitive function and body weight were successfully restored. The supplement Amyloban®3399 was also evaluated by Okamura *et al*. [101] for the treatment of sleep disorders in eight female undergraduate students through the daily intake of six tablets for 4 weeks, measuring sleep quality and general well-being status by the General Health Questionnaire (GHQ-28) and PSQI. Anxiety and insomnia showed a decreasing trend, as well as the PSQI scores. Furthermore, after 4 weeks of mushroom intake, increased levels of salivary free 3-methoxy-4-hydroxyphenylglycol (free MHPG) were detected, with this considered an accurate index of chronic stress and depressive symptoms, thus reflecting sympathetic nervous system activity. Although this pilot study showed the ability of *H. erinaceus* to improve well-being, sleep disturbance, and negative mood, as recognized by the authors themselves, caution should be exercised in making conclusions, as the sample size was too small. Besides, the selected subjects were recruited in the month before a major national examination, so all complaints probably lacked the severity and chronicity of

clinically diagnosed anxiety and mood disorders. A more recent randomized clinical trial was performed to assess how *H. erinaceus* can act on depression, anxiety, sleep disorders, and binge eating [113].

Seventy-seven subjects affected by overweight or obesity and with one or more mood disorders were randomized to receive three capsules of an HE dietary supplement for two months daily and under a low-calorie diet regimen. The supplement administered ("Mycotherapy Hericium") consisted of 80% bulk mycelium and 20% fruiting body extract. Before treatment, after the first month, and after the second month, the abovementioned complaints were assessed by using the Symptom Checklist-90, Zung's Self-Rating Depression Scale, Zung's Self-Assessment Anxiety Scale, and Binge Eating Scale (BES). All evaluations revealed significant improvements in depression, anxiety, and sleep quality in the HE-treated group. Moreover, with reference to the serum balance between brain-derived neurotrophic factor (BDNF) and its precursor pro-BDNF, an increase in circulating pro-BDNF levels was detected, but without fully clarifying whether these neurotrophins can actually be used as biomarkers of mood disorders.

2.5.4 Medicinal Mushrooms in Clinical Studies on Other Medical Topics

In order to ascertain the optimal dose of *A. bisporus*, or white button mushroom (WBM), to achieve in humans the effects of suppressing aromatase activity and inhibiting breast cancer cell proliferation already established *in vitro* and in preclinical tests, Palomares *et al*. [114] carried out the following dose-finding study: a 12-week course of treatment with 5, 8, 10, or 13 g daily of the fungal extract in postmenopausal women previously diagnosed with breast cancer but no longer undergoing treatment and who are recurrence free. Aromatase inhibition by aromatase activity (AA) tests and a decrease in free estradiol (FE2) and cytokine levels were the counted parameters. No subject met the predefined response criterion, but despite this, it was observed that FE2 tended to increase over the 12-week treatment period for the 5 g and 8 g dose groups, while it remained stable for the 10 g and 13 g groups; in the first two groups, AA testing also revealed a substantial increase in post-prandial peak, which decreased in the 10 g group and disappeared in the 13 g group. In addition, albeit without a clear dose–effect correlation, there were increases in IL-1β and IL-2, and a decrease in IL-6. The authors concluded that antiaromatase bioactive compounds were present in plasma at a consumption level of 10–13 g of extracts (corresponding to 100–130 g of the whole mushroom), but not in sufficient concentration to cause a significant reduction in estrogen, at least over the time period evaluated.

A different approach was used to evaluate another aspect of WBM, namely as a food rich in prebiotics. Health effects and the gut microbiota were assessed in an open-label crossover

trial by subjecting 32 healthy adults to the consumption of protein-matched amounts of mushrooms or meat twice daily for ten days [115]. No differences were found between the two groups in breath hydrogen, stool frequency, consistency, fecal pH, or Short Chain Fatty Acid (SCFA) concentrations.

On the other hand, the composition of the fecal microbiota of WBM-fed subjects was different, with a higher abundance of Bacteroidetes and a lower abundance of Firmicutes. Besides, the impact of mushroom consumption on laxation and consequently on gut health can be deduced from the increase in stool weight and the presence of undigested mushrooms in the stool. Still dealing with oyster mushroom, an interesting study by Jesenak *et al*. [116] highlights the efficacy of pleuran in preventing morbidity in children due to recurrent respiratory tract infections (RRTIs), while also outlining its complex immunomodulatory activity. In this double-blind, placebo-controlled, randomized, multicenter trial, 175 children with more than five respiratory infections were randomized to receive 5 mL/5kg Imunoglukan P4H® (Nové Záhrady I č., Bratislava, Slovak Republic) syrup (10 mg pleuran and 10 mg vitamin C in 1 mL of syrup) or placebo (only vitamin C, 10 mg/mL) for 6 months. Blood samples and questionnaires were taken for 12 months. A higher proportion of the treated group compared to the placebo group did not suffer from any respiratory infection throughout the treatment. Furthermore, in the P4H group, there were significant reductions in the frequency of flu and flu-like disease and the number of lower respiratory tract infections, as well as a statistically significant modulation of humoral and cellular immunity. In particular, in the treated group, the concentration of IgG and IgM increased during the treatment period and remained heightened throughout the study; IgA increased more in the P4H group than in the placebo one; Immunoglukan was also associated with an increase in NK cells and prevented the decline in CD8+ T cytotoxic lymphocytes.

Pleuran has also been tested for the management of herpes simplex virus type I infection, as the typical remedy acyclovir can cause viral resistance if used for long periods. Urbancikova *et al*. [117] conducted a randomized, placebo-controlled clinical trial to assess the effect of this compound on the duration and intensity of herpes symptoms and the incidence rate and duration of acute respiratory symptoms and intercurrent diseases in HSV-1-positive patients. This was done in 90 patients randomly assigned to receive pleuran or placebo, and the study was divided into two phases: an acute treatment phase, lasting 10 days, in which either Immunoglukan P4H® ACUTE (300 mg pleuran, 160 mg vitamin C, 10 mg Zn) or placebo (160 mg vitamin C, 10 mg Zn) was administered daily; and a subsequent 120-day preventive phase, with daily administration of either Immunoglukan P4H® (100 mg pleuran, 100 mg

vitamin C) or placebo (100 mg vitamin C). Active treatment resulted in a significant shortening of the duration of herpes symptoms.

During the preventive phase, the duration and severity of respiratory symptoms were lower. In both phases, no side effects were observed.

2.6 Discussion

Medicinal mushrooms have been shown to have many different pharmacological properties and are the subject of increasing interest. Many of them are already being used, particularly in the field of oncology, for their immunomodulatory and antitumor actions, which complement traditional treatments, improving their action and reducing their side effects [1,3,5].

The medicinal properties of mushrooms are due to the numerous and diverse secondary compounds and metabolites present in the mycelial and/or carpophore structures, which can act, in a synergistic or non-synergistic manner, on various biological functions of the human organism. All these properties were initially detected, demonstrated, and elucidated by *in vitro* tests, the first necessary step in highlighting the relevant potential of a given fungus for therapeutic purposes, followed by *in vivo* tests on animal models, usually murine, for the first investigations of the effects of the substances in living cells and systems. Nevertheless, although this research aims to develop drugs and natural products for human health care, few studies have been conducted within the framework of clinical trials, a domain that is still rather unstructured, with numerous shortcomings and gaps. As there is no standard for the procedure and evaluation of results, the value of such studies is undermined in several respects. First of all, the basic experimental design, which often examines a sample that is too small and therefore not sufficiently representative, and can lead to false positive results [103,118]. Moreover, not all trials are randomized or have a placebo control [104,105], nor are double-blinded, and there is generally a lack of uniqueness of purpose, so only some parameters are taken into account and others are neglected, such as safety and side effects. Several, then, are not double-blinded, so the variable of uncontrollable human bias comes into play. Some studies also rely on the methodology of subjective assessments of certain parameters, such as quality of life, so the result examined may not have any real scientific value [66,69,87].

Again, in most cases, the so-called pilot or phase I studies, which are normally designed to assess the efficacy of a preparation, identify its dosage range, and test its safety, and thus see whether a given research project can be continued, have no follow-up. The results thus remain only preliminary, far from a medical application procedure.

All these factors also make it difficult to compare the results obtained in separate clinical studies. In this regard, it must be emphasized that another factor also comes into play, namely the preparation used to carry out the trial. Even if the same fungal species is tested, sometimes opposite or different results are obtained, as in the case of Yoshimura *et al*. [68] and Ohno *et al*. [69] with *A. blazei* Murrill. Alternatively, a fungus tested for its specific action, which has already been proven in other trials, turns out to be completely ineffective, if not actually deleterious, worsening the clinical picture [77,78]. This is also explained by the type of extract used, either because the extraction method influences the substances present or their activity, or because the concentration of a given metabolite in the extract cannot be ascertained. Furthermore, even under the same conditions, the medicinal properties of a given mushroom can vary enormously depending on the strain, the geographical area, the growing conditions and substrate used, the part of the mushroom used, and the growing stage at the moment of processing. All these parameters change the composition of the mushroom and, consequently, its bioactive capacity.

2.7 Concluding Remarks

In recent years, research into medicinal mushrooms has progressed exponentially, but much remains to be done. Many species remain unstudied or underestimated in terms of their pharmacological properties. The primary urgency is also to identify the molecules present in the extracts, the metabolites responsible for their effects, their chemical characterization, and their mechanism of action. There is also an urgent need to fully understand both their individual and synergistic actions, with particular attention paid to *in vivo* dynamics, as well as refining the design of *in vivo* and clinical studies. It is also necessary to standardize the production of mushroom supplements throughout the supply chain, from cultivation to the extraction and preparation of the commercial formulation, as well as precise monitoring and regulation to ensure high quality levels.

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Chapter 3. Mycochemicals in wild and cultivated mushrooms: nutrition and health

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3.1 Abstract

The mushrooms have contributed to the development of active ingredients of fundamental importance in the field of pharmaceutical chemistry as well as of important tools in human and animal health, nutrition, and functional food. This review considers studies on the beneficial effects of medicinal mushrooms on the nutrition and health of humans and farm animals. An overview of the chemical structure and composition of mycochemicals is presented in this review with particular reference to phenolic compounds, triterpenoids and sterols, fatty acids and lipids, polysaccharides, proteins, peptides, and lectins.

The nutritional value and chemical composition of wild and cultivated mushrooms in Italy is also the subject of this review which also deals with mushrooms as nutraceuticals and the use of mushrooms in functional foods. The nutraceutical benefits of UV irradiation of cultivated species of basidiomycetes to generate high amounts of vitamin D_2 is also highlighted and the ability of the mushrooms to inhibit glycation is analyzed. Finally, attention is paid to studies on bioactivities of some Italian wild and cultivated mushrooms with particular reference to species belonging to the genus *Pleurotus*. The review highlights the potential of medicinal mushrooms in the production of mycochemicals that represent a source of drugs, nutraceutical, and functional food.

3.2 Graphical abstract

Sample	Molecular Weight (kDa)	Monosaccharide Composition (%)[a]						
		Glc	Rham	Gal	Xyl	Ara	Man	Fru
PEPS-A	—	94.8	—[b]	—	—	—	5.2	—
PEPS-B	—	60.8	—	—	—	—	3.0	36.2
PEPS-A1	68	100	—	—	—	—	—	—
PEPS-A2	43	100	—	—	—	—	—	—

[a]Individual components were identified by comparison with standard sugars.
[b]Not detected.

3.3 Introduction

3.3.1 Definition of mycochemicals

For millennia, mushrooms were well known as a nutritional and pharmaceutical resource especially in traditional oriental therapies, but after the discovery of Penicillin (Fleming, 1929), they became a prominent source of natural antibiotics and other bioactive compounds. The subject of mycochemistry, has developed as a distinct discipline that is concerned with the enormous variety of chemical substances, named ‘‘mycochemicals’’, elaborated and accumulated by mushrooms. It deals with the isolation and structure elucidation of the chemical structures of these substances, their biosynthesis, metabolism, turnover, their natural distribution, and their biological properties (Dewick, 2009). The mycochemicals play an important role in human and animals health, nutrition, and as functional food (Scheme 1). Obviously, in all these applications, methods are needed for separation, and identification of the many different mycochemicals present in mushrooms. Thus, advances in our understanding of mycochemistry are directly related to the application of known techniques together with the continuing development of new analytical techniques to solve outstanding problems as they appear (Ruthes *et al*. 2015).

The characterization of mycochemicals is carried out using one or other, or a combination, of different chromatographic techniques that include thin layer chromatography (TLC), gas and/or liquid chromatography (CC, GC, HPLC). FT-IR, mass spectrometry and NMR experiments [1D 1H, 13C NMR and 2D NMR (H–H COSY, TOCSY, HMQC, HMBC and NOESY)] are useful in providing information for the mycochemical structural elucidation. Beneficial effects of mushrooms on human and animals health and their nutrition The use of mushrooms in Chinese folk medicine and the Eastern countries has been known for a long time while only in recent decades, especially in Europe, there has been interesting in studies on their effects on human health (Wasser, 2014; Grundemann *et al*., 2020). Moreover, the consumer’s attention is increasingly shifting to the role that adding mushrooms to the diet can promote health and prevent the risk of disease, thanks to the effects of bioactive compounds on the human body. In Asian countries, mushrooms have always been a primary source of food and medicine, due to the benefits they bring to physical well-being in general and the preventive and curative effects on various diseases such as cancer, cardiovascular diseases,

Scheme 1 The role of mycochemicals. Partially modified from www.dreamstime.com

hypertension, neuropathies, etc. Numerous studies carried out in Asian countries, and more recently also in Europe, have demonstrated the multiple effects that the different chemical components of mushrooms have on the organism, not only humans but also animals. As reported by Fernandes *et al*. (2015) and Cheung (2013), dietary fiber of mushrooms helps to prevent constipation, haemorrhoids, colon diseases, diabetes, and cardiovascular diseases, improves intestinal tract function and insulin and cholesterol metabolism. It also strengthens the immune system and has anti-tumor activity. But the bioactive compounds in mushrooms are numerous and varied, as well as their possible uses. Wasser (2014) suggested medicinal mushroom drugs (MM drugs) in immunosuppressed patients.

The antitumor MM drugs, called biological response modifiers (BRMs), are used in different types of cancer and patients undergoing chemo- and radiotherapy, improving their quality of life as they reduce side effects and help overcome cancer growth. To date, several MM products have been developed for therapeutic and commercial purposes, especially from species widespread and used in the East. The most important polysaccharides which characterize mushroom extracts are Lentinan, isolated from *Lentinula edodes* (Berk.) Pegler, Schizopyllan (Sonifilan, Sizofiran, or SPG) from *Schizophyllum commune* Fr., Ganoderan from *Ganoderma lucidum* (Curtis) P. Karst., Krestin (PSK), and PSP (polysaccharide peptide) from *Trametes versicolor* (L.) Lloyd, Grifolan from *Grifola frondosa* (Dicks.) Gray, Befungin from *Inonotus obliquus* (Fr.) Pilàt, and Imunoglukan P4H (pleuran) from *Pleurotus ostreatus* (Jacq.) P. Kumm. (Giavasis, 2014; Wasser, 2014).

The daily intake of MMs part of a healthy di*et al*so produces beneficial effects. Food supplements such as fruit bodies powders and extracts; biomass or extracts from mycelium harvested from a submerged liquid culture in fermentation tanks or bioreactors; dried and pulverized preparations of the combined substrate, mycelium, and mushroom primordial; spores and their extracts; dried mushrooms in tablets or pills are available on the market (Wasser, 2014; Reis *et al*. 2017). Mushroom bioactive compounds have an enormous potential for use as performance-enhancing natural additives for livestock animals. A survey, carried out by Bonanno *et al*. (2019), reveals how the integration in the diet of dairy ewes of

mushroom myceliated grains (a mixing of *L. edodes*, *Cordyceps* spp., *G. lucidum, P. ostreatus*) improves production both in terms of quantity, with a higher milk yield, and quality (less intense yellow colour of cheese, lower secondary lipid oxidation, greater oxidative stability and antioxidant content of the cheese). BederskaŁojewska *et al*. (2017) showed how adding edible Basidiomycetes to feed improves the productive and physiological performance of broiler chickens and laying hen.

Considerable benefits are also obtained from by-products of mushroom production, which are also rich in interesting bioactive compounds for the production of beneficial animal feed, fertilizers, cosmetics, and cosmeceuticals. (He *et al*., 2016; Taofiq *et al*., 2016; Antunes *et al*., 2020). Mycochemicals structures and composition Several mycochemicals are present in mushrooms with different chemical structures and composition such as phenolic compounds, terpenoids, lipids, polysaccharides and proteins, which are easily separated from other constituents by their high molecular weights.

3.3.2 Phenolic compounds

The term "phenolic compounds" includes a wide range of mycochemicals that are characterized by an aromatic ring bearing one or more hydroxyl groups. Phenolic substances are water-soluble since they most frequently occur in combination with sugar as glycosides, but also as esters and polymers. These compounds belong to different classes based on the number of phenol rings and of the functional groups linked to these moieties. Thus, a classification comprises simple phenols, phenolic acids, phenylpropanoids, flavonoids, flavonols, flavones, stilbenes, and lignans. Phenolic acids are the main phenolic substances found in mushrooms (Ferreira *et al*., 2009); they are classified into two groups; hydroxybenzoic acid (HBA) and hydroxycinnamic acid (HCA). Hydroxybenzoic acid derivatives are in the bound form and are part of more complex structures as hydrolyzable tannins, lignins, sugars and organic acids. Hydroxycinnamic acid derivatives are also present mainly in the bound form, attached to cell-wall structural elements, such as lignin, cellulose, proteins or linked to organic acids, through ester bonds, such as quinic or tartaric acids (Manach *et al*., 2004). The most widespread are the HCAs, which are useful not only as providing the building blocks of lignin but also concerning disease resistance and growth regulation. Five HCAs are common, in fact almost ubiquitous in mushrooms: ferulic, sinapic, caffeic, and p-/o-coumaric acids. HCAs usually are present in mushrooms in combined form as esters; and they are obtained in best yield by mild alkaline hydrolysis, since with hot acid hydrolysis material is lost for the decarboxylation to the corresponding hydroxystirenes. Caffeic acid occurs in mushrooms regularly as a quinic acid ester (3-o-caffeoylquinic, 4-o-caffeoylquinic, 5-o-caffeoylquinic). Besides, tannic and ellagic acids are

observed (Ferreira *et al*., 2009). In mushrooms, the most prevalent HBAs derivatives are reported to be gallic, protocatechuic, gentisic, homogentisic, p-hydroxybenzoic, 5-sulphosalicylic, syringic, veratric, vanillic (Ferreira *et al*., 2009) (Table 1). HBA and HCA compounds are derived biosynthetically from the shikimate pathway. L-phenylalanine and -tyrosine are the crucial amino acids and the building blocks in this pathway. Flavonoids are another large group of naturally occurring phenolic compounds that are all structurally derived from the parent substance flavone consisting of two benzene rings (A and B) combined with a pyran one (C). Different classes of flavonoids are recognized, such as anthocyanidins, flavonols, flavones, isoflavones, flavanones, and flavonols (Manach *et al*., 2004).

The flavonoids are present in nature as glycosides or aglycones. It was reported that mushrooms do not synthesize flavonoids, however, the presence of flavonoids was found in various edible mushrooms, e.g. catechin, myricetin, chrysin, hesperetin, naringenin, naringin, formometin, biochanin, resveratrol, quercetin, pyrogallol, rutin, and kaempferol (Gil-Ramirez *et al*., 2016; Ferreira *et al*., 2009). Phenolic acids and flavonoids identification and quantification from some selected mushrooms [*P. ostreatus, P. eryngii* (DC.) Que´l., *Agaricus bisporus* (J.E. Lange) Imbach, *Cyclocybe aegerita* (V. Brig.) Vizzini, *Russula cyanoxantha* (Schaeff.) Fr., *R. virescens* (Schaeff.) Fr., *Macrolepiota procera* (Scop.) Singer, *Boletus edulis* Bull., *Lactarius deliciosus* (L.) Gray, *Coprinus comatus* (O.F. Mu¨ ll.) Pers., *Tuber melanosporum* Vittad.] were done by high performance liquid chromatography coupled with mass spectrometry (HPLC–MS) (Table 1). The compounds identification derives from their retention times, their UV–Vis absorption spectra and mass spectra data and also by comparison with available data (Fogarasi *et al*., 2018). 4-Hydroxybenzoic acid and 5-feruloylquinic acid were found to be the major compounds in *P. ostreatus* and *A. bisporus* with concentrations of 75.042 mg/100 g_fw and 35.040 mg/100 g_fw for *P. ostreatus* and 79.50 mg/ 100 g_fw and 71.01 mg/100 g_fw for *A. bisporus*, respectively. *B. edulis* extract is characterized by high concentrations of cinnamic acid 168.614 mg/100 g_fw and catechin 145.566 mg/100 g_fw (Fogarasi *et al*., 2018).

Hasnat *et al*. (2014) reported content of phenolic compounds for *R. virescens* of 8.74 and 2.21 mg gallic acid/100 g_fw, and flavonoid compounds were 2.83 and 1.02 mg catechin/100 g_fw for the water and ethanol extracts, respectively. Among phenolic acids, the major amount of protacatechuic acid was found in *M. procera* (5.19 mg/Kg_dw) (Nowacka *et al*., 2014). Kalogeropoulos *et al*. evaluated the content of individual phenolic compounds for *L. deliciosus*; p-OH-benzoic acid (24.5 µg/100 g fw) and p-OH-phenylacetic acid (18.3 µg/100 g fw) were the more abundant among the hydroxyl-benzoic acids, o-coumaric acid (30.2 µg/

100 g fw) among the hydroxycinnamic acids, and chrysin (16.5 µg/100 g fw) among the flavonoids. As concerns *C. comatus*, among the phenolic compounds, the highest content was detected for quinic acid (14.6 mg/100 g_dw) and quercetin (3.01 mg/100 g fw), where the lowest amount was detected for the isoflavonoids genistein (0.023 mg/ 100 g_dw) and daidzein (0.061 mg/100 g_dw) (Nowakowski *et al*., 2020). Besides, Comatin (4, 5-Dihydroxy- 2-methoxy-benzaldehyde) isolated and identified from *C. comatus* has shown hypoglycaemic properties on alloxan-induced-diabetic rats (Ding *et al*., 2010) (Table1). In the literature, it is common to find the total phenolic content (TPC) found in mushrooms methanolic extract by the Folin-Ciocalteu assay. However, this assay has some limitations since other readily oxidized compounds such as amino acids, ascorbic acid, and sugars could interfere overestimating the total phenolic content (Arbaayah and Umi, 2013). Phenolic compounds possess antioxidant properties to scavenge free radicals, to prevent lipid peroxidation, and to chelate ferrous ions (Kumar and Pandey, 2013).

Table 1 Phenolic compounds of some selected mushrooms species

Mushroom species	**Phenolic compounds**	**References**
Pleurotus ostreatus	4-HBA, 2,4-dihydroxybenzoic acid, 4-hydroxy phenylacetic acid, pirocatechuic acid, protocatechuic acid, catechin, gallocatechin, *o*-coumaric acid, cinnamic acid, 5-feruloylquinic acid, 3,5-dicaffeoylquinic acid, chlorogenic acid, syringic acid, vanillic acid, caffeic acid, ferulic acid, 2,6- dimethoxyphenol	Sarma *et al*. (2018), Fogarasi *et al*. (2018), Koutrotsios *et al*. (2017) and Palacios *et al*. (2011)
Pleurotus eryngii	4-HBA, *p*-coumaric acid, cinnamic acid, protocatechuic acid, gallic acid, phenol	Souilem *et al*. (2017) and Reis *et al*. (2012)
Pleurotus cornucopiae	Gallic acid, protocatechuic acid, chlorogenic acid, vanillin, ferulic acid, naringin, naringenin, hesperitin, formononetin, biochanin-A	Nuhu *et al*. (2011)
Agaricus bisporus	4-HBA, 2,4-dihydroxybenzoic acid, 4-hydroxy phenylacetic acid, protocatechuic acid, catechin, gallocatechin, phydroxybenzaldehyde, p-aminophenol, catechol, coumaric acid, cinnamic acid, 4- and 5-feruloylquinic acid, 3,5-dicaffeoylquinic acid	Weijn *et al*. (2013), Fogarasi *et al*. (2018), Palacios *et al*. (2011)
Cyclocybe aegerita	Protocatechuic acid, 4-HBA, chlorogenic acid, gallic acid, caffeic acid, vanillic acid, *p*-coumaric acid, ferulic acid, sinapic acid, *t*-cinnamic acid, rutin, quercetin, kaempferol	Gasecka *et al*. (2016)

Russula cyanoxantha	Quercetin, quercetin-3-o-rutinoside, catechin, epicatechin	Butkhup *et al*. (2018)
Russula virescens	Catechin, ferulic acid, kaempferol, luteolin, vanillic acid, apigenin	Hasnat *et al*. (2014)
Macrolepiota procera	Protocatechuic acid	Nowacka *et al*. (2014)
Boletus edulis	4-HBA, 2,4-dihydroxybenzoic acid, gallic acid, 4-hydroxy phenylacetic acid, protocatechuic acid, caffeic acid, catechin, chlorogenic acid, gallocatechin, *p*-coumaric acid, sinapic acid, *o*-coumaric acid, cinnamic acid, 3,5-dicaffeoylquinic acid, gentisinic acid, homogentisinic acid, myricetin, protocatechuic acid	Fogarasi *et al*. (2018) and Palacios *et al*. (2011)
Lactarius deliciosus	4-HBA, 4-hydroxy phenylacetic acid, 3,4-dihydroxy phenylacetic acid, syringic acid, vanillic acid, caffeic acid, cinnamic acid, chlorogenic acid, ferulic acid, *o*-coumaric acid, *p*-coumaric acid, tyrosol, vanillin, chrysin, kaempferol, resveratrol, gallic acid, gentisinic acid, homogentisinic acid, myricetin, protocatechuic acid, pyrogallol	Kalogeropoulos *et al*. (2013) and Palacios *et al*. (2011)
Coprinus comatus	Flavones, flavonols, flavanones, flavanols, biflavonoids, isoflavonoids, hydroxybenzoic acids, hydroxycinnamic acids, coumarins, chlorogenic acids 4,5-Dihydroxy-2-methoxy-benzaldehyde (comatin)	Nowakowski *et al*. (2020) and Cayan *et al*. (2018) Ding *et al*. (2010)
Tuber melanosporum	Homogentisic acid, 4-HBA, 3,4-dihydroxybenzaldehyde Flavonoids, phenols	Villares *et al*. (2012) Li *et al*. (2019)

3.3.3 Terpenoids

The general term ’terpenoid’ includes all such substances with a common biosynthetic origin. Terpenoids arise from the isoprene molecule CH2=C(CH3)-CH=CH2 and their carbon skeletons originate from the union of two or more of these C5 units. Their classification is according to whether they contain two (C10), three (C15), four (C20), six (C30), or eight (C40) such unit. Essential oils, volatile mono and sesquiterpenes (C10 and C15), including the less volatile diterpenes (C20), the involatile triterpenoids and sterols (C30), and the carotenoids pigments (C40) are terpenoids. Each of these different classes of

terpenoid is of importance in mushroom growth, metabolism, or ecology (Dewick, 2009). Chemically, terpenoids are generally lipid-soluble and are extracted from mushrooms with dichloromethane, light petroleum, or ether and can be separated by flash chromatography on silica gel or alumina using some solvents. Isomerism and the presence of different geometric conformations are common among terpenoids. It depends on the substitution around the cyclohexane ring, twisted in the so-called 'chair' form. The stereochemistry of the cyclic terpenoids is highly involved. During purification procedures, structural re-arrangement and isomerization may occur and lead to artifact formation. Essential oils. The mainly terpenoid essential oils include the volatile fraction responsible for the characteristic odor and scent found in many mushrooms. They are commercially important as the basis of skincare in cosmetics and flavorings in the food industry. Fogarasi *et al*. (2018) reported the presence of α-pinene, β-phellandrene, β-pinene, β-myrcene, and D-limonene in *A. bisporus* and *B. edulis* as main terpenoids. The intube extraction headspace coupled with gas chromatography- mass spectrometry (HS-ITEX/GC–MS) permits to obtain the volatile profile of selected mushrooms. The volatile constituents strongly influence the aroma profile of each mushroom variety.

3.3.4 Triterpenoids and sterols

Triterpenoids are compounds with a carbon skeleton based on six isoprene units. They biosynthetically derived from squalene, an acyclic C10 hydrocarbon. They have relatively complex cyclic structures, most being either alcohols, aldehydes, or carboxylic acids. Sterols are triterpenes which are based on the cyclopentane perhydrophenantrene ring system. So, one example is ergosterol, ubiquitous in occurrence in mushrooms. Ergosterol is a component of the fungal cell membrane, which under the influence of UV irradiation is converted to vitamin D_2. Besides, ergosterol shows several healthy beneficial properties such as antihyperlipidemic, anti-inflammatory, antioxidant and the effect for inhibiting fungi and bacteria growth (Koutrotsios *et al*., 2017). All types of triterpenoids are isolated by very similar procedures, based mainly on column chromatography, GLC and TLC. Identities are confirmed by melting point, rotation, FT-IR, GLC-MS, and NMR experiments. Table 2 includes the triterpenoids and sterols found in some selected mushroom species. Different *P. ostreatus* strains were evaluated for their sterol composition. In all mushroom samples analyzed ergosterol dominated, comprising 51.9–87.4% of sterols, followed by its metabolites ergosta-7-enol (12.7%), ergosta-5,7-dienol (7.6%), and ergosta-7,22-dienol (6%) (Koutrotsios *et al*., 2017). The ergosterol content in *P. eryngii* was reported as 20 mg/100 g_dw, although a higher value was measured in commercial samples (Souilem *et al*., 2017). Kikuchi *et al*. (2017, 2018) reported the isolation and structure elucidation of ergostane type sterols and bisabolane-type sesquiterpenes from *P. eryngii* with aromatase and nitric oxide production inhibitory effects, respectively (Table 2). Wang *et al*. (2013a) reported the identification of novel and rare perhydrobenzannulated 5,5-spiroketal sesquiterpenes, named pleurospiroketals A-E from the edible mushroom *P. cornucopiae* with inhibitory activity against nitric oxide production in lipopolysaccharide- activated

macrophages with IC50 values between 6.8–20.8 μM. From *M. procera* were isolated and identified 12 lanostane-type triterpenoids characterized by the presence of a rare '1-en-1,11-epoxy' moiety, namely lepiotaprocerins A-L. Lepiotaprocerins A-F showed significant inhibitions of nitric oxide (NO) production, while lepiotaprocerins G-L, showed cytotoxicity effects against different human cancer cell lines, and lepiotaprocerin I displayed anti *Tuber*cular activity against *Mycobacterium Tuberculosis* H37Ra with a MIC of 50 μg/mL (Chen *et al*., 2018).

Table 2 Triterpenoids of some selected mushrooms species

Mushroom species	**Triterpenoids**	**References**
Pleurotus ostreatus	Ergosterol, ergosta-5,7-dienol, ergosta-7-enol, ergosta-7,22-dienol, oleanolic acid, ursolic acid	Sarma *et al*. (2018), Fogarasi *et al*. (2018), Koutrotsios *et al*. (2017)
Pleurotus eryngii	Ergosterol Ergostane-type sterols Strophasterols E and F Bisabolane-type sesquiterpenes Eryngiolide A, pentacyclic triterpenoids	Souilem *et al*. (2017) Kikuchi *et al*. (2017) Kikuchi *et al*. (2019) Kikuchi *et al*. (2018) Fu *et al*. (2016)
Pleurotus cornucopiae	Ergosterol, Ergosta-5,8,22-trien-3-ol, 5,6-Dihydro-ergosterol, Ergosta-7-enol, Ergosta-7,22-dienol, Ergosta-14,22-dien-3-ol, Campesterol Pleurospiroketals A-E, Perhydrobenzannulated 5,5-spiroketal sesquiterpenes Monoterpenoids, sesquiterpenoids Ergostane-type sterols	Parmar and Kumar (2015) Wang *et al*. (2013a) Wang *et al*. (2013b) Lee *et al*. (2017)
Agaricus bisporus	Ergosterol Terpenoid spiro ketals	Alshammaaa (2017) Grothe *et al*. (2013)
Cyclocybe aegerita	Bovistols A-C, Protoilludane Pasteurestin C	Surup *et al*. (2019)
Russula cyanoxantha	Ergosta-4,6,8(14),22-tetraen-3-one	Zhao *et al*. (2011)
Macrolepiota procera	Lanostane triterpenoids (Lepiotaprocerins A-L)	Chen *et al*. (2018)
Boletus edulis	Botryane sesquiterpenoids (Boledulins A-C)	Feng *et al*. (2011)
Lactarius deliciosus	Ergosterol, Ergosta-5,7-dienol, Ergosta-7-enol, Ergosta-7,22-dienol, Lanosterol, Lanosta-8,24-dienol, 4α-Methylzymosterol Azulene-type sesquiterpenoids	Kalogeropoulos *et al*. (2013) Tala *et al*. (2017)
Coprinus comatus	Terpenoids	Dulay *et al*. (2015)

Tuber magnatum	Ergosterol, Ergosta-7,22-dienol, Ergosta-5,8-dien-3-ol, Brassicasterol, 5-Dihydroergosterol, Campe-sterol, 24(28) -Dehydroergosterol, Barrigenol R1, Fungisterol, Lanosterol, Dehydroepiandrosterone	Tejedor-Calvo *et al*. (2020) and Yeh *et al*. (2016)
Tuber melanosporum	Ergosterol, Ergosta-7,22-dienol, Brassicasterol, 5-Dihydroergosterol, Campesterol, 24(28)-Dehydroergosterol, Barrigenol R1, Fungisterol, Lanosterol, β-Sitosterol, Dehydroepiandrosterone	Tejedor-Calvo *et al*. (2020) and Yeh *et al*. (2016)
Tuber borchii	Ergosterol, Ergosta-7,22-dienol, Brassicasterol, Campesterol, 24(28)-Dehydroergosterol, Dehydroepiandrosterone	Tejedor-Calvo *et al*. (2020) and Yeh *et al*. (2016)

Three non-isoprenoid botryane sesquiterpenoids, named boledulins A-C were isolated from the cultures of *B. edulis* Bull. with moderate inhibitory activity against five human cancer cell lines (Feng *et al*., 2011), while from the edible mushroom *L. deliciosus*, azulene- type sesquiterpenoids were characterized (Tala *et al*., 2017). Many sterols such as campesterol, lanosterol, brassicasterol, β-sitosterol, ergosterol were analyzed in the fruiting bodies of different *Tuber* species (Table 2). The main sterols found in *Tuber magnatum* Picco and *T. melanosporum* fruiting bodies were ergosterol and brassicasterol, which amounted to 63.1–66.7% and 15.7–21.3% of the total sterols, respectively. Also the mycelia of *T. borchii* Vittad. are a rich source of ergosterol (90.3%). The complex composition profile of the truffle sterols might be taken as the fingerprint for the identification of the truffle species (Yeh *et al*., 2016).

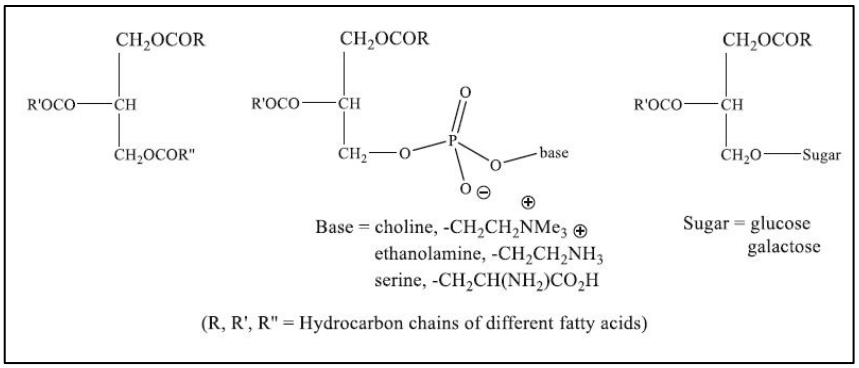


Fig.1 Chemical structures of mushroom lipids

3.3.5 Fatty acids and lipids

Mushrooms are an essential source of fatty acids that occur mainly in bound form, esterified to glycerol, as fats or lipids. They are crucial as membrane constituents in the mitochondria

and chloroplasts and provide mushrooms with a storage form of energy. The content of total lipids ranges mostly from 1 to 4% of the dry weight. Besides, mushroom fats are rich in unsaturated fatty acids (PUFA) and particularly in linoleic acid (Koutrotsios *et al*., 2017). Lipids are known by their distinct solubility properties and are extracted with alcohol, ether or dichloromethane from mushrooms. The general structures for the three main classes of mushrooms lipids are reported in Fig. 1. Structural variation within each class is due to the different fatty acid residues that may be present. The identification of lipids mainly requires the determination of their fatty acid components. Fatty acids are determined as methyl esters (FAMEs) after hot saponification of the sample, followed by reaction with BF3/MeOH. The resulting FAMEs are analyzed by GC–MS by comparison with standard FAMEs and confirmed utilizing mass spectra library (Helrich, 1990). In some selected mushrooms species, the fatty acid composition is characterized by a prevalence of polyunsaturated linoleic acid (C18:2 ω6), monounsaturated oleic acid (C18:1 ω9), and saturated palmitic acid (C16:0) (Table 3). The fatty acids are divided into saturated (SFA), monounsaturated (MUFA), and polyunsaturated (PUFA). In particular, the ratio between the single components of PUFA is fundamental in preventing cardiovascular diseases. PUFAs are a family of so-called "essential" fatty acids that are converted to tissue hormones useful to prevent blood clotting and hypertension (Pietrzak-Fiecko *et al*., 2016). Koutrotsios *et al*. (2017) evaluated the fatty acid profile of different *P. ostreatus* strains, collected in Greece, including saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), ω3 and ω6 fatty acids. PUFA was the major fatty acid class detected; linoleic acid (C18:2ω6) dominated in all samples (56.8–80.5%) followed by oleic (C18:1ω9) and palmitic (C16:0) (6.3–19.5 and 7.5–12.1%, respectively) (Table 3). Jing *et al*. (2012) reported a selective method where fatty acids from cultivated mushrooms *P. eryngii, C. aegerita and C. comatus* were derivatized with BAETS as the labeling reagent and identified by high-performance liquid chromatography with fluorescence detection and online mass spectrometry (HPLC-FLD-MS/MS). Total fatty acids (TFAs) values for *P. eryngii, C. aegerita* and *C. comatus* (dw) were 42.60, 48.95, and 79.21 mg 10 g^{-1}, respectively, while UFA:SFA ratio were 3.23, 3.29, and 3.03, respectively. Linoleic (C18:2ω6) and oleic (C18:1ω9) acids were the main FA found and their content was between 27.17–49.34 mg 10 g^{-1} and 4.08–22.15 mg 10 g^{-1}, respectively.

Table 3 Fatty acids of some selected mushrooms species

Mushroom species	**Fatty acids**	**References**

Pleurotus ostreatus	SFA, MUFA, PUFA, n − 6, n − 3	Koutrotsios *et al*. (2017) and Fogarasi *et al*. (2018)
Pleurotus eryngii	SFA (C10:0, C11:0, C12:0, C13:0, C14:0, C15:0, C16:0, C17:0, C18:0, C19:0, C20:0, C21:0, C22:0), MUFA (C16:1ω7, C16:1ω9, C18:1ω9), PUFA (C18:2ω6, C18:3ω3, C20:4ω6, C22:6ω3)	Jing *et al*. (2012) and Rodrigues *et al*. (2015)
Pleurotus cornucopiae	SFA (C14:0, C15:0, C16:0, C17:0, C18:0, C20:0, C24:0), MUFA (C16:1ω7, C16:1ω9, C18:1ω9), PUFA (C18:2ω6, C18:3ω3, C20:4ω6, C22:6ω3)	Rodrigues *et al*. (2015)
Agaricus bisporus	SFA, MUFA, PUFA, C16:1ω7, C16:0, C18:0, C18:1ω9, C18:2ω6, C20:0	Sande *et al*. (2019) and Fogarasi *et al*. (2018)
Cyclocybe aegerita	SFA (C10:0, C11:0, C12:0, C13:0, C14:0, C15:0, C16:0, C17:0, C18:0, C19:0, C20:0, C21:0, C22:0), MUFA (C16:1ω7, C16:1ω9, C18:1ω9), PUFA (C18:2ω6, C18:3ω3, C20:4ω6, C22:6ω3)	Jing *et al*. (2012)
Russula cyanoxantha	SFA (C6:0, C8:0, C10:0, C12:0, C13:0, C14:0, C15:0, C16:0, C17:0, C18:0, C20:0, C21:0, C22:0, C23:0, C24:0), MUFA (C16:1, C18:1ω9, C20:1, C24:1), PUFA (C18:2ω6, C18:3ω3, C20:2, C20:3ω3, C20:5ω3)	Grangeia *et al*. (2011)
Russula virescens	SFA (C16:0, C18:0), MUFA (C18:1ω9), PUFA (C18:2ω6)	Leal *et al*. (2013)
Macrolepiota procera	SFA (C16:0, C18:0), MUFA (C18:1ω9), PUFA (C18:2ω6)	Yilmaz *et al*. (2013)
Boletus edulis	SFA (C6:0, C8:0, C10:0, C12:0, C14:0, C15:0, C16:0, C17:0, C18:0, C20:0, C22:0, C23:0, C24:0), MUFA (C16:1, C17:1, C18:1ω9, C20:1, C22:1ω9, C24:1), PUFA (C18:2ω6, C18:3ω3, C18:3ω6, C20:2, C20:4ω6, C20:3ω3,C21:0,C20:5ω3)	Heleno *et al*. (2011) and Pietrzak-Fiećko *et al*. (2016)
Lactarius deliciosus	SFA (C10:0, C11:0, C12:0, C13:0, C14:0, C15:0, C16:0, C17:0, C18:0, C20:0, C21:0, C23:0, C24:0), MUFA (C16:1ω9, C16:1ω7, C18:1ω9, C20:1ω9), PUFA (C18:2ω6, C18:3ω3, C20:2ω6, C20:3ω6, C20:4ω6 + C22:0, C20:3ω6, C20:5ω3, C22:2ω6)	Kalogeropoulos *et al*. (2013) and Ergönül *et al*. (2012)
Coprinus comatus	SFA (C10:0, C11:0, C12:0, C13:0, C14:0, C15:0, C16:0, C17:0, C18:0, C19:0, C20:0, C21:0, C22:0), MUFA (C16:1ω7, C16:1ω9, C18:1ω9), PUFA (C18:2ω6, C18:3ω3, C20:4ω6, C22:6ω3)	Jing *et al*. (2012) and Ergö nül *et al*. (2012)

Tuber melanosporum	SFA (C14:0, C15:0, C16:0, C17:0, C18:0, C20:0, C21:0, C22:0, C23:0, C24:0), MUFA (C16:1, C17:1, C18:1ω9, C20:1ω9, C22:1ω9, C24:1ω9), PUFA (C18:2ω6, C20:2, C20:4ω6, C22:6ω3)	Jiang *et al.* (2018)

SFA saturated fatty acids, *MUFA* monounsaturated fatty acids, *PUFA* polyunsaturated fatty acids

Also, for *P. cornucopiae* the linoleic acid (C18:2 ω9) was the main FA, with a composition characterized by a higher content of mono (MUFA) and polyunsaturated FA (PUFA) than of saturated FA (SFA) (Rodrigues *et al*., 2015). The lipids analyzed for *A. bisporus* showed a high content of unsaturated acids with linoleic acid (C18:2 ω6) as the main constituent of fruiting bodies (33.3%) and stems (39.4%). The total saturated fatty acid (SFA) content was between 22.1 and 26.5% of total lipids, palmitic acid (C16:0) was the major SFA at about 14% followed by stearic acid (C18:0) at about 4%. Oleic acid (C18:1ω9) was the major monounsaturated fatty acid (MUFA) present at about 1.5% of total lipids (Sande *et al*., 2019). As concerns *R. cyanoxantha* the major fatty acid found was linoleic acid (C18:2ω6) (43.65%) followed by oleic acid (C18:1ω9) (28.39%) and palmitic acid (C16:0) (12.95%) (Grangeia *et al*., 2011). The fatty acid composition of different wild *Boletus* species collected in Portugal was reported by Heleno *et al*. (2011). (Table 3). The major fatty acid found in *B. edulis* was oleic acid (C18:1ω9) (42.5%) followed by linoleic acid (C18:2ω6) (41.32%) and palmitic acid (C16:0) (9.57%). A very similar profile of fatty acid composition was reported for 33 samples of wild *B. edulis* in the form of caps and stems, collected from selected regions of Poland. The dominant fatty acids in all samples analyzed were C18:2ω6, C18:1 ω9, and C16:0 (Pietrzak-Fiecko *et al*., 2016).

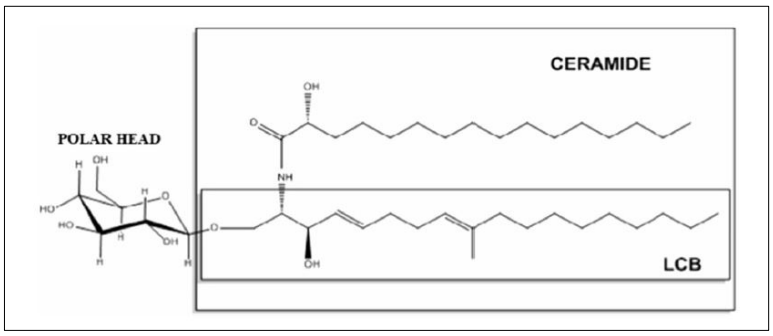


Fig. 2 Chemical structure of mushroom glycosphingolipids

Kalogeropoulos *et al*. (2013) reported the fatty acid composition of wild *L. deliciosus* from Greece. The prevalent fatty acids were linoleic acid (C18:2ω6) (31.78%), followed by stearic acid (C18:0) (29.83%) and oleic acid (C18:1ω9) (21.82%) (Table 3). Another class of lipids found in mushrooms is glycosphingolipids (GLSs) and the cerebrosides in particular. A polar head (usually a monosaccharide or a carbohydrate chain) and a fatty acyl group are linked

to a long-chain aminoalcohol called a long-chain base (LCB). The fatty acyl chain is amide-linked to the LCB and together they make up the ceramide; the monosaccharide or oligosaccharide group is linked to the primary alcoholic function of the ceramide (Fig. 2). GLSs are ubiquitous membrane constituents of mushrooms and are believed to possess a wide range of biological activities, including modulation of growth and regulation of differentiation. They are involved in membrane phenomena, such as cell–cell recognition, cell–cell adhesion, antigenic specificity, and other kinds of transmembrane signaling. β-Glucosylceramide is by far the most common GLS from mushrooms. A peculiarity of glucosylceramides from mushrooms is the frequent occurrence of a di-unsaturated C18 sphingosine with a methyl branching at C-9. Structure determination was based on carbohydrate analysis, methylation analysis, chemical degradation, and extensive use of FAB-MS (Itonori *et al*., 2004). Three cerebrosides with different lengths of the fatty acid portion have been isolated and identified from *Pleurotus cornucopiae* (Paulet) Rolland (Lee *et al*., 2017). Furthermore, purified acidic glycosphingolipids (AGLs) from *P. eryngii* were reported to induce interleukin-2 (IL-2) release from invariant natural killer T (iNKT) cells inducing prolonged retention of IL-4 in serum *in vitro* and *in vivo* (Fu *et al*., 2016). So through iNKT cell activation AGLs isolated from *P. eryngii* might be involved in the maintenance of immunohomeostasis. An important secondary metabolite from mushrooms is lovastatin, a polyketide employed as a cholesterol-lowering drug that inhibits (3S)-hydroxy- 3-methylglutaryl-coenzyme A (HMG-CoA) reductase. This is a key enzyme in the synthesis of mevalonate, since it is the immediate precursor of cholesterol and lovastatin is the lead compound of all of the drugs classified as statins. Lovastatin was discovered from *Aspergillus terreus* and *Monascus ruber* in the 1970s and is a natural product in oyster mushrooms (Chen *et al*., 2012) (Fig. 3).

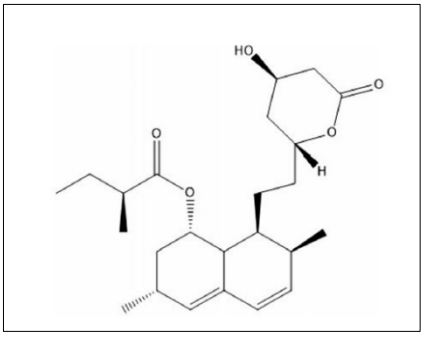


Fig. 3 Chemical structure of lovastatin

3.3.6 Polysaccharides

Mushrooms are a significative source of polysaccharides. The structural complexity of polysaccharides is ascribed to the linkage between two sugar units, through an ether linkage,

in several different ways. The reducing end of one sugar (C1) can condense with any hydroxyl group of a second sugar (at C2, C3, C4, or C6) so that during polymerization some sugars may be substituted in two positions, leading to branched chains structures. Besides, the ether linkage can have either a α- or β-configuration, due to the stereochemistry of simple sugars, and both kinds of linkage canco-exist in some molecule. Generally, the polysaccharides are present in the mushroom cell wall and include α-glucans and β-glucans. These macromolecules are composed of glucopyranose units linked with glycosidic bonds of the type (1→6)-β, (1→3)-β, or (1→3)-α. Mushrooms are characterized by different kinds of polysaccharides that include not only glucans but also heteroglycans and proteoglycan classes. Polysaccharides that include residues of only one type of monosaccharide unit are known as homoglycans, while residues of two or more types of monosaccharide molecules are categorized as heteroglycans (Kozarski *et al*., 2014). As concerns, the extraction and purification procedures, usually the polysaccharides are isolated by successive hot-water extractions followed by ethanol precipitation. Chromatographic methods such as size exclusion (SEC) and ion-exchange chromatography (IEC) are used as purification procedures of the crude polysaccharides, while chemical reactions of hydrolysis and derivatization together with NMR experiments are useful in providing information for their structural elucidation (Sun *et al*., 2010a). Polysaccharides isolated and identified from mushrooms differ in their physical–chemical properties such as in their water solubility, molecular weight, size of the molecule, and structure (Table 4). Recently, polysaccharides isolated from mushrooms have attracted increasing attention for their wide spectrum of biological properties, such as antioxidative, antitumor, immunomodulation (BRMs), and anti-inflammatory effects (Selvamani *et al*., 2018). The major pharmaceutical properties of mushrooms, i.e. antitumor activities and immunity potentiation, are ascribed to β-glucans. Many fungal β-glucans stimulate both innate and adaptive immunity. They activate innate immune system components such as natural killer (NK) cells, neutrophils, macrophages, and cytokines. These cytokines, in turn activate adaptive immunity with the stimulation of B-cell for antibodies production and promotion of T-cell differentiation to T-helper cells, which mediate cell and humoral immunities (Oloke and Adebayo, 2015). Pleuran, a water-soluble polysaccharide [β-(1,3/ 1,6)-D-Glucan], is the best-known β-glucan isolated from *P. ostreatus* with a molecular weight of 762 KD.

It is composed of a backbone (1 → 3) linked β-D-glucose with a side chain of a β-(1 → 6) or β-(1 → 4)-D-glucosyl residue of ever fourth glucose unit. The compound exhibit anti-neoplastic properties against different cells, including breast cancer MCF-7, prostate cancer cells PC-3 and colorectal HT-29 cancer cells. It possesses also antiviral and antioxidative

properties (Golak-Siwulska *et al*., 2018). The purified polysaccharides PEPE-A1 and PEPEA2 from *P. eryngii* are characterized by a β-(1 → 3)- glucan as the backbone accompanied by α-(1 → 6)-D-glucosyl residues side chains. They showed a strong inhibitory effect on lipid accumulation (Fu *et al*. 2016). Recently, a mannogalactan with the main chain of (1 → 6)-linked-α-D-galactopyranosyl and 3-Omethyl-α-D-galactopyranosyl residues, both partially substituted at OH-2 by β-D-Manp units was isolated from *P. eryngii* and tested against murine melanoma cells (Biscaia *et al*., 2017). Zhang *et al*. (2014) isolated three subfractions of intracellular zinc polysaccharides (IZPS) from *P. cornucopiae*. All the subfractions have shown antioxidant activities *in vitro* and *in vivo*. They were found able to act as upregulatation of the superoxide dismutase, GSH peroxidase and catalase, and significantly decreased the contents of malondialdehyde and lipid peroxidation *in vivo*. PCPS from *P. cornucopiae* mushroom extract is a β-(1 → 6)-glucan possessing a proinflammatory effect on innate immune cells (Minato *et al*., 2017). From *A. bisporus* a new heteropolysaccharide consisting of ribose, rhamnose, arabinose, xylose, mannose, glucose, and galactose with 1 → 2 and 1 → 4 glycosidic bonds and probably 1 → 3 glycosidic bonds were isolated and identified with high *in vitro* immunobiological activity (Liu *et al*., 2020a, b, c). Motoshima *et al*. (2018) identified a fucogalactan from *C. aegerita* (FG-Aa) characterized by (1 → 6)- linked α-D-galactopyranosyl main chain, substituted at O-2 by non-reducing end units of α-L-Fucp, on the average of one to every second residue of the backbone. The obtained fucogalactan was evaluated against arginase from *Leishmania amazonensis*.

Table 4 Polysaccharides of some selected mushrooms species

Mushroom species	**Polysaccharides**	**References**
Pleurotus ostreatus	Pleuran [β-(1,3/1,6)-D-Glucan] α-(1- 3)-glucans Mycelium polysaccharides 2 (POMP2), POPS-1	Selvamani *et al*. (2018) Golak-Siwulska *et al*. (2018) Sarma *et al*. (2018)
Pleurotus eryngii	PEPE-A1, PEPE-A2 Partially methylated mannogalactan	Fu *et al*. (2016) Biscaia *et al*. (2017)
Pleurotus cornucopiae	Intracellular zinc polysaccharides (IZPS)	Zhang *et al*. (2014)
Agaricus bisporus	β-glucan (PCPS) [β-(1- 6)-Glucan] β-glucan [β-(1-6)-Glucan], mannogalactan Heteropolysaccharide ABP Ia	Minato *et al*. (2017) Smiderle *et al*. (2011) Liu *et al*. (2020a, b, c)
Cyclocybe aegerita	Fucogalactan (FG-Aa) Ac-MPS, AI-MPS	Motoshima *et al*. (2018) Jing *et al*. (2018)

Russula cyanoxantha	β-glucan	Butkhup *et al*. (2018)
Russula virescens	(1-3)-β-D-glucan, RVP SRVPs	Sun *et al*. (2010b) Li *et al*. (2020)
Macrolepiota procera	Polysaccharides	Nowak *et al*. (2018)
Boletus edulis	Polysaccharides (BEBP-1, BEBP-2 and BEBP-3) Polysaccharids (BEPF30, BEPF60 and BEPF80)	Luo *et al*. (2012) Zhang *et al*. (2011)
Lactarius deliciosus	Polysaccharide (LDG-M) Polysaccharide (LDG-A) Polysaccharide (LDG-B) Polysaccharide (LDGO-A)	Su *et al*. (2019) Hou *et al*. (2019) Hou *et al*. (2016) Ding *et al*. (2015)
Coprinus comatus	Modified polysaccharide (MPCC) Polysaccharide (CCPP-1) Polysaccharide (CC30w-1)	Zhao *et al*. (2019) Liu *et al*. (2013) Zhou *et al*. (2013)
Tuber magnatum	(1-3)-β-D-glucan (1-3)-β-D-glucan	Tejedor-Calvo *et al*. (2020) Tejedor-Calvo *et al*. (2020)
Tuber melanosporum	Exo-polysaccharides (TP1, STP1, STP2)	Liu *et al*. (2020a, b, c)
Tuber borchii	(1-3)-β-D-glucan	Tejedor-Calvo *et al*. (2020)

A water-insoluble (1 → 3)-β-D-Glucan was firstly isolated from the fresh fruiting bodies of *R. virescens*, and then the sulfated derivative was synthesized with sulfur trioxide-pyridine complex. The sulfated derivative exhibited enhanced anti-tumor activities against Sarcoma 180 tumor cell (Li *et al*., 2020). Besides, a water-soluble poaysaccharide (RVP) with antioxidant properties was isolated from the fruiting bodies of *R. virescens* consisting of (1 → 6)-linked-α-D-galactopyranosyl and (1 → 2,6)-linked-α-D-galactopyranosyl residues that terminated in a single nonreducing terminal (1 →)-α-D-mannopyranosyl residue at the O-2 position of each (1 → 2,6)-linked-α-D-galactopyranosyl residues along the backbone (Sun *et al*., 2010a). Also RVP was sulfated and *in vitro* activity test data indicated that the SRVPs showed better antioxidant, anticoagulant, antitumor and antibacterial activities compared with RVP. Three crude polysaccharides (BEPF30, BEPF60, and BEPF80) were isolated from the fruiting bodies of *B. edulis* and investigated for their antioxidant activities. BEPF60 showed significant reducing power and chelating activity together with the highest inhibitory effects on hydroxyl and superoxide radicals (Zhang *et al*., 2011). Other crude water-soluble polysaccharides (BEBPs) were extracted from *B. edulis* and evaluated for their antioxidant activities. BEBP-3 showed a significant antioxidant activity (Luo *et al*., 2012). *Lactarius deliciosus* is an important source of polysaccharides. Su *et al*. (2019) reported the structural

characterization and immune regulation activity of a novel polysaccharide (LDG-M) from *L. deliciosus* Gray. LDG-M was composed of β-D-glucose and α-D-lyxose with ratio 2:1. The proposed structure of LDGM was a backbone of 1,6-linked-β-D-glucose and 1,4,6-linked-β-D-glucose, with branches composed of one (1 → 4)-linked-α-D-lyxose residue (Table 4). The structural elucidation of LDG-A indicated a backbone of 1,6-disubstituted-α-L-mannopyranose with branches at O-2 mainly composed of a (2 → 3)-α-D-xylopyranose residues. LDG-A exhibited marked antitumor activities *in vivo*. A new heteropolysaccharide (LDG-B) with a backbone of (1,6)-linked-D-galactose and (1,2,6)-linked-D-galactose with branches composed of 4-linked-D-glucose and 6-linked-D-galactose residue was identified from *L. deliciosus*. Cell cycle test data showed that LDG-B could promote the proliferation of B cells and macrophage cells by affecting G0/G1, S and G2/M phases (Hou *et al*., 2016). Besides, also the structure elucidation and anti-tumor activity of water-soluble oligosaccharides (LDGO-A) were reported by Ding *et al*. (2015).

A modified polysaccharide named MPCC was obtained by snailase hydrolysis from *C. comatus* with antioxidant and hepatoprotective properties (Zhao *et al*., 2019). The structural investigation of CCPP-1 from *C. comatus* has shown that CCPP-1 was α-D-(1 → 4)-glucan with branches at C-6 consisting of non-reducing terminal approximately every fourteen residues. While the crude polysaccharide fractions CCPF showed significant hypoglycemic activity, CCPP-1 was not useful on reducing blood sugar (Liu *et al*., 2013). As concerns *Tuber* fruiting bodies and fermentation system, the structure, the physicochemical and biological properties of the polysaccharides have not been thoroughly investigated. Tejedor-Calvo *et al*. (2020) reported a preliminary screening of the main bioactive compounds for *T. magnatum*, *T. melanosporum* and *T. borchii* by using pressurized liquid extractions (PLE). The polysaccharide composition of the obtained extracts was investigated by NMR analysis and their immunomodulatory activity tested *in vitro* with cell cultures. NMR investigation revealed that the extracted polysaccharides were β-(1 → 3)-glucans and a heteropolymer consisting of galactose and mannose.

3.3.7 Proteins, peptides and lectins

Other macromolecular mycochemicals isolated from mushrooms with high molecular weight are proteins, peptides, and lectins. The proteins in mushrooms, as in other plants, are high molecular weight polymers of amino acids. The amino acids are arranged in a particular linear order and each protein has a specific amino acid sequence. Proteins are usually purified according to molecular weight so they are subjected to gel filtration on a column of Sephadex. Separation of proteins by gel electrophoresis is also partly determined by their molecular size since their mobility on the gel is closely related to their charge properties

(Oloke and Adebayo, 2015). The composition of mushroom proteins seems to be of higher nutritional value concerning most plant proteins. Mushrooms proteins contain all nine essential amino acids required by humans and can be used as a substitute for meat (Kakon *et al*., 2012). High contents of proteins 38.9 and 36.9% were observed in *A. bisporus* and *B. edulis*, respectively (Nagy *et al*., 2017). Mushrooms are a rich source of proteins with several properties for biotechnological and medicinal applications. Immunomodulatory proteins (FIPs) are a group of fungal proteins able to alter the cytokine response (Oloke and Adebayo, 2015). Proteins isolated from selected mushrooms exhibited antiviral, antitumor, antifungal, and antibacterial properties (Table 5). Moreover, the fruiting bodies and mycelium of several mushrooms are an abundant source of ergothioneine, an unusual sulfur-containing derivative of histidine, with antioxidant properties (Chen *et al*., 2012).

Table 5 Proteins, peptides and lectins of some selected mushrooms species

Mushroom species	Proteins, peptides and lectins	References
Pleurotus ostreatus	Cibacron blue affinity purified protein (CBAEP) Pleurostrin Dimeric lectin Laccase Concanavalin A	Sarma *et al*. (2018) Erjavec *et al*. (2012) Oloke and Adebayo (2015) Golak-Siwulska *et al*.(2018) Sarma *et al*. (2018)
Pleurotus eryngii	Eryngin Laccase Protease (Pleureryn) PEP 1b	Erjavec *et al*. (2012) Fu *et al*. (2016) Fu *et al*. (2016) Hu *et al*. (2018)
Pleurotus cornucopiae	Oligopeptides Laccase Lectin (PCL-M)	Golak-Siwulska *et al*.(2018) Wu *et al*. (2014) Oguri (2020)
Agaricus bisporus	Lectin (ABL) Protein FIIb-1	Verma *et al*. (2019) Verma *et al*. (2019)
Cyclocybe aegerita	Ribotoxin-like protein (Ageritin) Lectin (AAL) Lectin (AAL-2)	Citores *et al*. (2019) Liu *et al*. (2017) Ren *et al*. (2015)
Russula virescens	Laccase Feruloyl esterase (FAE)	Zhu *et al*. (2013) Wang *et al*. (2014b)
Macrolepiota procera	β-Trefoil lectin (MpL)	Zurga *et al*. (2017)
Boletus edulis	β-Trefoil lectin (MpL)	Zurga *et al*. (2017)
Lactarius deliciosus	Laccase	Khaund and Joshi (2014)
Coprinus comatus	Protein Y3 Laccases	Nowakowski *et al*. (2020) Nowakowski *et al*. (2020)

Tuber borchii	Lectin (Cyanovirin-N)	Matei *et al*. (2011)

Many proteins are also enzymes, catalyzing particular steps in either primary or secondary metabolism, and possess health-promoting effects. Laccases were isolated from *P. ostreatus* and *P. cornucopiae* with antiviral effect against the hepatitis C virus and HIV-1 reverse transcriptase, respectively (Table 5). Lectins are another group of mycochemicals that include polysaccharide-protein and polysaccharide-peptide complexes. Lectins derived from mushrooms exhibit antiproliferative, immunomodulatory, antitumor, HIV-1 reverse transcriptase inhibiting, cell growthregulating, and many more properties (Oloke and Adebayo, 2015). Some proteins, peptides, and lectins isolated from various selected mushrooms are reported in Table 5. From *P. ostreatus* a Cibacron blue affinity-purified protein (CBAEP) was isolated with potent antitumor, anticancer and immunomodulatory activity against Sarcoma-180, Dalton lymphoma (DL)-bearing mice, and B16FO melanoma tumor-bearing mice (Sarma *et al*., 2018).

Besides, pleurostrin and eryngin are two proteins isolated from *P. ostreatus* and *P. eryngii* mushrooms with antibacterial and antifungal properties (Erjavec *et al*., 2012). The laccase isolated from *P. ostreatus* exhibited an antiviral effect against the hepatitis C virus (Golak-Siwulska *et al*., 2018). A dimeric lectin, composed of subunits with a molecular weight of 40 and 41 KDa, isolated from fresh fruiting bodies of *P. ostreatus* exerted antitumor activity in mice bearing sarcoma S-180 and hepatoma H-22 (Table 5). Fu *et al*. (2016) reported the isolation of a laccase from *P. eryngii* with antiviral activity against HIV. The laccase was active against HIV-1 growth with an IC50 of 2.2 μM by inhibiting HIV-1 reverse transcriptase. Also a protease named pleureryn, extracted from fresh fruiting bodies of *P. eryngii*, showed 23.1 ± 0.6% and 91.4 ± 3.2% inhibition of HIV-1 reverse transcriptase at 3 and 30 mM, respectively (Table 5). Hu *et al*. (2018) reported the functional characterization of a *P. eryngii* protein (PEP 1b). PEP 1b is an immunomodulatory protein with 21.9 KDa able to induce the M1-polarization of the macrophage cell line RAW 264.7 cells through the activation of the TLR4-NF-jB and MAPK signal pathways. Two types of angiotensin I-converting enzyme (ACE) inhibitory oligopeptides were obtained from the basidioma of *P. cornucopiae*. The amino acid sequences of the two purified oligopeptides were found to be RLPSEFDLSAFLRA and RLSGQTIEVTSEYLFRH. Besides, from the fermentation broth of *P. cornucopiae* was isolated a new laccase with a molecular mass of 67 KDa. It inhibited proliferation of the hepatoma cells HepG2, the breast cancer cells MCF-7, and the activity of HIV-I reverse transcriptase with IC50 values of 3.9, 7.6 and, 3.7 μM, respectively (Wu *et al*., 2014). Besides, a divalent cation-dependent GalNAc-specific lectin (PCL-M) was purified from the mycelia of *P. cornucopiae*. It is a multimeric glycoprotein composed of 40 KDa subunits linked by disulfide bonds (Oguri, 2020). A lectin, isolated from *A. bisporus* (ABL) showed antiproliferative effects on different cell types and might be useful for glaucoma. Besides, the fruiting bodies of *A. bisporus* are associated with a protein, named FIIb-1, characterized as tyrosinase (Verma *et al*., 2019).

Recently, a ribotoxin-like protein, named Ageritin was isolated from the basidiomycetes *C. aegerita*. Several biological activities are ascribed to Ageritin such as antibacterial, antiviral, endonuclease,

nuclease, antifungal, and cytotoxicity to COLO 320, HeLa and, Raji cells by promoting apoptosis (Citores *et al*., 2019). The lectin (AAL), isolated from *C. aegerita* exhibited antitumor activity by inducing apoptosis (Liu *et al*. 2017), while lectin-2 (AAL-2) and its complexes with GlcNAc and GlcNAcb1-3Gal b1- 4GlcNAc revealed the structural features of specific recognition of non-reducing terminal N-acetylglucosamine (Ren *et al*., 2015).

A novel laccase was purified and characterized by *R. virescens*. Its N-terminal amino acid sequence was AIGPTAELVV and it was able to degrade various phenolic compounds and to decolorize several dyes (Zhu *et al*., 2013). Zurga *et al*. (2017) isolated novel ricin B-like lectin with a β-trefoil fold from *M. procera*, designated as MpL with nematocidal activity indicating a function in protecting fruiting bodies against parasites. MpL was studied for potential delivery of peptidase protein inhibitors to lysosomes showing that it is a promising carrier of protein drugs to intracellular targets. An antiviral protein Y3 isolated from *C. comatus* showed an inhibitory effect on the tobacco mosaic virus. Y3 has shown anticancer potential inducing caspase-dependent apoptosis in Jurkat cells of human T-cell leukemia. Besides, also laccases from mycelia of *C. comatus* have shown antiproliferative and antiviral properties (Nowakowski *et al*., 2020) (Table 5).

3.4 Nutritional value of mushrooms

The consumption of mushrooms as food has ancient origins. There is evidence of their inclusion in the diet, in fact, already in the civilizations of the Greeks and Romans, who considered them “the food of the Gods” (Valverde *et al*., 2015). The enormous alimentary potential of mushrooms lies not only in their rich aroma and flavor, which make them an authentic delicacy but also in their high nutritional value so that they are considered functional foods (Barros *et al*., 2008; Tsai *et al*., 2009; Wani *et al*., 2010; Wang *et al*., 2014c; Kumar, 2015; Correa *et al*., 2016; Rathore *et al*., 2017; Reis *et al*., 2017; Antunes *et al*., 2020). The fungal fruiting body is composed mostly of water, so the caloric intake provided by it is very low (about 350–400 kcal kg^{-1}) (Kalac, 2012). Dry matter (DM) represents only 5–15%, with variable contents of carbohydrates and proteins, but also fibers and minerals, depending on the fungal species (Barros *et al*., 2008; Wani *et al*., 2010; Reis *et al*., 2012; Cheung, 2013; Kalogeropoulos *et al*., 2013; Wang *et al*., 2014c; Heleno *et al*., 2015). Of all the species of mushrooms cultivated or available in Italy, those belonging to the genus *Pleurotus* are among the most appreciated for their high nutritional value. Studies have shown that the content of *P. eryngii* carbohydrates, the main components of the fungal fruiting body, is very high (75.4%), even comparable to that of wheat grains and oat bran (Venturella *et al*., 2015; Carrasco-González *et al*., 2017). *C. aegerita* has an even higher content, around 84% (Petrovic´ *et al*., 2015), in the wild *L. deliciosus* is 66.61 g/100 g_dw, while that of *C. comatus*, *M. procera* and *B. edulis* is significantly lower (58.4%, 54.70% and 46.95%, respectively) (Tsai *et al*., 2007; Ayaz *et al*., 2011; Xu *et al*., 2019). Lower is

also the carbohydrate content of *A. bisporus* (51.05%) (Atila *et al*., 2017). Particular is the case of the wild mushrooms *R. cyanoxantha* and *R. virescens*, which, although belonging to the same genus, may show significantly different, and in any case rather low, carbohydrate contents (9.56 and 24.40%, respectively) (Srikram and Supapvanich, 2016). Most of the fungal carbohydrates are not digestible and include dietary fiber, cell wall polysaccharides as chitin, ß-glucans and mannans, and oligosaccharides. Mushroom dietary fiber is composed by insoluble fiber: mostly chitin and ß-glucans, but also other structural polysaccharides such as hemicelluloses. Soluble fiber (mainly pectines) is generally less than 10% DM. *Pleurotus* genus has a high content of crude fiber (10.2%), as well as *C. comatus* (12.5%), and ß-glucans (25.9%); in particular, the highest amount of ß-glucans is found in *P. ostreatus* (up to 50%) (Tsai *et al*., 2007; Correa *et al*., 2016; Carrasco-Gonzalez *et al*., 2017; Bulam *et al*., 2019). This makes this genus one of the main and most interesting sources of ß-glucans, including pleuran, currently commercialized as a natural immunostimulant (Imunoglukan P4H®) due to its bioactivity (Carrasco-González *et al*., 2017; Reis *et al*., 2017; Golak-Siwulska *et al*., 2018; Bulam *et al*., 2019). On the contrary, the chitin level is significantly higher in *A. bisporus* than in *P. ostreatus* (Atila *et al*., 2017). Of the total free sugars, the most abundant is mannitol (80% ca, enough to be called ‘‘the mushroom sugar’’), except in *C. aegerita* and *C. comatus*, where the dominant sugar is trehalose (12.49 g/100 g_dw and 169.14 mg/g_dw, respectively) (Tsai *et al*., 2007; Wani *et al*., 2010; Petrovic *et al*., 2015; Atila *et al*., 2017). Another important component of the fungal dry matter (19–35% DM) are proteins, which confer mushrooms a nutritional value comparable to some foods such as meat, eggs and, milk products (Barros *et al*., 2008; Kalac, 2009; Wani *et al*., 2010; Wang *et al*., 2014c; Khatun *et al*., 2015; Correa *et al*., 2016; Rathore *et al*., 2017). In fact, not only these are highly digestible proteins (e.g. the digestibility of *Pleurotus* proteins is even higher than plants, that is 90%, hence only slightly below the meat and comparable with casein and eggs), but they also include all the essential amino acids usually found in animal proteins: tryptophan, isoleucine, valine, phenylalanine, leucine, threonine, lysine, histidine, methionine. There are, also, non-essential amino acids such as arginine, glutamic acid, aspartic acid, tyrosine, serine, aspargine, and many others (Tsai *et al*., 2009; Wani *et al*., 2010; Caglarirmak, 2011; Erjavec *et al*., 2012; Kakon *et al*., 2012; Kalac, 2012; Kivrak *et al*., 2014; Wang *et al*., 2014c; Kumar, 2015; Correa *et al*., 2016; Atila *et al*., 2017). Excellent protein content was found by Srikram and Supapvanich (2016) in *R. cyanoxantha* (49.20%, while it was 29.50% in *R. virescens*), by Ayaz *et al*. (2011) in *B. edulis* (32.50 g/100 g_dw) and a good one by Xu *et al*. (2019) in *L. deliciosus* (17.19 g/100 g_dw), rather low (4.22%), instead, in *M. procera*. Recent studies have shown as *P. ostreatus*

(protein content 23%) meets the nutritional requirements for all essential amino acids, or even doubles or triples for some of them (Correa *et al*., 2016; Carrasco-González *et al*., 2017; Bulam *et al*., 2019), and that *A. bisporus* has significant amounts of numerous essential and non- essential amino acids, with an overall protein content of 29.14% (Kakon *et al*., 2012; Atila *et al*., 2017). Also relevant is the fact that mushrooms are the major food source of ergothioneine, especially *B. edulis*, and some species also of Glutathione (mainly *C. aegerita* among the national mushrooms, followed by *B. edulis* and *P. ostreatus*), amino acid compounds that are important antioxidants (Kalaras *et al*., 2017). Interesting is also the content of γ-aminobutyric acid (GABA), a hypotensive agent, in *C. comatus*, as well as in *B. edulis* (Tsai *et al*., 2007). Therefore, mushrooms are a viable dietary alternative for vegetarians and vegans and also an ideal component of healthy food especially for child development. One more advantage of mushrooms as nutrients is their low crude fat content (2–6% of DM), making them suitable for a low-calorie diet. Among the species of the genus *Pleurotus*, the lowest lipid levels are found in *P. nebrodensis* (Inzenga) Que´l. (1.6%), while the highest *in P. eryngii* and *P. ostreatus* (3.5% and 3.4%, respectively) (Venturella *et al*., 2015; Carrasco-González *et al*., 2017; Sande *et al*., 2019). The crude fat content of *C. comatus* is 3.11% (Tsai *et al*., 2007), while lower are that of *B. edulis* and *M. procera* (2.85% and 2.40%, respectively) (Ayaz *et al*., 2011), whereas *L. deliciosus* show a slightly higher content (4.82 g /100 g_dw) (Xu *et al*., 2019). A higher fat content was found in *R. virescens* (12.54%) and *R. cyanoxantha* (7.87%) (Srikram and Supapvanich, 2016). Generally, in mushrooms, the unsaturated fatty acid prevail over the saturated ones. In *Pleurotus* spp., for example, monounsaturated fatty acids prevail over others, accounting for up to about 70% of the total, and their content is considerably higher than other species such as *A. bisporus* (Correa *et al*., 2016). In this species, the essential polyunsaturated linoleic acid is the most abundant, 5-folds more than in *P. ostreatus*, followed by palmitic, stearic, oleic acids, and others (Atila *et al*., 2017; Sande *et al*., 2019). As reported by Reis *et al*. (2012), however, the total content of monounsaturated fatty acids is higher in *P. eryngii* and *P. ostreatus*; in the latter, the oleic acid seems to be the prevailing monounsaturated fatty acid while linoleic acid is the major polyunsaturated one, whereas palmitic acid is the most abundant among the saturated ones (Correa *et al*., 2016). Also in *C. aegerita* linoleic acid is the most abundant (78.4%), followed by palmitic, oleic and, stearic acids (Petrovic *et al*., 2015). In *L. deliciosus*, on the other hand, the prevalent fatty acid is palmitic, followed by stearic, oleic, and linoleic acid (Xu *et al*., 2019). Thus, mushrooms can play an important role in nutrition as a source of essential fatty acids for humans as linoleic and linolenic. The content of primary vitamins such as riboflavin, niacin, thiamine, tocopherol, vitamin of D complex, and

folates is noteworthy (La Guardia *et al*., 2005). Mushroom is, thus, the only non-animal-based food containing vitamin D. As regards niacin, a significantly high content (5.9 mg/kg) was found in *P. eryngii* var. *eryngii* (DC.) Quél., hence sufficient to satisfy 55–82% of the recommended dietary allowance (RDA) of nicotinic acid, and higher than that of other mushroom species such as *P. ostreatus* (4.95 mg), *A. bisporus* (3.8 mg) and *Boletus* spp. (0.8 mg); the riboflavin content (0.2 mg/kg) is similar for all these species, while the values of biotin are higher for *P. eryngii* (7.45 μg) (Venturella *et al*., 2015; Atila *et al*., 2017). A high vitamin B_{12} and riboflavin content has been reported for *P. nebrodensis* (La Guardia *et al*., 2005; Venturella *et al*., 2015). *B. edulis* is the mushroom species with significant ascorbic acid content (4.11 g/kg_dw) as found by Ayaz *et al*. (2011). If compared with vegetables, mushrooms have riboflavin content significantly higher. The bioavailability of folates is good, content in ergosterol (a precursor of vitamin D_2) is high. For this reason, mushrooms are particularly suitable for those who need to take ergocalciferol from foods of non-animal origin, such as vegetarians and vegans. Also of note is the vitamin C content in *Pleurotus* spp. (Kalac, 2009, 2012; Caglarirmak, 2011; Feeney *et al*., 2014; Kumar, 2015; Atila *et al*., 2017; Rathore *et al*., 2017; Papoutsis *et al*., 2020). Compared with vegetables, mushrooms have a higher or similar content of micro- and macro-elements, mostly K and P, followed by Ca, Mg, and Fe (La Guardia *et al*., 2005; Wani *et al*., 2010; Ayaz *et al*., 2011; Kakon *et al*., 2012; Kalac, 2012; Wang *et al*., 2014c; Correa *et al*., 2016; Atila *et al*., 2017; Carrasco-González *et al*. 2017). Particularly interesting is the iron content of *P. ostreatus*, which overcomes that of pork and beef liver (23.3 and 4.9 mg Fe/100 g) (Carrasco-Gonzalez *et al*., 2017). Thanks to the lower Na content that characterizes them, mushrooms are recommended for the prevention of hypertension and particularly for the diet of whose suffer from this medical condition (Vetter, 2003; Kalac, 2012; Rathore *et al*., 2017). No less important is the characteristic and excellent aroma of edible mushrooms, which, together with the texture of their flesh, makes them a valid and delicious substitute for meat and an ideal enrichment for many dishes. Mushrooms are appreciated for their umami or savory flavor, deriving from non-volatile (taste) and volatile (smell) components, such as terpenes, aldehydes, lactones, free amino acids, aromatic alcohols, 50-nucleotides, soluble sugars, ketones, octanes, and octenes (Kalac, 2009, 2012; Tsai *et al*., 2009; Feeney *et al*., 2014; Wang *et al*., 2014c; Atila *et al*., 2017; Rathore *et al*., 2017). Also in *Tuber* species, there is interesting nutrient composition, which changes qualitatively and quantitatively at various stages of maturation. Basically, their composition reflects that of the most commonly described fungi, with the exception of two characteristics: the absence of mannitol and the presence of melanin. As reported by Harki *et al*. (2005) and Lee *et al*. (2020), *T.*

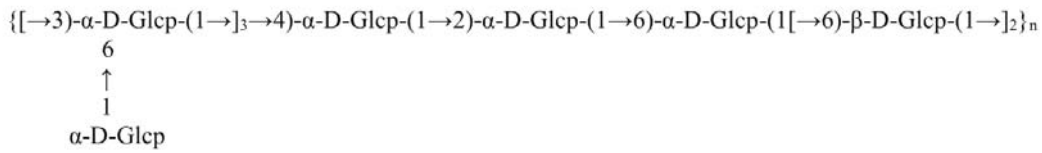
melanosporum and *T. magnatum*, two common species in Italy and among the most appreciated among truffles, are rich in proteins, K and P, sulfur amino acids and unsaturated fatty acids such as oleic and linoleic acid (more than 60% of total FA content). More specifically, the mature (stage VI) ascocarps of *T. melanosporum* contain 30.6% carbohydrates (lower than many species of basidiomycetes), 29.7% proteins, and 5.4% lipids, of which linoleic acid prevails (55.9%), followed by oleic and palmitic acid. According to Patel *et al*. (2017) and Wang and Marcone (2011), truffles are reached in free (particularly the sulfur-containing cysteine and methionine) and essential amino acids (methionine, phenylalanine, valine, serine, isoleucine, and threonine), metals (Fe, Ca, K, P, Cu, Zn, and Mn), contain rhamnose, ergosterol (especially in *T. melanosporum*, 1.90 mg/ g DM), as well as being rich in melanin (up to 15% dry weight). Also important are their volatile organic compounds such as aldehydes, alcohols, ketones, and organic acids (ascorbic acid), responsible for their typical umami and aroma.

3.4.1 Chemical composition of Italian wild mushrooms

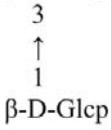
The consumption pattern from Europe shows a greater preference for wild mushrooms than for cultivated ones (Peintner *et al*., 2013). In Italy, gathering wild mushrooms is a common practice due to the favorable geographic conditions where the Alps, the Apennine mountains and the forests of southern Italy are ideal grounds for the growth of the most popular mushrooms. The knowledge of edible species is necessary since non-edible ones may have toxic effects. The peak season for mushroom gathering in most areas of Italy is from April to early November, with variations from region to region. Weather conditions are key factors for an abundant mushroom season, which requires a perfect combination of sun, rain, humidity, and warmth. With the aim of evaluating the chemical composition of mushrooms widely consumed in Italy, different species have been examined to determine their proximate composition. *Pleurotus eryngii* var. *eryngii* the "cardoncello" mushroom, is a highly prized and widely distributed edible mushroom throughout Italy. Protein acidic extracts of Mediterranean culinary-medicinal Oyster mushrooms *P. eryngii* var. *eryngii, P. eryngii* var. *ferulae* (Lanzi) Sacc., *P. eryngii* var. *elaeoselini* Venturella, Zervakis & La Rocca and *P. nebrodensis* were tested for their *in vitro* growth inhibitory activity against *Staphylococcus aureus* ATCC 25,923, *Stphylococcus epidermidis* RP 62A, *Pseudomonas aeruginosa* ATCC 15,442 and *Escherichia coli* ATCC 10,536. All the *Pleurotus* species analyzed inhibited the tested microorganisms in varying degrees (Schillaci *et al*., 2013). From the basidiomata of the edible mushroom *P. eryngii* var. *elaeoselini* three water-soluble glucans (PELPS-A1, PELPS-A2 and PELPS-A3) were obtained from the hot water extract by chromatography on

DEAE-cellulose 32 and Sephadex G-100 column. Acid hydrolysis, periodate oxidation and NMR experiments (1H-, 13C-NMR, DQF-COSY, TOCSY, ROESY, HMQC and HMBC) were useful in providing information for their structural elucidation. Based on the data obtained, the structures of the repeating unit of the three isolated polysaccharides were established as follows:

(1) PELPS-A1:



(2) PELPS-A2: [→6)-β-D-Glcp-(1→6)-β-D-Glcp-(1→6)-β-D-Glcp-(1→]n



(3) PELPS-A3: [→6)-α-D-Glcp-(1→6)-α-D-Glcp-(1→6)-α-D-Glcp-(1→]n

PELPS-A1 is a new polysaccharide, isolated and identified for the first time from *P. eryngii* var. *elaeoselini*. The crude extract of *P. eryngii* var. *elaeoselini* was tested for the antioxidant activity by DPPH and hydroxyl radical scavenging assays showing an SC50 of 1.4 mg/mL and SC50 of 5.7 mg/mL, respectively. *In vitro*, antioxidant tests showed that the three isolated polysaccharides exhibited moderate and similar hydroxyl radical scavenging activity (Cateni *et al*., 2020). Costa *et al*. (2015) developed a headspace-solid- phase microextraction (HS-SPME) method coupled with GC–MS and GC-FID to evaluate the volatile profiles of ten wild mushroom species including *C. aegerita* and *L. deliciosus* collected in south Italy. The mushroom *C. aegerita* showed consistent amounts of ethanol (34%), isopropyl acetate (10%) and isopentanol (30%), while *L. deliciosus* presented not only an abundant fraction of 3-octanone but also consistent amounts of terpenoids, such as limonene (5%), linalool (8%), and dihydrocitronellol (4%). *C. aegerita*, commonly known as “Pioppino”, an edible wild species of the Campania Region (southern Italy), was screened for its bio-chemical composition, nutritional values, and antioxidant effect. GC–MS analysis showed that the most abundant unsaturated acid in Pioppino was linoleic acid (C18:2; 0.618 g kg^{-1}), while palmitic acid (C16:0; 0.107 g $kg^{-1}$was the major of saturated fatty acids. The alcoholic extracts of three different samples of Pioppino were analyzed by liquid chromatography- high resolution mass spectrometry (LC-HRMS) in full scan mode (Landi *et al*., 2017a). Pioppino was mainly constituted of disaccharides, hexitol derivatives and malic acid. Other metabolites as saccharopine, agaritine, pentosylhexitol, ergothioneine, γ-

glutaminyl-4-hydroxybenzene, pentosyl xanthosine, homogentisic acid, malic acid, pentos-2-ulose, fumaric acid, veratric acid, *p*-cumaric acid, *o*-cumaric acid, δ-tocopherol and, γ-tocopherol were identified by comparison of their relative retention times and MS/ MS spectra with those of reference pure compounds. Wild mushrooms [*Fistulina hepatica* (Schaeff.) With., *Infundibulicybe geotropa* (Bull.) Harmaja, *Laetiporus sulphureus* (Bull.) Murrill, *Macrolepiota procera* var. *procera* (Scop.) Singer and *Suillus granulatus* (L.) Roussel] collected in different forests of Sicily (southern Italy) were analyzed for the content of protein, fat, carbohydrate and, vitamins showing their importance from a nutritional point of view (Palazzolo *et al*., 2012). A lectin was isolated from the wild mushroom *B. edulis* (porcini mushroom) collected in Italy. This protein is a dimer and each monomer folds as a β- trefoil domain. Its X-ray structure, the interaction with galactose, lactose, N-acetylgalactosamine, Galb1- 3GalNAc and, T-antigen disaccharide were studied together with its antiproliferative properties on human cancer cells (Bovi *et al*., 2013). *B. edulis* is a culinary mushroom highly appreciated for its aroma, but fresh mushrooms are very perishable products with a limited shelf life of 1 to 3 days at room temperature. Thus, dehydration is one of the significant preservation methods used for the storage of mushrooms. The composition of volatile compounds of dried porcini mushroom during commercial shelf- life (up to 12 months) at the storage temperature of 20 °C and under stressed conditions at 37 °C was investigated using two mass spectrometry (MS)-based techniques. 66 volatile compounds were identified, 36 of which reported for the first time. Alcohols, aldehydes, ketones, and monoterpenes diminish during the storage while carboxylic acids, pyrazines, lactones and, amine increase. The storage temperature influences the final quality of the dried porcini (Aprea *et al*. 2015). The mycochemical studies regarding truffles are mainly focused on the complex mixture of volatile organic compounds (VOCs) released from their ascomata that in addition to their biological value deter- mine their economic value. T. magnatum grows in some regions in Italy (Tuscany, Piedmont, Marche and, Umbria) and its volatile organic compounds were analyzed by PTR-TOF–MS experiments comparing samples from different regions of Italy and different seasons (Vita *et al*., 2015). The chemical composition of the aroma has led to the identification of 111 compounds divided into six different chemical classes as follows: hydrocarbons, aromatic hydrocarbons, phenols, sulfur compounds, terpenes, and other compounds. The VOCs profiles vary within the different seasonal and geographical productions. A further study of the VOCs generated by *T. magnatum* fruiting bodies from different regions of Italy with different environmental conditions was reported by Vita *et al*. (2018). The white truffle's aroma is frequently correlated to sulfur-containing volatiles

which can be used to trace the origin of truffle fruiting bodies. Dimethyl sulfide, dimethyl disulfide, bis (methylthio) methane were detected in all samples, dimethyl trisulfide in some samples while S-allyl- thiopropionate and 3-methylthio-propionaldehyde were found for the first time in the aroma. Aldehydes [e.g. (4Z)-decenal, (2E)-butenal, 4-methylpent-2-enal, 2-methylpent-2-enal], alcohols [e.g. 1-octen-3-ol, dodecanol, (4Z)-decen-1-ol], ketones [e.g. 3-octanone, 2-octanone, 6-methyl-5-hepten-2-one, 2-decanone, 2-undecanone], terpenes [e.g. limonene, α- and β- pinene, α-terpinene, eucalyptol, camphor], hydrocar- bons and esters [e.g. (2E)-hexenyl-acetate, ethyl lactate, 3,5,5-trimethylhexyl-acetate, isobutyl pentanoate, 3-acetoxyoctane] were also detected. Besides, since the quality of the fruiting bodies of *T. magnatum* varies significantly based on of the origin area due to the differences in environmental growth conditions, a proteomic analysis was reported for samples collected in different areas of Italy (Vita *et al*., 2017). As concerns the black truffle T. *melanosporum* Vitt. the volatile organic compounds from samples collected in middle Italy and the variation induced by the storage temperature was reported (Bellesia *et al*., 1988). The major volatile compounds of *T. melanosporum* are butan-2,3-dione, 2- and 3-methylbutanal, 2- and 3-methylbutanol. The two aldehydes (2- and 3-methylbutanal) and two alcohols (2- and 3-methylbutanol) play an important role, while sulfur compounds are present at trace levels. On storage, all these compounds are lost, but at 0 °C, an increase of the 2- and 3-methyl butanal and of 2- and 3-methyl butanol occurs. In many cases, *T. borchii* is illegally used as a substitute of the more appreciated *T. magnatum*. The composition of the volatile organic fraction of *T. borchii* was analyzed by gas–solid extraction and purge and trap injection in GC–MS, together with the variations during storage (Bellesia *et al*., 2001). In fresh samples the aroma mainly consists of a mixture of alcohols, the most important one is 1-octen-3-ol together with aldehydes and 2- and 3-methylthiophenes as sulfur compounds. Also for *T. borchii* the best preservation conditions seem to be at 0 °C, while comparing *T. borchii* with *T. magnatum*, the absence in the volatile fraction of dimethyldisulfide, dimethyl- trisulfide and 2,4-dithiapentane seems to be the distinguishing feature. D'Auria *et al*. (2012) reported a further study on volatile organic compounds of samples of *T. borchii* and *T. asafoetida* Lesp., collected in woodlands of the Basilicata region (southern Italy). Solid-phase microextraction-gas chromatography–mass spectrometry analysis of the samples showed the presence of 2-methyl-1,3-butadiene as the significant component in both truffles. In *T. borchii* 3-methylbutanal, 3-methyl-1-butanol and tetradecane were present in low amounts. Besides, a lectin named TBFL-1 was isolated and identified from *T. borchii*. The fruiting body that is able selectively to bind the exopolysaccharides produced by ascoma-associated *Rhizobium* spp. TBFL-1 is a 11.9-KDa phase-specific protein, it is a non-glycosylated

polypeptide chain localized on the hyphal cell wall and is the main soluble protein in the fruiting body aqueous extract. Studies of the related gene *tbf-1* demonstrated the presence of an N-terminal signal peptide of 12 amino acids (Cerigini *et al*., 2008).

3.4.2 Chemical composition of Italian cultivated mushrooms

A study conducted on different mushroom strains cultivated in an Italian farm (Italmiko, Senise-Potenza, Italy) was carried out by solid-state 13C CPMAS NMR (Pizzoferrato *et al*., 2000). This technique can investigate the chemical composition in the solid-state of a food sample. This property was useful to study mushrooms of different species [*P. ostreatus, P. eryngii, Pleurotus pulmonarius* (Fr.) Que´l. and *L. edodes*] to obtain the quantitative evaluation of the protein/polysaccharide ratio. The value of the protein/ polysaccharide ratio has been correlated with the results obtained by chemical analysis and a good correlation (R2 = 0.93; R2 = 0.81) has been obtained. As concerns *P. ostreatus* the resonances are quite similar and only slight changes in the relative intensity can be observed. The *P. eryngii* samples analyzed show a similar pattern with a high content of polysaccharides and a low amount of proteins. *P. ostreatus, P. eryngii* and *C. aegerita* were investigated for their β-1–3-glucan synthase activity and its induction by olive mill wastewaters (OMW) (Reverberi *et al*., 2004). In the control medium, although with different degrees, all fungal strains displayed β-1,3-glucan synthase activity. When the isolates grew on OMW an increase of about 12-fold was observed for *P. ostreatus*, while no differences were reported for *P. eryngii* and *C. aegerita*. Two different polysaccharides (PEPS-A1 and PEPS-A2) were isolated from the cultivated edible mushroom, *P. eryngii* C-142-c strain. The chemical structures of the repeating unit of PEPS-A1 and PEPS- A2 were established based on acid hydrolysis, methylation analysis, and NMR experiments as follows:

(1) PEPS-A1 (α-glucan):

$$[\rightarrow 6)-\alpha-D-Glcp-(1\rightarrow 6)-\alpha-D-Glcp-(1\rightarrow 6)-\alpha-D-Glcp-(1\rightarrow]_n$$

(2) PEPS-A2 (β-glucan):

$$[\rightarrow 6)-\beta-D-Glcp-(1\rightarrow 6)-\beta-D-Glcp-(1\rightarrow 6)-\beta-D-Glcp-(1\rightarrow]_n$$

The antioxidant activity of PEPS-A1 and PEPS-A2 was evaluated by hydroxyl radical scavenging activity test showing SC50 values of 400 µg/mL and 122 µg/ mL, respectively. Both polysaccharides affected cell viability after 48 and 72 h of treatment, inducing the death of 50% of HT-29 cells between 0.25 and 1 µg/ mL and 0.5 and 1 µg/mL, respectively for PEPS-A1 and PEPS-A2 (Cateni *et al*., 2018). Punelli *et al*. (2009) reported the molecular characterization and enzymatic activity of laccases in *P. eryngii* and *P. eryngii* var. *ferulae*. Using a PCR- based approach, four putative laccase genes (lac1, lac2, lac3 and lac5-like gene) have been isolated and identified in both *P. eryngii* and *P. eryngii* var. *ferulae*.

Multiple headspace-solid phase microextraction (MHS-SPME) followed by gas chromatography/ mass spectrometry (GC–MS) and flame ionization detection (GC-FID) was applied to the identification and quantification of volatiles from the mushroom *A. bisporus* (Costa *et al*., 2013). 1-Octen-3-ol, 3-octanone, 3-octanol, 1-octen-3-one and benzaldehyde are key compounds of mushroom samples analyzed. Quantitative differences among the samples were observed, in particular for 1-octen-3-ol when fresh mushrooms were differently pretreated (0.75 and 3.30 μg^{-1}, in chopped and homogenized samples, respectively). It seems that from 1-octen-3-ol breakdown other 8 carbon compounds are formed: 3-octanone (3.34 vs. 2.01 μg^{-1}) and 3-octanol (0.19 vs. 0.07 μg^{-1}) were found in high amount in chopped samples, balancing the reduced presence of 1-octen-3-ol. *A. bisporus* is the most cultivated mushroom in Italy. Samples of the cultivated species *A. bisporus* in Sicily (South Italy) were analyzed by the head- space-solid-phase microextraction (HS-SPME) method coupled with GC–MS and GC-FID to evaluate compositional changes occurring during storage (Costa *et al*., 2015). 51 compounds were identified, with high amounts of C8 compounds such as 3-octanone, 3-octanol and (2E)-octenol. Besides, compounds with the aromatic ring were determined at significative amounts, such as benzaldehyde and benzyl alcohol. In Italy, *A. bisporus*, when purchased in the supermarkets, is found in refrigerated counters. So, 10-day-old mushrooms, kept in the refrigerator were analyzed. After 10 days of storage, a reduction of about 3.5% of the volatile fraction was observed. Ethanol, (2E)-octenol and phenylacetaldehyde were not detected in the stored mushrooms. As concerns compounds with aromatic ring a drastic decrease was observed. The amount of terpenoids was constant, while a reduction of C8 compounds, 3-octanol and (2E)-octenol was observed. Landi *et al*. (2017a) reported the nutritional value, chemical composition, and anti-radical properties of cultivated *A. bisporus* purchased in Campania (South Italy). As concerns fatty acid composition analysis the most abundant unsaturated acids in Champignon were linoleic (C18:2; 0.858 g kg^{-1}) and γ-linolenic (C18:3; 0.243 g kg^{-1}), which represented 67% and 19% of the total, respectively. The prevalence of polyunsaturated fatty acids (PUFA) showed linoleic acid (C18:2) as the major fatty acid, while palmitic acid (C16:0) was the major of saturated fatty acids (SFA). The tentative identification of constituents from Champignon alcoholic extract by liquid chromatography-high-resolution mass spectrometry (LC- HRMS) showed the presence of mannitol, saccharopine, trehalose, agaritine, pentosylhexitol, ergothioneine, γ-glutaminyl-4-hydroxybenzene, malic acid, fumaric acid, ferulic acid, sinapic acid, and cinnamic acid.

3.4.3 Mycochemicals as nutraceuticals

Mushrooms are an excellent food not only from a culinary point of view, as unique sensory experiences, but also for well-being because of the many positive effects they have on the human body, helping it to maintain a good state of health and defend it against illness. This aspect is becoming increasingly important in a society more and more threatened by an unhealthy lifestyle, pollution, radiation and many other stress factors. Thereby in the last decades, the gaze on food has changed, and new concepts of it were developed. The term ’nutraceuticals’ was first used by de Felice (1989) who, combining the words nutrition and pharmaceuticals, defined them as ’a food (or part of a food) that provides medical or health benefits, including the prevention and treatment of a disease’. The term “mushroom nutraceuticals” refers to refined or partially refined extracts, single compounds or nutrients, or dried biomass obtained from either mushroom mycelium or fruiting body, usually included in dosed, concentrated, and purified form in different pharmaceutical formulations such as capsules, tablets, pills, etc., and consumed as a dietary supplement and has potential therapeutic applications (Reis *et al*., 2017). Nonetheless, being considered non- specific biologic therapies, nutraceuticals differ from pharmaceuticals in that they are not currently subject to medical prescription and their therapeutic proper- ties are not recognized from a legal point of view. Since 2011, in fact, in EU the registration and marketing of ’botanical medicinal products’ is no longer permitted and, therefore, despite their pharmacological properties, they can only be classified as food supplements, falling under EU Regulation no. 1924/2006 (Pirillo and Capatano, 2014). Therefore, several aspects of their preparation and marketing remain still unresolved, such as standardization of the production chain, safety parameters, regulation, efficacy, and mechanism of action. Although the market does not have production standards, it is mainly developed in Asian countries; Western countries, on the other hand, are used to buy from the East finished products for resale or raw materials (powders and extracts not always of ascertained origin) and then make the final pipeline. In these areas, therefore, the potential for exploitation and investment is enormous. Several recent studies have demonstrated the multiple nutraceutical properties of mushrooms by the presence of numerous bioactive molecules that give them antioxidant, antimicrobial, antitumor, immunomodulating, anti-hypercholesterolemia, anti- inflammatory, antiviral, radical scavenging, hypolipidemic, antithrombotic, hepatoprotective, anti-hypercholesterolemia, hypotensive, and anti-diabetic activities, antinociceptive and cardiovascular beneficial effects (Barros *et al*., 2008; Carrasco-González *et al*., 2017; Gargano *et al*., 2017; Rathore *et al*., 2017; Reis *et al*., 2017; Ma *et al*., 2018; Islam *et al*., 2019). These bioactive compounds, contained in different quantities depending on the fungal

species and growing conditions, are the most varied, including polysaccharides and especially ß-glucans, dietary fibers, phenolics, peptides, terpenes, glycoproteins, ergosterols, alcohols, unsaturated fatty acids (UFA), lectins, tocopherols, ascorbic acid, carotenoids and others (Barros *et al*., 2008; Rathore *et al*., 2017; Reis *et al*., 2017; Ma *et al*., 2018; Islam *et al*., 2019). As for the genus *Pleurotus*, many species belonging to it have shown activity against various chronic diseases in various studies, thus with a wide spectrum for potential biotechnological applications. They possess numerous bioactive compounds such as polysaccharides, lipopolysaccharides, proteins, peptides, glycoproteins, nucleosides, triterpenoids, lectins, lipids, and their derivatives (Patel *et al*., 2012; Talkad *et al*. 2015; Golak-Siwulska *et al*., 2018). Fruiting bodies possess higher concentration of antioxidants than other commercial mushrooms (Talkad *et al*., 2015); the AOX properties of different kinds of *Pleurotus* extracts efficiently contrast reducing the occurrence of age-associated disorders like stroke, Parkinson's disease, atherosclerosis, diabetes, cancer, and cirrhosis (Patel *et al*., 2012); they help also to reduce the severity of inflammatory skin disease and regulate hyperpigmentation disorders (Taofiq *et al*., 2016). Pleuran is the polysaccharide isolated from *Pleurotus* spp. A variety of properties such as immunomodulatory, antitumor, AOX, antiviral and antimicrobial, and anti-inflammatory. It was also found that by including 100 mg of Imunoglukan in the diet of elite athletes, the suppressed immune system responses induced by short-term high-intensity exercise decreased (Bobovcàk *et al*., 2010). Studies have remarked also on the importance of proteins isolated from *P. ostreatus* and *P. eryngii* (pleurostrin and eryngin) as an effective antifungal and antibacterial agents (Carrasco-González *et al*., 2017). Many bioactive compounds of *Pleurotus* spp. and their properties are reported in Table 6. *A. bisporus* is also of increasing importance thanks to the innumerable medicinal properties of its bioactive extracts and compounds, which make it suitable against many human diseases such as coronary heart diseases, diabetes mellitus, bacterial and fungal infections, disorders of the human immune system, and cancers (Ozturk *et al*., 2011; Atila *et al*., 2017). Even the Canadian Cancer Society recommends its consumption because of its beneficial effects against various diseases (Atila *et al*., 2017). The high dietary fiber and antioxidant content of this mushroom, including vitamins C, D, and B_{12}, as well as folate and polyphenols have positive effects on diabetes and cardiovascular diseases (Atila *et al*., 2017). Moreover, the prebiotics contained in the fruiting bodies has a positive influence on gut health. A study conducted by Hess *et al*. (2018) has shown that, compared to meat, the consumption of mushroom may impact laxation in healthy adults. It is demonstrated by the increase in stool weight and presence of undigested mushrooms in stool and by the different fecal microbiota composition, with a greater abundance of *Bacteroidetes* and lower presence

of *Firmicutes*. *In vivo* tests on mice have shown analgesic and antipyretic properties comparable to that of the common drug diclofenac (Bose *et al*., 2019). As reported by Ismaya *et al*. (2020), recently a new molecule from *A. bisporus* has been discovered; is a mannose-binding protein (Abmb) that might be employed as a drug carrier for oral administration due to its capability to permeate a dialysis bag made of fresh jejunum *ex vivo*, that does not suffer alterations in a bio conjugation with a drug model, and to its resistance to the harsh gastrointestinal tract (Ismaya *et al*., 2020). Some activities and compounds of *A. bisporus* are reported in Table 7. Other mushrooms already mentioned, which can be found spontaneous or cultivated in Italy, are still less studied compared to the previous ones. This is also due to the fact that their consumption is often smaller in quantitative terms or less widespread. Some of the studies carried out to date are reported in Table 7. They reveal the enormous therapeutic potential of these mushrooms, as well as the benefits that their more frequent inclusion in the diet would bring to the individual's state of health. As far as truffles are concerned, most of the studies carried out refer to the culinary aspect, analyzing their composition and focusing mainly on the volatile components responsible for their particular aroma and flavor. In recent years, studies on the therapeutic potential of this type of food are increasing. It has been seen, in fact, as *Tuber* spp. have numerous bioactive compounds, with properties ranging from anti-tumor to anti-inflammatory, antioxidant, hepatoprotective, anti-cholesterolemic, and even antidepressant. In Table 7 some bioactivities of this genus are reported. A more recent topic concerns the nutraceutical benefits of UV irradiation of cultivated *A. bisporus* and *P. ostreatus* to generate high amounts of vitamin D_2 and to maintain the ability of the fungus to inhibit glycation of a target protein (Gallotti and Lavelli, 2020). Besides polysaccharides from cultivated *Rubroboletus sinicus* (W.F. Chiu) Kuan Zhao & Zhu L. Yang showed high inhibitory effects on glycation (Liping *et al*., 2016). A medium-molecular-weight fraction obtained by sclerotia of *Lignosus rhinocerus* (Cooke) Ryvarden contain bioactive compounds which exhibit potent anti-glycation activity and is eligible for preventing diabetic complications by Advanced Glycation End Products (AGE) (Yap *et al*., 2018).

Table 6 Bioactivities of *Pleurotus* spp

Activity	**Bioactive compound or extract**	***Pleurotus* species**	**Mechanisms of action**	**References**
Anti-oxidative	Lectins	*P. ostreatus*	Activation of Toll-like receptor 6 signal pathway of dendritic cells	Ma *et al.* (2018)

	Polysaccharides	*Pleurotus spp.*	Improved activity after polysaccharides sulphonation,	Li and Shah (2016)
		P. ostreatus	Increase of the activity of SOD and consequent inactivation of superoxide radicals;	Islam *et al*. (2019)
			Increase of CAT activity by upregulating gene expression and consequent prevention of the cells from hydrogen peroxide toxicity;	Islam *et al*. (2019)
			Reduction of GPx activity and increase of GR, GST and APx activity	Islam *et al*. (2019)
		P. eryngii *P. ostreatus*	Activation of SOD, CAT and GPx and decreasing ALT in mice with CCI4-induced liver injury;	Carrasco-González *et al*. (2017)
			Inhibition of lipid peroxidation on porcine brain homogenates	Carrasco-González *et al*. (2017)
		P. eryngii	Inhibition of cell viability in colorectal adenocarcinoma cell line (HT29)	Cateni *et al*. (2018)
	Phenols	*P. ostreatus*	Inhibition of the growth of HL-60 cells by inducing apoptosis	Patel *et al*. (2012) and Vanamu (2012)
	Flavonoids, ßcarotene,	*P. ostreatus*	Inhibition of the growth of HL-60	Patel *et al*. (2012) and

	Ascorbic acid		cells by inducing apoptosis;	Vanamu (2012)
			Reduction of ascorbate radicals	Islam *et al*. (2019)
	α-tocopherol (Vitamin E)	*P. ostreatus*	Prevention of lipid peroxidation in cell membranes	Islam *et al*. (2019)
	Glutathione	*Pleurotus spp.*	Prevention of GSH oxidation and assurance of the safety of its redox enzymes	Islam *et al*. (2019)
	Statins (lovastatin)	*P. ostreatus*	Inhibition of the plasma and hepatic lipid peroxidation and increase of the hepatic catalase activity in high-cholesterol fed rabbits	Jeon *et al*. (2001)
Immunomodulatory	Polysaccharides	*Pleurotus spp.* *P. ostreatus*	Macrophage stimulation in children with RRTIs, pleuran (Imunoglukan P4H) increases immunoglobulin isotypes, slows down the decline of T-cytotoxic lymphocytes, and e increases the NK cell number	Correa *et al*. (2016)
		P. nebrodensis	PN-S evaluated in RAW264.7 macrophage; improved phagocytosis of macrophages, enhanced production of interleukin-6 (IL-6), nitric oxide (NO),	Correa *et al*. (2016)

			interferon gamma (INF-c), and tumor necrosis factor-a (TNF-a) in the acrophages, with upregulation of mRNA expressions of interleukin6 (IL-6), inducible nitric oxide synthase (iNOS), interferon gamma (INF-c) and tumor necrosis factor-a (TNF-a)	
		P. ostreatus	Immunomodulatory activity against infectious bursal disease (IBD) in broilers;	Islam *et al*. (2019)
			Decrease of the toxicity of cyclophosphamide in mice	Islam *et al*. (2019)
	Triterpenoids (i.e. ergosterol) and steroids	*Pleurotus spp.*	Not specified	Gargano *et al*. (2017)
Anti-inflammatory	Polysaccharides (ß-glucans)	*P. ostreatus*	Synergistic effect with methotrexate in arthritis induced rats	Carrasco-González *et al*. (2017)
	Aqueous extract	*P. ostreatus*	Inhibition of DNA-binding activity of AP-1 and NF-kB in RAW264.7 cell line and suppression of the secretion of TNF and IL-6 in a mice model;	Carrasco-González *et al*. (2017) and Patel *et al*. (2012)
			Reduction of NO and TNF-a production in murine macrophage cell line RAW264.7	Carrasco-González *et al*. (2017)

	Pleuran	*P. eryngii*	Suppression of inflammation in delayed type (type IV hypersensitive) allergy response in mice	Patel *et al*. (2012); Talkad *et al*. (2015)
	Phenols (ethanolic extract)	*P. eryngii*	Suppression of induced dermatitis and decrease of serum level of IgE and TARC as well as expression of cytokines related with inflammation (TNF-a, INF-g, IL-4, IL-5 and IL-13) and severe skin lesions in mice	Carrasco-González *et al*. (2017) and Ma *et al*. (2018)
Antihypercholesterolemic	Statins (Lovastatin)	*P. ostreatus*	Inhibition of 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase that catalyzes the conversion of HMGCoA to mevalonic acid in the cholesterol synthesis pathway; pleiotropic actions in the cardiovascular, immune and nervous systems;	Talkad *et al*. (2015)
			Inhibition of the plasma and hepatic lipid peroxidation and increase of the hepatic catalase activity in high-cholesterol fed rabbits;	Jeon *et al*. (2001)

			Acceleration of HDL, reduction of production of VLDL, LDL, cholesterol; reduction of cholesterol absorption and of HMG-CoA reductase activity in the liver;	Patel *et al*. (2012)
			Positively affect the coagulation system and fibrinolysis	Golak-Sivulska *et al*. (2018)
	Flavons (Chrysin)	*P. ostreatus*	Decrease in mean blood/serum levels of glucose, lipid profile parameters, and hepatic marker enzymes and a concomitant increase in enzymatic and nonenzymatic antioxidant parameters in hypercholesterolemic rats	Anandhi *et al*. (2013)
Anti-cancer and anti-tumor	Cold-water extract	*P. eryngii var. ferulae and P. nebrodensis*	On human colon cancer cells: inhibition of viability of HCT116 cells; promotion of apoptosis; increase of Bax-to- Bcl-2 messenger RNA ratio; inhibition of cell migration and effect on homotypic and heterotypic cell–cell adhesion; negative influence on protein tyrosine and	Fontana *et al*. (2014)

			phosphorylation levels of extracellular signal regulated kinase 1/2	
	Methanolic extract	*P. ostreatus*	In breast cancer: suppression of different cell lines proliferation (MCF-7, MDA-MB-231);	Chaturvedi *et al.* (2018)
			Induction of expression of tumor suppressor p53 and cyclindependent kinase inhibitor p21	Carrasco-González *et al*. (2017)
	Polysaccharides	*P. eryngii*	In mice with renal cancer: increase of relative thymus and spleen lymphocytes proliferation by elevated activity of NK cells and CTL in spleen; increase of serum concentration level of TNF-a and IL-2;	Chaturvedi *et al.* (2018)
			Inhibition of tumor growth and increased relative thymus and spleen indices	Zhang *et al*. (2016)
		P. ostreatus	Inhibition the development of Ehrlich Tumor (ET) and Sarcoma 180 (S-180)	Carrasco-González *et al*. (2017)
		P. nebrodensis	Apoptosis induction by reduction of mitochondrial membrane potential and changes in migration cell rate	Carrasco-González *et al*. (2017)

		P. ostreatus	Cytotoxic activity towards HeLa cell lines	Golak-Sivulska *et al*. (2018)
		P. citrinopileatus Singer	Cytotoxic activity to cervical cancer cells (and no to normal cells)	Golak-Sivulska *et al*. (2018)
		Pleurotus spp.	Thymus-dependent immune mechanism, which involves the activation of cytotoxic macrophages, monocytes, neutrophiles, natural killer cells, dendritic cells, and chemical messengers (cytokines, such as interleukins, interferons., and colony stimulating factors) which triggers the complementary and acute phase responses	Rathore *et al*. (2017)
	Pleuran (ß-glucan)	*P. ostreatus*	Anti-neoplastic properties against different cells, including breast cancer MCF-7, prostate cancer cells PC-3 and colorectal HT-29 cancer cells	Golak-Sivulska *et al*. (2018)
	Proteins	*P. ostreatus*	Therapeutic effect towards the colorectal cancer cell line SW 480 and monocytic leukaemia	Golak-Sivulska *et al*. (2018)

			THP-1 by inducing their apoptosis	
	Proteins (hemolysin)	*P. nebrodensis*	Strong growth inhibition (IC50\40 mg/mL) against five cancer cell lines (Lu-04, Bre-04, HepG2, L929 and HeLa) and apoptosis induction in L929 and HeLa cell lines	Carrasco-González *et al.* (2017)
	Lectins	*P. ostreatus*	Reduction of tumor burden in Sarcoma S180 (88.4%) and hepatoma H-22 (75.4%) inoculated mice and increase of the survival time	Carrasco-González *et al.* (2017)
	Ethanolic extract	*P. eryngii var. ferulae*	Inhibition of growth and proliferation of stomach (BGC 823) and melanoma (B16F10) cancer cells;	Carrasco-González *et al.* (2017)
			Induction of cell cycle arrest in G0/G1 of stomach and melanoma cancer cell lines;	Carrasco-González *et al.* (2017)
			Delay and reduction of melanoma tumor growth in a murine model	Carrasco-González *et al.* (2017)
	Laccase	*P. cornucopiae*	Inhibition of proliferation of the hepatoma cells HepG2, the breast cancer cells MCF-7	Wu *et al.* (2014)
	Monoterpenes and sesquiterpenoids (Pleurospiroketal)	*P. cornucopiae*	Cytotoxicity against cancer line	Rathore *et al.* (2017)

	Triterpens	*P. eryngii*	Inhibitory activity against breast cancer MCF-7 cell lines *in vitro*	Zhang *et al*. (2016)
Antihypertensive	Aqueous extract	*Pleurotus spp.*	High angiotensin l-converting enzyme (ACE) inhibition	Carrasco-González *et al*. (2017)
	Hot water extract	*P. cornucopiae*	ACE inhibition *in vitro* and antihypertensive effect on spontaneously hypertensive rats	Carrasco-González *et al*. (2017)
	Oligopeptides	*P. ostreatus*	Pressure lowering activity	Patel *et al*. (2012)
Antiviral, antimicrobial	Nebrodeolysin	*P. nebrodensis*	Inhibition of the viral cytopathic effect of HIV-1	Carrasco-González *et al*. (2017)
	Laccase	*P. ostreatus*	Antiviral effects against hepatitis C	Golak-Sivulska *et al*. (2018)
	Ubiquitin-like protein	*P. ostreatus*	Antiviral effects against HIV-1 viruses	Carrasco-González *et al*. (2017)
	Lectin	*P. citrinopileatus*	Potent effect against HIV-1 reverse transcriptase activity	Carrasco-González *et al*. (2017)
	Water-soluble sulfonated polysaccharides	*P. eryngii*	Inhibition in growth of pathogenic *Escherichia coli*, *Staphylococcus aureus* and *Listeria monocytogenes*	Carrasco-González *et al*. (2017)
	Nanoparticles synthesized through mixing aqueous extract with silver nitrate	*P. cornucopiae*	Remarkable antifungal effects	Carrasco-González *et al*. (2017)

	Nanoparticles synthesized through mixing a silver solution with aqueous extract	*P. ostreatus*	Inhibition in Gram negative bacteria growth	Carrasco-González *et al*. (2017)
	Aqueous extract	*P. ostreatus*	Inhibition in replication of Herpes simplex virus type 1 *in vitro*	Carrasco-González *et al*. (2017)
	Polysaccharides	*Pleurotus spp.*	Activation of the microbial autolytic system of eight strains: seven autolyzing strains with intensity values ranging from 2.7% in *Candida* spp. To 36.1% in *Saccharomyces cerevisiae*, while autolysis was of 1.8% in one non-autolyzing strain (*Bacillus cereus*)	Correa *et al*. (2016)
	Methanolic extract	*Pleurotus spp.*	Inhibition in growth of *Bacillus megaterium, S. aureus, E. coli, Klebsiella pneumoniae, C. albicans, C. glabrata*, species of *Trichophyton* and *Epidermophyton*	Patel *et al*. (2012)
	Ether and acetone extract	*P. ostreatus*	Effective against *B. subtilis, E. coli* and *S. cerevisiae*	Patel *et al*. (2012)
	Ethanolic extract	*P. ostreatus*	Inhibition in growth of Gram positive bacteria (*Listeria innocua, B. cereus, S. aureus*), Gram	Vanamu (2012)

			negative bacteria (*E. coli, Pseudomonas aeruginosa*), and yeast (*C. albicans*, *Candida* sp.)	
	Ribonucleases	*P. ostreatus*	Potentiality to neutralize HIV through degradation of viral genetic material	Patel *et al*. (2012)
	Protein (hemolysin)	*P. nebrodensis*	Anti-HIV-1 activity in CEM cell culture	Patel *et al*. (2012)
Hyperglycemic	Guanide	*Pleurotus spp.*	Anti-hypoglycemic effect	Patel *et al*. (2012)
	Polysaccharides	*P. citrinopileatus*	Elevation of the activity of glutathione peroxidase	Patel *et al*. (2012)
Hepatoprotective	Polysaccharopeptides	*P. ostreatus*	Alleviation of thioacetamide induced alterations, inflammation, steatosis, necrosis and fibrosis	Patel *et al*. (2012)
	Hot-water extract	*P. ostreatus*	Less leakage of alkaline phosphatase, less pronounced increase in hepatic malondialdehyde concentration, less notable reduction in hepatic total protein, RNA and DNA contents; in contrast, increase in hepatic superoxide dismutase, glutathione peroxidase and glutathione reductase activities	Patel *et al*. (2012)

Anti-Ageing	Aqueous, methanolic, and acetonic extracts	*Pleurotus spp.*	Anti-tyrosinase, antihyaluronidase, anti-collagenase and anti-elastase activity	Taofiq *et al*. (2016)
	Mushroom powder	*P. ostreatus and P. eryngii*	Significant bifidogenic effect and strong lactogenic effect, respectively	Mitsou *et al*. (2020)
	Extracts	*P. ostreatus*	Lowered levels of malondialdehyde, a polyunsaturated lipid and an electrophilic mutagen, on administration of mushroom extract to aged rats, and subsequent reaction with deoxyadenosine and deoxyguanosine in DNA, forming a DNA adduct	Patel *et al*. (2012)

3.4.4 Mycochemicals in functional foods

As mentioned, today's concept of food is changing, becoming more complex. "Functional food" namely conventional food is consumed as part of the daily diet. This type of food positively affects one or more physiological functions of the human body is proven; therefore, in addition to the nutritional intake, they contribute to maintaining the state of wellness, improving health, and reducing the risk of disease. This concept is flanked by that of ''food supplements'' which, compared to the previous ones, constitute a concentrated source of nutrients or substances with nutritional and/or physiological effect; they are marketed in various dose forms, including tablets, capsules, gummies, and powders, as well as drinks and energy bars and aim to provide nutrients to fulfil the nutritional requirement of an individual. Mushrooms are functional food, because of their nutritional features: they are hypocaloric and a good source of high-quality dietary fiber. Their carbohydrate content as glycogen (and not of starch) is low. They also have significative digestible proteins and all the essential amino acids required by an adult and often deficient in plants, as well as various vitamins and mineral elements in content often at higher levels than vegetables. Therefore, in addition to taking a leading role in diseases such as hypertension, cholesterol, obesity, etc.) and they provide an efficient alternative in areas with widespread malnutrition.

Mushrooms show potential for obtaining fortified foods, improving nutrition, and adding health benefits. Although knowledge about the therapeutic proper- ties of mushrooms is now quite extensive, their incorporation into foods to produce fortified foods is not so widespread today. However, various research has been undertaken in recent years in this direction, demonstrating how the addition of extracts or compounds of medicinal mushrooms, such as *Pleurotus* spp., into processed food, increases their sensory, nutritional, functional, or nutraceutical features (Carrasco-González *et al*., 2017; Reis *et al*., 2017; Lavelli *et al*., 2018; Salehi, 2019).

The potential of mushroom powder to enrich baked (bread, biscuits, and cakes) and extruded (breakfast cereals, snacks) cereal products with fiber for the production of fitness-promoting foods (low in calories, cholesterol, and fat) is remarkable. Gaglio *et al*. (2019) evaluated the effect of partially replacing wheat flour with *P. eryngii* powder (5 and 10% w/w) in baked bread; the fermentation process has not undergone any alterations, the final product had positive physical and organoleptic characteristics with the advantage of having higher concentrations of thiamin, riboflavin and pantothenic acid and, more importantly, supplied biotin, cobalamin, and cholecalciferol generally absent in wheat bread. Another study on *P. eryngii* (Kim *et al*., 2010) demonstrated how biscuits supplemented with mush- room powder showed significantly increased total phenol compound content, ferric reducing antioxidant power (FRAP), and DPPH radical scavenging activity, maintaining appreciable organoleptic and rheological properties. Also, *A. bisporus* powder was evaluated by Kumar and Barmanray (2007) as a supplement for fortified biscuits, that showed a significantly higher protein content with good overall acceptability. *P. eryngii* β-glucan-rich fractions (BGRFs) have been tested as an ingredient of wheat semolina pasta (Kim *et al*., 2016), obtaining the best results in terms of qualitative, textural, and sensory characteristics with a concentration of 4%, in addition to higher beneficial properties. Studies carried out by Lu *et al*. (2016, 2018) have shown that the inclusion of powdered *A. bisporus* and *B. edulis* mushrooms in wheat semolina allows to obtain a pasta with more fiber and less starch, therefore with a lower glycemic power and higher antioxidant properties. Equally positive results have been achieved in snack products supplemented with *A. bisporus* and *B. edulis* powder (Singla *et al*., 2009; Lu *et al*., 2020). Mushrooms have also been tested to enrich other types of foods. Exploiting the high fiber and protein content, *A. bisporus* powder has been used for the production of functional meat products with better emulsion characteristics and textural properties (Kurt and Gencelep, 2018). Barros *et al*. (2011) demonstrated that *B. edulis* extracts protect beef burgers from lipid peroxidation and also give them greater antioxidant potential, while a study by Stojakovic´ *et al*. (2015) revealed that the methanolic

extract of *B. aureus* helps to increase the shelf-life of meat, protecting it from food contaminating bacteria. An interesting application of *Pleurotus* spp. concerns fortified dairy foods. A study carried out by Pelaes Vital *et al*. (2015) showed how adding *P. ostreatus* aqueous extract to milk leads to the production of yogurt with an increased *Streptococcus thermophiles* and *Lactobacillus bulgaricus* CFU, polyphenols content, and enhanced antioxidant activity, and improved rheological properties. Soy milk added with polysaccharide extract of *P. eryngii* shows an increased vitality of *Bifidobacterium longum* and reduced pH during yogurt fermentation (Li and Shah, 2016). The incorporation of *P. ostreatus* in the cheese mixture as a fresh and dried mushroom has resulted in cream cheese with higher ash, protein, and mineral contents, as well as an increase in lipolytic and proteolytic bacteria and excellent storage performances (Khider *et al*., 2017). The incorporation of *C. aegerita* powder has also proven to increase the antioxidant properties of cream cheese, as well as giving it more appreciated sensory characteristics (Petrovic *et al*., 2015). The extract of *A. bisporus* has proved effective in preserving yogurt from the pathogen *Listeria monocytogenes* (Stojkovic *et al*., 2014). Moreover, a study conducted by Proserpio *et al*. (2019) involved the addition of *P. ostreatus* powder in vegetable soups, resulting in a product with a higher content of bioactive ß-glucans and good palatability at a concentration of 2%. Although the results obtained so far are remarkably promising, much remains to be done; in addition to enlarging the fans of mushroom species potentially valuable as food fortifiers, further study is needed on various parameters such as the bioaccessibility of bioactive compounds, especially considering the different production steps that a processed product undergoes, their bioavailability, possible interactions with the food matrix and possible interferences with the bioavailability or absorption of the various nutrients. This path is even necessary so that their relevance and effectiveness can be recognized and thus ruled also from a legislative point of view, in order to achieve the important objective of large-scale marketing of healthy food products that promote physical well-being.

Table 7 Bioactivities of some Italian wild and cultivated mushrooms

Mushrooms species	**Bioactive compound or extract**	**Activity and mechanisms**	**References**
Agaricus bisporus	Polysaccharides	Scavenging activity, metal chelating activity, reducing power; antihypoxic activity	Li *et al*. (2015)
	Lectin (ABL)	Anticancer - Inhibition of proliferation of cancerous human epithelial colon cells (HT29) *in vitro*	Ismaya *et al*. (2020)

		Inhibition of MCF-7 (breast cancer cells) and Caco-2 cancer cell proliferation *in vitro*	
		Antineoplastic - Suppressed proliferation of retinal pigment epithelium (RPE) cells, and subsequent lowering of proliferative vitreo retinopathy; Slows down proliferation of human ocular fibroblast and reduces collagen lattice contraction *in vitro*	Ismaya *et al*. (2020)
		Antiviral - Strong inhibition against human immunodeficiency virus type-1 (HIV-1) reverse transcriptase (IC50 of 8 μM) *in vitro*;	Ismaya *et al*. (2020)
	Mannose-binding protein (Abmb)	Anticancer - Inhibits proliferation of MCF-7 breast cancer cells at 12.5 μM and arrests growth at lower concentrations *in vitro*	Ismaya *et al*. (2020)
	Methanolic and aqueous extracts	Anti-inflammatory, analgesic, antipyretic, antioxidative and antimicrobial (in mice and/or *in vitro*)	Bose *et al*. (2019)
	Polysaccharide	Immunostimulatory and antitumor bioactivity *in vivo* and *in vitro*	Atila *et al*. (2017)
	Fruiting body extracts	Immunostimulating - On activated human peripheral blood mononuclear cells (PBMCs) and induced synthesis of interferon gamma (IFN-c)	Atila *et al*. (2017)
		Antitumor - Inhibition on cell proliferation of HL-60 leukemia cells and other leukemia human cell lines via the induction of apoptosis;	Atila *et al*. (2017)
		Suppression of aromatase activity, inhibition on breast cancer cell proliferation, and decrease in mammary tumor formation *in vivo*	Atila *et al*. (2017)
	UFA	Antitumor - Inhibition on aromatase activity	Atila *et al*. (2017)
	Arginine	Antitumor - Delay of tumor growth and metastasis	Atila *et al*. (2017)
	Lovastatin	Antitumor Anti-cancer effects in the triple-negative breast cancer cell line MDA-MB-231	Atila *et al*. (2017)
		Antihyperlipidemic - Reduction of cholesterol level in serum and/or liver	Atila *et al*. (2017)

	Sterols	Antihyperlipidemic - Reduction in cholesterol absorption and thereby lowered plasma cholesterol and LDL cholesterol	Atila *et al.* (2017)
	Fruiting body Extracts	Antidiabetic Decreased severity of streptozotocin-induced diabetes in rat	Atila *et al.* (2017)
	a-glucans	Antidiabetic - Lowered producing lipopolysaccharide-induced TNFa	Atila *et al.* (2017)
	Polysaccharides and phenolics	Scavenging of superoxide, hydroxyl and DPPH radicals and hydrogen peroxide, enhancement of the activities of antioxidant enzymes in sera, liver, and heart of mice	Zhang *et al.* (2016)
	Proteoglycan	Antitumor Involvement of NK cells and induction of gene expression of nitric oxide by transcription factor and NF-kappa B downstream signalling, interferon-c and interleukin, that activate NK cells	Chaturvedi *et al.* (2018)
Boletus edulis	Polysaccharide(BEP)	Immunomodulatory - Reduction of tumor mass in Renca tumor bearing mice; stimulation of splenocytes proliferation, increase in NK cell and CTL activities in spleen	Wang *et al.* (2014a)
	Polysaccharides	Antioxidant activity	Zhang *et al.* (2018)
	Lectin	Hemagglutinating activity; Mitogenic activity in mouse splenocytes; Antiviral Inhibition of human immunodeficiency virus-1 reverse transcriptase	Zheng *et al.* (2007)
	Phenolics	Antioxidative - Inhibition of lipid oxidation	Ma *et al.* (2018)
	Prepared for consumption mushrooms	Antioxidative High antioxidant activity against ABTS, DPPH and in FRAP assay	Jaworska *et al.* (2015)
	Ethanolic and hot water extracts	Antioxidative	Tsai *et al.* (2007)
	Polysaccharide (BPS)	Antidiabetic Inhibition of oxidative stress and inflammation in rats liver	Xiao *et al.* (2019)
Coprinus comatus	Ethyl acetate extract	Antitumor - Activity against ovarian cancer cell lines SKOV-3 and SW-626 and reduced viability of human ovarian cancer cells;	Venturella *et al.* (2019)

		Apoptosis induction in ovarian cancer cells (ES- 2) via both extrinsic and intrinsic pathways	
	Aqueous suspension	Antioxidative - increase of antioxidative status of liver homogenate and prevention of histological changes in liver cross sections in oxidative stressed rats	Popovic *et al*. (2010)
	Fruiting body extract	Antiaggregant - Inhibition of platelet aggregation induced by ADP via a P2Y12 receptor	Poniedziałek *et al*. (2019)
	Ethanolic and water extract	Antioxidant and scavenging property	Li *et al*. (2010)
	Laccase	Antiviral - Inhibition of human immunodeficiency virus type 1 (HIV-1) reverse transcriptase	Ma *et al*. (2018)
		Antitumor - Suppression of proliferation of tumor cell lines HepG2 and MCF7	Ma *et al*. (2018)
	γ-aminobutyric acid (GABA)	Hypotensive	Tsai *et al*. (2007)
Cyclocybe aegerita	Polysaccharides	Anti-ageing - Increased cell viability and β-Gal viability, prevention of G1-phase cell-cycle arrest, decreased mitochondrial membrane potential	Liu *et al*. (2020c)
		Antidiabetic - Inhibition of iNOS expression, reduction of blood glucose level	Liu *et al*. (2020c)
	Water extract	Antiangiogenic - *In vitro* inhibition of vascular endothelial growth factor (VEGF)-induced proliferation in HUVECs; down-regulation of intracellular reactive oxygen species (ROS) level and VEGF secretion in Caco-2 cells; decrease in the migration of endothelial cells (ECs)	Lin *et al*. (2017)
	Proteins	Antitumor - Against different tumor cell lines; stimulation of immune response; enhanced splenocyte cytotoxic activity and mRNA level of cytokines in mice	Liang *et al*. (2011)
	Ageritin (ribotoxinlike protein)	Antitumor - Cytotoxicity and cell death promoting effects towards CNS model cell lines (SK-N-BE(2)-C, U-251 and C6);	Landi *et al*. (2017b) and

		extrinsic apoptotic pathway by initially activating caspase-8	Ruggiero *et al*. (2018)
	Galectin (AAL)	Antitumor - Anti-metastasis activity in breast cancer, antiproliferation activity against 4T1 cells	Yang *et al*. (2018)
	Ceramide	Antitumor - Inhibition of the proliferation of stomach, breast and CNS cancer cell lines *in vitro*	Diyabalanage *et al*. (2008)
		Anti-inflammatory - Inhibition on cyclooxygenase enzymes COX-1 and -2	
	Hot-water and ethanolic extracts	Antioxidative	Tsai *et al*. (2006, 2007)
	Methanolic extract (FAF)	Antioxidative and cyclooxygenase (COX) enzyme inhibitory activity	Zhang *et al*. (2003)
Lactarius deliciosus	Methanolic extract	Antioxidant and free radical scavenging activity; Antimicrobial - Inhibition of bacteria (*Bacillus cereus, B. subtilis, Proteus mirabilis, E. coli, Staphylococcus aureus*) and fungi (*Aspergillus niger, Penicillium expansum, P. chrysogenum, Alternaria alternata, Trichoderma viride,Cladosporium cladosporioides, Mucor mucedo, Fusarium oxysporum, Candida albicans*); Anticancer - Growth inhibition in HeLa, A549 and LS174 cell lines	Kosanic´ *et al*(2016).
	Aqueous and/or ethanol extract	Antioxidative; Antihyperglycemic Inhibitory effects on α-amylase and α-glucosidase	Xu *et al*. (2019)
Macrolepiota procera	Methanolic extract	Antioxidant and free radical scavenging activity; Antimicrobial - Inhibition of bacteria (*Bacillus cereus, B. subtilis, Proteus mirabilis*) and fungi (*Aspergillus niger, Penicillium expansum, Alternaria alternata, Trichoderma viride,Cladosporium cladosporioides, Fusarium oxysporum, Candida albicans*); Anticancer Growth inhibition in HeLa, A549 and LS174 cell lines	Kosanic *et al*. (2016)

	Mushroom extract	Antioxidant activity	Islam *et al*. (2019)
	Powder of freeze, dried and irradiated mushrooms	Antioxidant activity	Fernandes *et al*. (2013)
Russula virescens	Polysaccharide (RVP) Phenolics	Antioxidant activity	Sun *et al*. (2010a, b)
Russula cyanoxantha	Phenolics	Antioxidant activity	
Tuber magnatum	Water and/or methanol extract	Anti-inflammatory Inhibition of COX-1 and 12-LOX pathway products synthesis Antitumor Cytotoxicity against some tumour cell lines (HeLa, MCF7, HT-29) Antioxidative	Beara *et al*. (2014)
Tuber melanosporum	Polysaccharides	Antitumor Activities against A549, HCT-116, HepG2, HL- 60, and SK-BR-3 cells lines	Lee *et al*. (2020) and Patel *et al*. (2017)
	Anandamide (endocannabinoid)	Antitumor - Inhibited on angiogenesis of highly invasive and metastatic breast cancer cells; Stimulation of non-apoptotic cell death in COX- 2 overexpressed colorectal cancer cells	Lee *et al*. (2020) and Patel *et al*. (2017)
	Methanolic extract	Antioxidative - Inhibition of lipid oxidation	Villares *et al*. (2012)
Tuber spp.	Flavonoids	Antioxidative, anti-inflammatory, anti-mutagenic, and anticancer	Lee *et al*. (2020)
	Ergosterol	Antioxidant, anti-inflammatory, and antihyperlipidemic	Lee *et al*. (2020)
	Oleic acid	Antitumor Suppression of overexpression of HER2; induction of cancer cell apoptosis	Lee *et al*. (2020)
	L-tyrosine	Hypocholesterolemic; Anti-depressant	Patel *et al*. (2017)

3.5 Conclusions

The review reveals the great potential of mushrooms in the production of mycochemicals that represent a rich source of drugs, nutraceutical, and functional food. The mycochemicals isolated and identified from mushrooms are bioactive compounds belonging to different chemical classes. The present study describes the chemical composition of Italian wild and

cultivated mushrooms as a source of bioactive metabolites for further development of drugs. The application of mushrooms for health purposes is recent in the Western areas but still slowly growing. In European markets, nutraceuticals are not yet a widespread and established product and, in most cases, imported from Asia. Due to the vacant and imprecise regulations, we often have to deal with nutraceuticals of dubious composition and without guaranteed quality standards. Most Western countries, moreover, follow the rules of the WHO, DSHEA (Dietary Supplement Health and Education Act), and EFSA (European Food Safety Agency) in which plant or MM extracts are dietary supplements. So clinical studies are not required before their introduction in the market. These markets, therefore, have enormous potential for development, which can only be achieved through intensive research and the spreading of knowledge to educate and raise awareness in this respect among consumers and society because very few people are still aware of the benefits and importance of MMs. The research on medicinal mushrooms in Italy needs to undertake more extensive studies to ascertain the medicinal properties of the mushroom species. The final objective of the newborn Italian Medicinal Mushrooms Society is to improve the quality of life and the state of health of people, also in the vision of an increasingly integrated medicine. The animal farming sector could also benefit from the inclusion of mushrooms, MMs supplements, or fortified feed in the animal diet, as well as, for example, from the possibility of using alternative and natural antibiotics and antivirals. Besides, mushrooms represent an economic crop that fits with the circular economy and the recycling of agro-industrial wastes. Finally, mushrooms are also able to provide nutritional support in areas with malnutrition and economically depressed areas.

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Chapter 4. Functional bread supplemented with *Pleurotus eryngii* powder: a potential new food for human health

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4.1 Abstract

The purpose of this study was to develop functional breads with powdered *Pleurotus eryngii*. The breads were produced applying the traditional Italian style sourdough technology. *P. eryngii* powder was added to flour of tender wheat varieties (Grano Dei Miracoli, Inalettabile, Mentana, Gentilrosso, Ardito and a mix of Rieti, Verna, and Mentana) or semolina of durum wheat landrace (Saragolla) and subjected to sourdough fermentation. Sourdough inoculum was produced with selected strains of lactic acid bacteria (LAB) belonging to the species *Levilactobacillus brevis*, *Weissella cibaria* and *Leuconostoc citreum*. The addition of *Pleurotus* powdered (PP) (10% w/w) did not influence the fermentation process, since LAB developed until 10^9 CFU/g after 8 h of leavening. The values of pH, TTA and organic acids of doughs prepared with PP were higher than those of control fermentation. All breads differed for height, weight loss, firmness, colour and void fraction. Sensory evaluation indicated that the bread produced with Mentana flour added with PP was mostly appreciated by the judges. Hence, only the bread processed from this wheat variety was investigated for vitamins and microelements showing an increase in B_1, B_2, B_3, and D vitamins, total polyphenols, and beta-glucans. This work provided evidence to perform PP supplementation to increase the functional aspects of bread.

4.2 Introduction

Cereal-based foods have been crucial components of the human diet for millennia and still remain the main source of nutrition, particularly in developing countries (Blandino et al., 2003). Wheat has been one of the most important crops in the Mediterranean basin and, after mass migrations, its cultivation has undergone a remarkable expansion, which has driven its cultivation all over the world (Toderi, 1989).

During the last century, consumption habits have changed considerably, leading to excessive ingestion of refined sugars and high-calorie foods. This change, combined with a sedentary lifestyle, has been correlated with the increasing incidence of chronic metabolic diseases (Alongi et al., 2019). Among these, one of the most alarming is type 2 diabetes, which could become the seventh leading cause of death worldwide by 2030 (WHO, 2003). Type 2 diabetes is characterized by chronic hyperglycemia; dietary changes have been suggested to limit its occurrence (WHO, 2003; American Diabetes Association, 2004). Several foods

have demonstrated anti-diabetic properties thanks to their bioactive compounds, which act mainly by modulating the activity of digestive enzymes and the intestinal transit rate, resulting in a lowering of the glycaemic index (Ríos et al., 2015). For this reason, the interest of research and food industries is being extensively focused on the development of functional foods able to prevent several diet-related diseases beyond the simple nutritional effects (Roberfroid, 2000; Menrad, 2003), as indicated in Regulation (EC) No. 1924/2006. A given food may naturally contain functional components or they can be exogenous and added to increase their concentrations.

In recent years, a re-discovery of wheat landraces has been registered (Ruisi et al., 2021). These old wheat genotypes are being used to produce cereal-based foods with beneficial effects on the human health (Bordoni et al., 2017; Dinu et al., 2018). These benefits can be attributed to a higher content of unsaturated fatty acids, soluble fibre, minerals, vitamins and phytochemicals than modern wheat varieties (Dinelli et al., 2007; Hidalgo & Brandolini, 2014). Clinical studies provided evidence that the ancient wheat varieties exert beneficial effects on various parameters linked to cardio-metabolic diseases including lipid and glycaemic profiles, inflammatory and oxidative status (Dinu et al., 2018).

Basidiomycetes and Ascomycetes possess different antitumor, antibacterial, antifungal, antiviral, cytotoxic, immunomodulating, anti-inflammatory, antioxidant, anti-allergic, antidepressant, antihyperlipidemic, antidiabetic, digestive, hepatoprotective, neuroprotective, nephroprotective, osteoprotective, and hypotensive properties (Gargano et al., 2017; Venturella et al., 2021). The enrichment of cereal-based foods with fungal mycelia or fruiting bodies increased the total availability of vitamins, minerals, fibre, beta-glucans, and antioxidants in bakery breads and cakes (Corey, et al., 2009; Regula et al., 2016; Salehi et al., 2016; Vlaic et al., 2019). Among the cultivated edible mushrooms, *Pleurotus eryngii* (DC.) Quél. (Pleurotaceae) is one of the most worldwide widely consumed species (Biao et al., 2020). This fungus is a natural source of bioactive compounds, including carbohydrates, peptides and, dietary fibre (Yuan et al., 2017a). Furthermore, different studies (Hu et al., 2019; Venturella et al., 2015) showed that *P. eryngii* exhibits health-promoting properties such as antioxidant, anti-inflammatory, anti-colon cancer, and anti-colitis properties. Furthermore, this species is characterized by a high vitamin contents (Cateni et al., 2021).

With the aim of functionalize bread, the main food common to almost all societies (Jenson, 1998), this work was performed to produce traditional style Italian breads, based on sourdough technology, adding to flours from *Triticum aestivum* L. and *Triticum durum* Desf. landraces the edible mushroom *P. eryngii*, characterized by medicinal properties, in powder

form. The breads were analysed for their main microbiological, chemical, physical, sensory and functional aspects.

4.3 Materials and methods

4.3.1 Raw materials

The culinary mushroom *Pleurotus eryngii*, known in Italy as "cardoncello" mushroom, was provided by Italmiko (Senise, Italy) and powdered (PP) as reported by Schillaci et al. (2013). Mushroom enriched breads were processed mainly from flours of tender (*T. aestivum*) wheat varieties, including Grano Dei Miracoli (Gm), Inalettabile (In), Rieti (Ri), Mentana (Me), Gentilrosso (Gr), Ardito (Ar), and a mix of Rieti, Verna, and Mentana (Mix). Furthermore, the durum wheat landrace Saragolla (Sa) was also included in this study. All flours were provided by Valmarecchia Foundation (Novafeltria, RN, Italy). Each flour and the corresponding flour + PP mixture was subjected to water absorption test to evaluate the effect of PP on the water amount needed to produce the doughs. The tests were performed using a Farinograph-E (Brabender GmbH & Co. KG, Duisburg, Germany) following the manufacturer's instruction and the results expressed as % at 500 farinograph units (FU).

4.3.2 Lactic acid bacteria strains and sourdough development

The obligate heterofermentative strains *Leuconostoc citreum* PON100290, *Weissella cibaria* PON10032, *Levilactobacillus brevis* PON100289 and GGS13 belonging to the Culture Collection of the Department of Agricultural, Food and Forestry Sciences (University of Palermo), were used as starter cultures. These strains were isolated from wheat matrices (Alfonzo et al., 2013) and applied for the production of sourdough breads using tender wheat flour (Settanni et al., 2013) and durum wheat semolina (Alfonzo et al., 2016). The strains were reactivated through overnight 30 °C incubation in modified-de Man-Rogosa-Sharpe (mMRS) broth, prepared from MRS (Oxoid, Milan, Italy) added with 10% (v/v) fresh yeast extract and 1% (w/v) maltose adjusted to pH 5.6 with 5 M lactic acid. Sourdough was prepared as reported by Alfonzo et al., (2016) using the direct liquid inoculum as starter. Doughs (300 g) were prepared using 187.5 g of flour inoculated with all the microbial suspension (112.5 mL), mixed by the SilverCrest Bread Maker SBB 850 A1 (Kompernass GMBH, Bochum, Germany) for 10 min and fermented at 30 °C for 24h. The back slopping protocol consisted of mixing mature sourdough with flour and sterile tap water in a ratio of 1:1:0.5. Back slopping was performed for 7 d before using sourdough as leavening agent for bread production.

4.3.3 Dough production and baking process

The experimental plan included two different dough productions per flour: "control dough" prepared from flour and sourdough; "*Pleurotus* dough" prepared from flour, 10% (w/w of flour) of PP and sourdough, based on the previous study by Gaglio et al. (2019). Following the recipe of "Pagnotta" bread produced in Sicilian bakeries (Corona et al., 2016), the doughs (507.5 g) were prepared as follows: 217.21 g of wheat flour or wheat flour added with PP, 187.5 mL of sterile tap water, 7.5 g of cooking salt and 95.28 g of mature sourdough. Fermentation was carried out for 8 h at 30 °C and 90% relative humidity (RH). The doughs (100 g) were transferred into rectangular stainless steel baking pans (143x79 mm, top inside; 129x64 mm bottom outside; depth inside 57 mm) following the indication of the American Association of Cereal Chemistry (Method 10-10B, AACC, 2000), and covered with aluminium foil. The remaining dough (average 200 g each) were placed into sterile glass beakers (volume 500 mL) and covered with parafilm. All pans and beaker were incubated at 30 °C for 8 h. After fermentation, all doughs were baked in the semi-industrial oven Compact Combi (Electrolux, Pordenone, Italy) by a 2-step baking program consisting of 5 min at 200 °C under a combination of air and steam and 15 min at the same temperature using only hot air. Control and *Pleurotus* productions were carried out in duplicate and repeated twice after one month.

4.3.4 Physicochemical and microbiological analysis of dough

Dough samples were collected after the mixing process (T_0) and at the end of fermentation (T_8). The values of pH was determined with the portable pH meter pH 7 - food (XS instruments, Carpi (MO), Italy). Total titratable acidity (TTA) was determined using the official AACC method (results expressed in terms of mL of NaOH 0.1 N/10 g of dough. pH and TTA were measured in triplicate. The concentration of acetic acid and lactic acid were determined by enzymatic assay performed with iMagic-M9 (Shenzhen iCubio Biomedical Technology Co., Shenzhen, China) automatic chemistry analyzer using Enzytec™ L-Lactic acid E8260 and Enzytec™ acetic acid E5226 (R-Biopharm, Darmstadt, Germany) following the protocol provided by the manufacturer for solid food matrices. Microbiological analyses were performed on raw materials, dough after ingredient mixing and dough at 8 h of fermentation. Ten grams of each sample were suspended in 90 mL of Ringer solution, homogenized with the BagMixer® 400 stomacher (Interscience, Saint Nom, France) for 2 min at maximum speed, and then subjected to the decimal serial dilution procedure. Cell suspensions were plated and incubated as follows: total mesophilic count (TMC) on plate count agar (PCA), incubated at 30 °C for 72 h in aerobic condition; sourdough LAB on SDB agar, incubated at 30 °C for 48 h in anaerobic condition; rod LAB on mMRS agar, incubated

at 30 °C for 48 h in anaerobic condition; members of *Enterobacteriaceae* family on violet red bile glucose (VRBGA) agar, incubated at 37 °C for 24 h in aerobic condition and yeast on yeast extract peptone dextrose (YPD) nutrient agar, incubated at 25 °C for 48 h. To inhibit bacterial growth chloramphenicol (0.05 mg/mL) was added to YPD, while cycloheximide (10 mg/mL) was added in mMRS and SDB to inhibit fungal growth. All microbiological counts were performed in triplicate.

4.3.5 Physical characteristics of breads

After baking, the breads were cooled at room temperature for 30 min and weighed to calculate weight loss during baking. The breads were then transversally cut into two pieces and the central slice was measured with a caliper (Schober et al., 2005). The central slices were also subjected to the image analysis as reported by Gaglio et al. (2020a). Briefly, each central slice was scanned (Epson Perfection 4180 Photo, Seiko Epson Co., Suwa, Japan) with high resolution (300 dpi) and the images saved in TIFF format. Each image was converted to an 8-bit grayscale image and cropped at 177 × 177 pixels (equal to 15 × 15 mm of the slice area) with ImageJ software (National Institutes Health, Bethesda, Maryland, USA). Void fraction (percentage of total bread slice area corresponding to the pores), mean cell area (in mm^2), and Cell density (number of cells/cm^2) were calculated by applying Otsu's threshold algorithm. The colorimetric parameters analysed for crumb (three points) and crust (four points) were lightness (L*), redness (a*), and yellowness (b*) of the Hunter's scale expressed according to International Commission on Illumination (CIE) L*a*b* system. To this purpose, a Chroma Meter (CR-300; Minolta, Osaka, Japan) was used. on four points of crust and three points of crumb. The determination of crumb's hardness was performed using the Instron-5564 (Instron® Co., Canton, Massachusetts, USA) as reported by Corsetti et al. (2000).

4.3.6 Sensory evaluation

A panel of 10 judges, including 5 women and 5 men (aged between 24 and 59 years old), were trained for evaluation of bread attributes. Sensory attributes of the final bread were analyzed following the guidelines of ISO 13299:2016. The judges evaluated 20 descriptors considering those suggested by Comendador et al. (2012), Rodrigues et al. (2014), and Martins et al. (2015) including visual elements (color, porosity, alveolation, and alveolation uniformity for the crumb, color, and thickness of crust), olfactory sensations (intensity, bread odor, and unpleasant odor), taste sensations (intensity, bread aroma, unpleasant aroma, salty, acid, astringent, bitter, taste persistency, adhesiveness in mouth, crispness), and overall assessment. The scores were given using a line scale as reported by Gaglio et al. (2020a),

with dislike/low quality on the left and with like/high quality on the right. The hedonic scale results were converted as a distance (mm) of the mark from the left end of the line.

4.3.7 Bread chemical composition

Proximate analysis, composition of microelements and vitamin profile were determined only for the *Pleurotus* bread reaching the highest scores at the sensory evaluation. Analyses were performed by CHELAB S.r.l. Sole Partner Company (ACCREDIA LAB N° 0051 L) subjected to the direction and coordination of Mérieux NutriSciences Corporation (Resana, Italy). Analyses were carried out following internal methods based on official methods: protein content was assessed by MP 1457 rev 3 2017 (based on AOAC 990.03 2002, AOAC 992.15 1992, AOAC 992.23 1992 and UNI EN ISO 14891:2002 methods); total fats content by ISTISAN 1996/34 method A; carbohydrates by MP 0297 rev 6 2018; fibre (total, soluble and insoluble fraction) by MP 2135 rev 4 2017 (based on AOAC 991.43 1994 method); ashes by MP 0297 rev 6 2018 (based on AOAC 945.46, ISTISAN 1996/34, REGCE 152/09 27/01/09 Annex III Method M, UNI 10590:1997, AOAC 923.03, DM 21/04/1986 PAR 10, DM 03/02/1989 Method 13, UNI EN 1135/97, AOAC 938.08, DM 06/01/1979 PAR 6, AOAC 920.93 A, DM 21/09/70 PAR 10 methods); humidity and dry matter by ISTISAN 1996/34 method B; β-d-glucans by AOAC 995.16 2005; total polyphenol content by MP 0468 rev 8 2014.

Regarding the composition of microelements, calcium, phosphorus, magnesium, potassium and sodium contents were analysed by MP 1289 rev 13 2020; iron, manganese, copper, zinc, chromium and selenium contents by MP 1288 rev 17 2020; total iodine by MP 1618 rev 3 2017; fluorides by MP 1479 rev 1 2016; chlorides by MP 0464 rev 9 2017. Vitamins profile were determined as follow: D vitamin by MP 1570 rev 2 2017; E vitamin by UNI EN 12822:2014; B_1 vitamin (thiamine) by MP 0851 rev 1 2007; B_2 vitamin (riboflavin) by MP 2251 rev 0 2017 (based on UNI EN 14152); B_3 and B_6 vitamins by MP 2327 rev 0 2019 (based on AOAC 2015.14); B_5 vitamin (pantothenic acid) by MP 2333 rev 0 2019 (based on ISO 20639); B_8 vitamin (biotin) by PNTA0164; B_9 vitamin (folic acid and folates) by PNTA0192; B_{12} vitamin (cobalamin) by MP 2347 rev 0 2019 (based on AOAC 2014.02); K_3 vitamin by MP 2347 rev 0 2019 (based on AOAC 2014.02); C vitamin (ascorbic acid) by MP 2174 rev 3 2019; A vitamin by UNI EN 128231:2014.

4.3.8 Statistical analyses

Chemical, physical, microbiological, and sensory data were tested for differences using the one-way analysis of variance (ANOVA; general linear model) followed by *post hoc* Tukey's multiple range test applied for pairwise comparison. Statistical significance difference for *p*

≤ 0.05 between control and *Pleurotus* samples are marked with different letters; "a" denotes the highest value. The statistical analysis was performed using XLStat® add-in ver. 2014.5.03 (Addinsoft, Paris, France) for Microsoft Excel®.

4.4 Results and discussion

4.4.1 Farinograph water absorption

In order to evaluate the effect of PP on the amount of water absorbed by each flour used in this study the Farinograph curves were determined (results not shown). The results on the eight flour samples processed before PP addition were in the range 47.9 – 53.3%, while water absorption in presence of PP increased to 52.4 – 57.0%. The lowest water absorption values were registered with Ar flour with and without PP, while the highest values were recorded with Mix sample before and after PP addition. On average, PP determined an increase of 3.8% of water absorption. Almost the same increase in water absorption was reported by Mashayekh et al. (2008) who tested the effect of fortification of defatted soy flour on sensory and rheological properties of wheat bread.

4.4.2 Microbiological counts

The results of the microbiological analyses of raw materials, sourdough and the experimental doughs are reported in Table 1. All flours, semolina, and PP were characterized by detectable levels of TMC, yeasts, and *Enterobacteriaceae*. LAB on mMRS were detected only in 3 flour samples (Ge, Gm and In) and PP, while none of the samples object of investigation showed the presence of sourdough LAB (on SDB) Previous works on raw materials showed that yeast and LAB levels are particularly variable (from below the detection limit up to 4.9 log CFU/g) in both flour and semolina (Alfonzo et al., 2013; Nachi et al., 2019; Gaglio et al., 2020b). Several factors influence the microbial community of flour/semolina; among these, plant growth conditions (Minervini et al., 2015), geographical area of production, genotypes, contamination of sowed seeds (Alfonzo et al., 2017), and storage time (Gaglio et al., 2020b) play defining roles. The sourdough developed from the direct inoculums of LAB was analysed at the 7th day, when it was used as leavening agent in bread production. LAB loads were almost at the same level on SDB (9.19 log CFU/g) and mMRS (9.24 log CFU/g), while yeast levels were 7.77 log CFU/g. Thus, the optimal LAB/yeast ratio of 100:1 typical for mature sourdoughs (Gobbetti et al., 1994) was reached. Viable counts were also determined for all dough samples before (T_0) and after (T_8) fermentation. Just after ingredients mixing TMC ranged between 7.21 and 8.05 log CFU/g and the differences were not significant. LAB loads were different on the two media used: their numbers ranged between 7.74 and 8.69 log CFU/g on SDB and were slightly lower on mMRS (7.00 – 8.16

log CFU/g). In this case, the differences among flour samples were statistically significant,

Sample	Type	Microbial loads (Log CFU/g)									
		PCA		mMRS		SDB		YPD		VRBA	
		T_0	T_8	T_0	T_8	T_0	T_8	T_0	T_8	T_0	T_8
Raw materials											
PP	*P. eryngii* powder	5.42 ± 0.16	n.d.	2.53 ± 0.18	n.d.	<2	n.d.	3.13 ± 0.23	n.d.	5.07 ± 0.16	n.d.
Sa	Semolina	5.26 ± 0.15	n.d.	<1	n.d.	<2	n.d.	2.65 ± 0.31	n.d.	4.42 ± 0.29	n.d.
Mix	Type 2 Flour	4.19 ± 0.11	n.d.	<1	n.d.	<2	n.d.	2.44 ± 0.22	n.d.	3.32 ± 0.11	n.d.
Ge	Type 1 Flour	4.00 ± 0.16	n.d.	2.48 ± 0.18	n.d.	<2	n.d.	2.27 ± 0.11	n.d.	3.36 ± 0.15	n.d.
Ar	Type 1 Flour	5.00 ± 0.23	n.d.	<1	n.d.	<2	n.d.	3.09 ± 0.25	n.d.	4.63 ± 0.05	n.d.
Me	Type 1 Flour	4.85 ± 0.18	n.d.	<1	n.d.	<2	n.d.	2.93 ± 0.28	n.d.	3.95 ± 0.25	n.d.
Gm	Type 1 Flour	5.43 ± 0.20	n.d.	3.00 ± 0.11	n.d.	<2	n.d.	3.11 ± 0.35	n.d.	4.58 ± 0.17	n.d.
In	Type 1 Flour	4.54 ± 0.22	n.d.	1.55 ± 0.09	n.d.	<2	n.d.	2.35 ± 0.22	n.d.	3.94 ± 0.09	n.d.
Ri	Type 1 Flour	4.20 ± 0.13	n.d.	<1	n.d.	<2	n.d.	2.19 ± 0.15	n.d.	3.40 ± 0.11	n.d.
Sour dough		9.20 ± 0.19	n.d.	9.24 ± 0.15	n.d.	9.19 ± 0.09	n.d.	7.77 ± 0.18	n.d.	<1	n.d.
Doughs											
Mix C		7.89 ± 0.36^{a}	8.55 ± 0.11^{abcd}	8.16 ± 0.38^{a}	8.99 ± 0.12^{a}	7.93 ± 0.32^{ab}	8.98 ± 0.34^{a}	5.22 ± 0.17^{ab}	6.37 ± 0.11^{bcdef}	1.30 ± 0.27^{h}	<1
Mix P		8.05 ± 0.34^{a}	9.19 ± 0.12^{ab}	7.99 ± 0.25^{ab}	8.57 ± 0.33^{a}	8.69 ± 0.39^{a}	9.23 ± 0.24^{a}	5.47 ± 0.31^{ab}	7.10 ± 0.33^{a}	2.12 ± 0.23^{efg}	<1
Ge C		7.35 ± 0.34^{a}	8.42 ± 0.18^{cd}	7.20 ± 0.42^{ab}	8.87 ± 0.17^{a}	8.09 ± 0.34^{ab}	9.23 ± 0.42^{a}	5.75 ± 0.34^{a}	6.21 ± 0.18^{cf}	1.20 ± 0.15^{h}	<1
Ge P		7.50 ± 0.23^{a}	8.37 ± 0.36^{cd}	7.57 ± 0.41^{ab}	8.60 ± 0.22^{a}	8.61 ± 0.42^{ab}	9.26 ± 0.25^{a}	5.81 ± 0.32^{a}	7.00 ± 0.36^{ab}	2.24 ± 0.13^{def}	<1
Ar C		7.21 ± 0.32^{a}	8.33 ± 0.24^{cd}	7.10 ± 0.36^{b}	8.56 ± 0.17^{a}	8.18 ± 0.17^{ab}	9.05 ± 0.34^{a}	5.32 ± 0.31^{ab}	6.74 ± 0.17^{abcde}	1.62 ± 0.12^{gh}	<1
Ar P		7.24 ± 0.34^{a}	8.98 ± 0.23^{abc}	7.41 ± 0.37^{ab}	8.85 ± 0.32^{a}	8.67 ± 0.16^{a}	9.21 ± 0.27^{a}	5.48 ± 0.33^{ab}	6.87 ± 0.22^{abc}	3.25 ± 0.19^{ab}	<1
Me C		7.24 ± 0.16^{a}	9.01 ± 0.36^{abc}	7.59 ± 0.34^{ab}	8.72 ± 0.39^{a}	8.42 ± 0.34^{ab}	9.34 ± 0.17^{a}	4.90 ± 0.40^{ab}	6.30 ± 0.23^{cdef}	1.70 ± 0.19^{fgh}	<1
Me P		7.44 ± 0.33^{a}	9.23 ± 0.24^{a}	7.86 ± 0.39^{ab}	8.81 ± 0.31^{a}	8.54 ± 0.32^{ab}	9.48 ± 0.24^{a}	4.89 ± 0.32^{ab}	6.15 ± 0.25^{cf}	1.30 ± 0.22^{h}	<1
Sa C		7.39 ± 0.32^{a}	8.21 ± 0.24^{d}	7.55 ± 0.23^{ab}	8.80 ± 0.41^{a}	8.12 ± 0.22^{ab}	9.20 ± 0.36^{a}	5.48 ± 0.42^{ab}	6.31 ± 0.16^{cdef}	1.28 ± 0.15^{h}	<1
Sa P		7.62 ± 0.36^{a}	8.36 ± 0.32^{cd}	7.74 ± 0.41^{ab}	8.94 ± 0.32^{a}	8.31 ± 0.31^{ab}	9.15 ± 0.23^{a}	5.82 ± 0.34^{a}	6.69 ± 0.12^{abcde}	2.24 ± 0.24^{def}	<1
Gm C		7.37 ± 0.21^{a}	8.19 ± 0.24^{d}	7.76 ± 0.34^{ab}	8.88 ± 0.12^{a}	8.30 ± 0.39^{ab}	9.07 ± 0.36^{a}	5.53 ± 0.24^{ab}	6.44 ± 0.24^{bcdef}	2.75 ± 0.17^{bcd}	<1
Gm P		7.63 ± 0.24^{a}	8.45 ± 0.36^{bcd}	7.68 ± 0.39^{ab}	8.74 ± 0.20^{a}	8.53 ± 0.11^{ab}	9.23 ± 0.21^{a}	4.77 ± 0.25^{b}	6.85 ± 0.13^{abcd}	3.48 ± 0.12^{a}	<1
In C		7.53 ± 0.42^{a}	8.29 ± 0.26^{cd}	7.00 ± 0.40^{b}	8.98 ± 0.39^{a}	8.00 ± 0.39^{ab}	9.36 ± 0.31^{a}	5.80 ± 0.34^{a}	6.23 ± 0.21^{def}	<1^{i}	<1
In P		7.87 ± 0.18^{a}	8.35 ± 0.21^{cd}	7.78 ± 0.16^{ab}	8.71 ± 0.40^{a}	7.88 ± 0.20^{ab}	9.07 ± 0.32^{a}	4.72 ± 0.32^{b}	5.91 ± 0.22^{f}	2.55 ± 0.36^{cde}	<1
Ri C		7.46 ± 0.15^{a}	8.32 ± 0.17^{cd}	7.84 ± 0.17^{ab}	9.00 ± 0.14^{a}	7.74 ± 0.31^{b}	9.00 ± 0.24^{a}	5.11 ± 0.38^{ab}	6.05 ± 0.13^{f}	2.56 ± 0.16^{cde}	<1
Ri P		7.42 ± 0.36^{a}	8.45 ± 0.19^{bcd}	7.55 ± 0.34^{ab}	8.63 ± 0.12^{a}	7.92 ± 0.32^{ab}	9.11 ± 0.36^{a}	5.17 ± 0.23^{ab}	6.00 ± 0.11^{f}	2.90 ± 0.17^{abc}	<1
		N.S.	***	*	N.S.	**	N.S.	***	***	***	

Abbreviations: PCA, plate count agar for total mesophilic count; mMRS, De Man-Rogosa-Sharpe modified agar for mesophilic rod LAB; SDB, sourdough bacteria agar for sourdough LAB; YPD, yeast peptone dextrose agar for yeast; VRBGA, violet red bile glucose agar for *Enterobacteriaceae*. Results indicate mean values ± SD of two determinations. Significance*:* *= $p \leq 0.05$; **= $p \leq 0.01$; ***= $p \leq 0.001$; N.S. = not significant. Significant differences among means at $p \leq 0.05$, measured by Tukey's multiple range test, are indicated by different letters within the same series (control or treated sample), with letter a denoting the highest value.

but the addition of PP did not significantly impacted the development of LAB between control and *Pleurotus* doughs for all flour tested. The levels of TMC were lower than those

Table 1. Microbial load of raw materials, sourdough and dough.

of LAB. This phenomenon has been registered in other works (Corona et al., 2016; Alfonzo et al., 2017; Gaglio et al., 2020a) and depends on the fact that PCA do not satisfy the nutritional requirements of LABs like richer media. At T_0 yeasts were present at 4.72 and 5.82 log CFU/g; between all samples were showed a significant difference but only between InC and InP trial statistical differences were found. *Enterobacteriaceae* were detected in

almost all dough samples at levels in the range 1.20 – 3.48 log CFU/g, but they were below the detection limit for InC dough. The levels of this bacterial group were significantly different among flours and after PP addition for the majority of samples, except those of the trials conducted with the flours Me and Ri. The highest values were registered in presence of PP for all samples except Me. After fermentation (T_8) the microbial levels generally increased of about 1 log cycle, except for *Enterobacteriaceae* whose levels decreased until being undetectable. Also after fermentation TMC levels were statistically different among flour samples, but they were not affected by PP addition. The levels of LAB reached 8.98 – 9.48 log CFU/g on SDB and 8.56 – 9.00 log CFU/g on mMRS and no statistical differences were registered. These data indicated that the fermentation process carried out by sourdough LAB was not affected by the flour used. At the end of fermentation, yeasts developed up to 5.91 – 7.10 log CFU/g with a statistically significant difference in samples and, in Mix and Ge, between trials. These data are similar to those reported in the literature for dough processed from semolinas (Corona et al., 2016; Alfonzo et al., 2017; Gaglio et al., 2020a).

4.4.3 Chemical parameters of dough

The main acidification parameters (pH, TTA, and concentration of lactic and acetic acid) of doughs are reported in Table 2. The initial pH value of dough samples was in the range 4.59 – 5.51. Dough pHs were statistically different for the trials carried out with the different flours and PP determined also significant differences between control and *Pleurotus* productions for the trials Ge, Me, Gm, and Ri. At the end of fermentation, these values dropped to 3.69 – 4.83. On the contrary, TTA levels increased from 4.85 – 11.25 ml NaOH 0.1N to 11.20 - 18.60 ml NaOH 1N. PP determined significant differences also between control and *Pleurotus* dough for the flours Sa and Gm at T_0 and Mix, Ge, Me, and Ri at T_8. The values of pH and TTA were both higher for *Pleurotus* doughs rather than those registered for control doughs at T_0 as well as at T_8. This can be explained by a buffering capacity of the protein content in PP as reported in a similar paper considering the fermentation of sourdough added with powdered legumes (Rizzello et al., 2014; Coda et al., 2017). The initial amount of lactic acid was 1.50 – 2.45 mg/g while that of acetic acid was 0.26 – 0.42 mg/g. As expected, at T_8 both organic acid concentrations increased (3.75 – 6.49 and 0.74 – 2.27 mg/g of lactic and acetic acid, respectively). The concentrations of organic acids in all *Pleurotus* trials were higher than those of control. Other investigations on breads fortified with pistachio powdered showed comparable amounts of lactic acid and slightly lower concentrations of acetic acid (Gaglio et al., 2020a). These data were used to calculate the molar ratio between lactic and acetic acid, known as fermentation quotient (FQ). This parameter provides information on how the main fermentation organic acids impact texture

and aroma profile of bread (Spicher, 1983). As reported by Spicher & Brümmer (2001) if FQ ranges between 1.5 – 4 sourdough technology influences positively the aromatic and textural characteristics of the final product. In this study, all trials were characterised by an FQ within the optimal range except InC trial whose value (4.55) was a little higher.

Table 2. Kinetics of acidification and organic acid production during fermentation.

Sample	pH		TTA		T_0			T_8		
	T_0	T_8	T_0	T_8	Latic acid (mg/g)	Acetic acid (mg/g)	FQ	Latic acid (mg/g)	Acetic acid (mg/g)	FQ
Mix C	4.86 ± 0.35^{bcde}	4.01 ± 0.05^{ef}	6.70 ± 0.57^{bcde}	11.90 ± 1.67^{cd}	1.66 ± 0.31^{ab}	0.33 ± 0.01^{abcde}	3.31	5.30 ± 0.33^{ab}	1.05 ± 0.02^{gh}	3.36
Mix P	4.94 ± 0.28^{bcde}	4.49 ± 0.21^{bc}	10.20 ± 1.68^{abc}	16.90 ± 1.69^{ab}	1.93 ± 0.25^{ab}	0.40 ± 0.03^{abcd}	3.21	5.24 ± 0.24^{ab}	1.85 ± 0.08^{c}	1.88
Ge C	4.73 ± 0.04^{de}	3.69 ± 0.01^{fg}	6.65 ± 1.05^{bcde}	11.65 ± 1.01^{d}	1.89 ± 0.33^{ab}	0.27 ± 0.05^{de}	4.62	5.33 ± 0.28^{ab}	1.14 ± 0.03^{fg}	3.12
Ge P	5.21 ± 0.02^{ab}	4.44 ± 0.09^{bcd}	10.70 ± 0.99^{ab}	16.55 ± 1.89^{ab}	2.22 ± 0.24^{ab}	0.36 ± 0.02^{abcde}	4.07	6.30 ± 0.30^{a}	2.13 ± 0.03^{ab}	1.97
Ar C	4.86 ± 0.06^{bcde}	3.86 ± 0.06^{fg}	6.75 ± 1.03^{bcde}	14.25 ± 1.87^{abcd}	2.13 ± 0.28^{ab}	0.34 ± 0.08^{abcde}	4.21	5.74 ± 0.74^{a}	1.21 ± 0.05^{f}	3.15
Ar P	4.97 ± 0.07^{bcd}	4.33 ± 0.04^{cd}	9.45 ± 1.89^{abcd}	18.60 ± 1.93^{a}	2.45 ± 0.30^{a}	0.45 ± 0.04^{a}	3.68	6.34 ± 0.92^{a}	2.03 ± 0.07^{b}	2.08
Me C	4.61 ± 0.02^{e}	3.83 ± 0.02^{fg}	5.25 ± 1.32^{de}	11.80 ± 1.36^{cd}	1.50 ± 0.11^{b}	0.41 ± 0.02^{abc}	2.43	3.75 ± 0.35^{b}	0.98 ± 0.02^{h}	2.56
Me P	5.07 ± 0.05^{bcd}	4.58 ± 0.21^{abc}	7.35 ± 1.62^{abcde}	16.80 ± 1.08^{ab}	1.66 ± 0.17^{ab}	0.42 ± 0.03^{ab}	2.62	3.83 ± 0.37^{b}	1.50 ± 0.03^{de}	1.70
Sa C	4.82 ± 0.04^{cde}	3.91 ± 0.01^{efg}	6.40 ± 1.69^{cde}	14.35 ± 1.18^{abcd}	1.98 ± 0.35^{ab}	0.29 ± 0.05^{bcde}	4.58	6.34 ± 0.18^{a}	1.54 ± 0.05^{d}	2.74
Sa P	5.13 ± 0.04^{bc}	4.66 ± 0.09^{ab}	11.10 ± 1.40^{a}	18.45 ± 1.59^{a}	2.16 ± 0.37^{ab}	0.32 ± 0.02^{abcde}	4.47	6.49 ± 0.26^{a}	2.27 ± 0.06^{a}	1.91
Gm C	5.03 ± 0.06^{bcd}	4.18 ± 0.05^{de}	6.35 ± 1.18^{cde}	12.50 ± 1.93^{bcd}	1.86 ± 0.18^{ab}	0.26 ± 0.08^{e}	4.80	5.81 ± 0.38^{a}	1.38 ± 0.03^{e}	2.80
Gm P	5.51 ± 0.08^{a}	4.83 ± 0.0^{a}	11.25 ± 1.76^{a}	16.65 ± 1.70^{ab}	2.18 ± 0.26^{ab}	0.31 ± 0.03^{bcde}	4.71	6.27 ± 0.93^{a}	2.06 ± 0.07^{b}	2.03
In C	4.73 ± 0.04^{de}	3.83 ± 0.03^{fg}	4.85 ± 1.75^{e}	11.85 ± 1.00^{cd}	1.80 ± 0.22^{ab}	0.28 ± 0.03^{cde}	4.32	5.08 ± 0.74^{ab}	0.74 ± 0.02^{i}	4.55
In P	5.05 ± 0.01^{bcd}	4.52 ± 0.05^{bc}	6.30 ± 1.39^{cde}	15.10 ± 1.07^{abcd}	2.09 ± 0.38^{ab}	0.30 ± 0.05^{bcde}	4.61	6.26 ± 0.54^{a}	1.54 ± 0.03^{d}	2.70
Ri C	4.59 ± 0.06^{e}	3.79 ± 0.02^{fg}	5.70 ± 1.38^{de}	11.20 ± 1.35^{d}	1.75 ± 0.12^{ab}	0.31 ± 0.02^{bcde}	3.78	4.91 ± 0.92^{ab}	0.92 ± 0.05^{h}	3.55
Ri P	5.08 ± 0.01^{bcd}	4.54 ± 0.08^{bc}	8.60 ± 1.21^{abcde}	16.35 ± 1.34^{abc}	1.97 ± 0.23^{ab}	0.39 ± 0.08^{abcde}	3.41	5.40 ± 0.61^{ab}	1.61 ± 0.06^{d}	2.23
Significance	***	***	***	***	*	***	n.d.	***	***	n.d.

Results indicate mean values ± SD of four determinations (carried out in duplicate for two different production).
Abbreviations: C samples were control production, made with wheat flour and sourdough; P samples were experimental production, made with wheat flour, *Pleutortus eryngii* powder (10% w/w) and sourdough; FQ, fermentation quotient; n.d. not determined.
Significance*:* *= $p \leq 0.05$; **= $p \leq 0.01$; ***= $p \leq 0.001$; N.S. = not significant. Significant differences among means at $p \leq 0.05$, measured by Tukey's multiple range test, are indicated by different letters within the same series (control or treated sample), with letter a denoting the highest value;

4.4.4 Bread quality and attributes

The characteristics of the breads are reported in Table 3. The final breads were characterized by a different height (22.49-32.97 mm); the highest values were registered for Me *Pleurotus* breads. Mix, Ge, and Ar breads added with PP showed a height lower that control breads. The decrease of bread volume due to the addition of mushroom powder was noticed also by other authors (Yuan et al., 2017b; Gaglio et al., 2019), but for the majority of samples, *Pleurotus* breads where characterised by a higher volume. A similar behaviour was reported by Lu et al. (2018) who tested porcini mushroom in bread production. Weight loss was affected by the type of flour used, while no statistical differences were registered between control and *Pleurotus* breads. Similar findings were reported also when the fermentation was carried out by bakers' yeast (Gaglio et al., 2019). *Pleurotus* breads showed a luminosity (L*) lower than that of control samples and differences were statistically significant both in crust and crumb. A similar trend was observed for yellowness (b*) of crust. Regarding redness (a*), crust and crumb of *Pleurotus* samples was higher than that of control samples and the differences were statistically significant. Other publications showed the same trend for all parameters (Gaglio et al., 2019) and only differences in a* of crust (Lee et al., 2009; Yuan et al., 2017b). The firmness of the breads was particularly variable among trials (10.72 and 42.79 N). In general, the addition of mushroom powders increase bread firmness (Lee et al., 2009; Yuan et al., 2017b; Gaglio et al., 2019), but our study showed a different behaviour: firmness increased in Mix and Me samples and decreased for the other trials. A decrease of firmness due to mushroom powder was observed by Salehi et al. (2016) in cake samples. Image analysis showed an overall increase of void fraction, cell density, and mean cell area due to the presence of PP. Increase of void fraction and cell density was previously reported by Gaglio et al. (2019) in breads with 10% (w/w) of PP. Only in Sa sample the presence of PP determines the reduction of void fraction and mean cell area but these differences were not statistically significant.

4.4.5 Sensory evaluation

Spider plots resulting from the sensory evaluation are reported in Fig. 1. Statistical significant differences were found for all attributes evaluated both control (Fig. 1A) and *Pleurotus* (Fig. 1B) breads. The addition of PP (Fig. 1B) impacted crumb and crust color, odor intensity, bread odor, crumb elasticity, aroma intensity, bread aroma, acid, bitter and taste persistency. Higher scores for odor and aroma intensities, crust and crumb colors, and taste persistency of *Pleurotus* breads were observed in previous surveys (Yuan et al., 2017b; Gaglio et al., 2019; Salehi, 2019). Sugar and phenolic compounds contained in PP influence

Table 3. Bread attributes.

Sample	Height (mm)	Weight loss (g)	Crust color			Crumb color			Firmness value (N)	Void fraction (%)	Cell density ($n.cm^{-2}$)	Mean cell area (mm^2)
			L*	a*	b*	L*	a*	b*				
Mix C	31,26 ± 0.48^{b}	14.75 ± 1.78^{c}	53.59 ± 3.50^{abcd}	8.36 ± 1.52^{abcd}	27.37 ± 3.31^{a}	58.64 ± 0.03^{abcd}	2.47 ± 0.23^{bcd}	16.01 ± 0.30^{cd}	14.05 ± 0.44^{j}	26.85 ± 0.62^{fg}	25.33 ± 1.76^{fg}	0.09 ± 0.01^{ab}
Mix P	28,16 ± 0.37^{cd}	14.78 ± 1.32^{c}	35.91 ± 2.52^{e}	11.31 ± 1.91^{a}	17.78 ± 2.34^{a}	46.50 ± 0.89^{f}	3.93 ± 0.24^{a}	17.67 ± 0.08^{abc}	30.97 ± 1.35^{d}	27.16 ± 0.27^{fg}	40.00 ± 1.93^{cd}	0.09 ± 0.01^{ab}
Ge C	29,85 ± 0.75^{b}	20.17 ± 0.76^{abcd}	56.24 ± 4.96^{abc}	7.12 ± 4.60^{abcd}	27.93 ± 4.95^{a}	64.33 ± 3.34^{a}	1.23 ± 0.18^{fg}	10.82 ± 0.00^{f}	37.35 ± 1.49^{b}	28.75 ± 0.84^{def}	26.37 ± 0.84^{f}	0.10 ± 0.01^{ab}
Ge P	26.71 ± 0.15^{ef}	19.97 ± 1.37^{abcd}	36.85 ± 3.33^{e}	11.61 ± 0.82^{a}	19.57 ± 3.66^{a}	57.49 ± 1.76^{bcd}	3.19 ± 0.11^{ab}	17.26 ± 0.62^{bc}	26.80 ± 0.35^{e}	30.99 ± 0.08^{bc}	45.33 ± 0.47^{b}	0.11 ± 0.01^{ab}
Ar C	25.50 ± 0.65^{f}	22.41 ± 1.09^{ab}	53.83 ± 4.74^{abcd}	8.66 ± 2.82^{abcd}	28.67 ± 1.49^{a}	63.00 ± 3.93^{ab}	1.95 ± 0.16^{def}	12.84 ± 0.13^{e}	34.78 ± 1.05^{bc}	28.72 ± 0.28^{def}	21.33 ± 2.61^{gh}	0.10 ± 0.02^{ab}
Ar P	23.61 ± 0.01^{g}	20.84 ± 0.95^{abc}	36.38 ± 3.03^{e}	10.74 ± 1.67^{ab}	19.34 ± 3.70^{a}	55.28 ± 1.43^{cde}	3.20 ± 0.33^{ab}	17.29 ± 1.85^{bc}	26.04 ± 0.62^{e}	30.67 ± 0.21^{bcd}	38.81 ± 1.85^{cd}	0.11 ± 0.01^{ab}
Me C	31.02 ± 0.05^{b}	17.94 ± 1.99^{cde}	58.49 ± 8.17^{a}	3.15 ± 1.78^{cd}	24.45 ± 5.52^{a}	62.76 ± 1.88^{ab}	1.49 ± 0.22^{efg}	11.17 ± 0.47^{ef}	10.72 ± 0.27^{j}	23.24 ± 0.72^{h}	28.59 ± 0.71^{f}	0.08 ± 0.01^{ab}
Me P	32.97 ± 0.71^{a}	16.66 ± 0.43^{de}	35.56 ± 2.95^{e}	11.99 ± 1.29^{a}	18.72 ± 3.71^{a}	53.44 ± 0.71^{de}	2.87 ± 0.07^{bc}	16.76 ± 0.23^{cd}	14.13 ± 0.43^{i}	30.16 ± 1.52^{bcd}	42.81 ± 0.81^{bc}	0.11 ± 0.02^{ab}
Sa C	22.49 ± 0.04^{g}	23.92 ± 0.52^{a}	43.82 ± 7.84^{bcde}	10.14 ± 3.57^{ab}	22.54 ± 5.58^{a}	55.21 ± 0.81^{cde}	2.72 ± 0.29^{bcd}	16.77 ± 0.30^{cd}	35.64 ± 1.33^{bc}	30.21 ± 0.42^{bcd}	28.00 ± 1.03^{f}	0.11 ± 0.02^{ab}
Sa P	23.44 ± 0.69^{g}	23.98 ± 1.60^{a}	33.39 ± 3.69^{e}	11.20 ± 2.40^{a}	16.12 ± 5.79^{a}	51.39 ± 1.73^{ef}	3.78 ± 0.66^{a}	18.95 ± 0.28^{ab}	26.63 ± 0.72^{e}	29.28 ± 0.22^{cde}	54.52 ± 1.81^{a}	0.10 ± 0.01^{ab}
Gm C	22.74 ± 0.01^{g}	20.65 ± 1.76^{abcd}	42.43 ± 5.08^{cde}	12.26 ± 2.67^{a}	23.13 ± 4.49^{a}	57.86 ± 1.49^{bcd}	2.14 ± 002^{cde}	15.26 ± 0.21^{d}	42.79 ± 1.52^{a}	27.49 ± 0.43^{efg}	41.04 ± 1.43^{bcd}	0.10 ± 0.01^{ab}
Gm P	25.88 ± 0.20^{f}	21.44 ± 1.04^{abc}	33.70 ± 1.65^{e}	10.80 ± 2.58^{ab}	14.95 ± 3.42^{a}	51.67 ± 2.61^{ef}	3.22 ± 0.48^{ab}	17.41 ± 0.42^{bc}	20.39 ± 0.79^{gh}	33.25 ± 1.33^{a}	56.15 ± 1.88^{a}	0.12 ± 0.01^{a}
In C	26.81 ± 0.40^{def}	19.41 ± 1.36^{bcd}	62.70 ± 3.25^{a}	2.17 ± 0.76^{d}	23.53 ± 6.60^{a}	58.17 ± 1.85^{bcd}	0.73 ± 0.42^{g}	10.83 ± 0.02^{f}	33.63 ± 0.82^{cd}	31.58 ± 0.79^{ab}	33.19 ± 1.73^{e}	0.11 ± 0.04^{a}
In P	28.33 ± 0.46^{c}	18.71 ± 1.16^{bcde}	41.64 ± 4.76^{de}	9.90 ± 1.36^{abc}	20.47 ± 4.75^{a}	49.86 ± 1.03^{ef}	3.84 ± 0.08^{a}	19.24 ± 0.47^{a}	23.03 ± 0.99^{fg}	33.13 ± 0.30^{a}	52.74 ± 1.49^{a}	0.12 ± 0.01^{a}
Ri C	26.62 ± 0.86^{ef}	19.41 ±1.72^{bcd}	57.47 ± 3.61^{ab}	4.17 ± 2.09^{bcd}	22.45 ± 6.24^{a}	60.56 ±1.81^{abc}	1.41 ± 0.01^{efg}	11.78 ± 0.15^{ef}	25.60 ± 1.34^{ef}	18.12 ± 0.47^{i}	17.19 ± 1.33^{h}	0.06 ± 0.01^{b}
Ri P	27.34 ± 0.24^{cde}	17.69 ±1.58^{cde}	36.21 ± 4.71^{e}	11.01 ± 0.88^{ab}	17.63 ± 4.64^{a}	50.82 ± 0.34^{ef}	3.21 ± 0.08^{ab}	16.81 ± 0.84^{cd}	18.82 ± 0.42^{h}	26.06 ± 0.15^{g}	37.78 ± 0.79^{d}	0.09 ± 0.02^{ab}
Significance	***	***	***	***	*	***	***	***	***	***	***	*

Results indicate mean values ± SD of four determinations (carried out in duplicate for two different production).
Abbreviations: C samples were control production, made with wheat flour and sourdough; P samples were experimental production, made with wheat flour, *Pleutortus eryngii* powder (10% w/w) and sourdough.
Significance: *= p≤0.05; **= p≤0.01; ***= p≤0.001; N.S. = not significant. Significant differences among means at p ≤ 0.05, measured by Tukey's multiple range test, are indicated by different letters within the same series (control or treated sample), with letter a denoting the highest value.

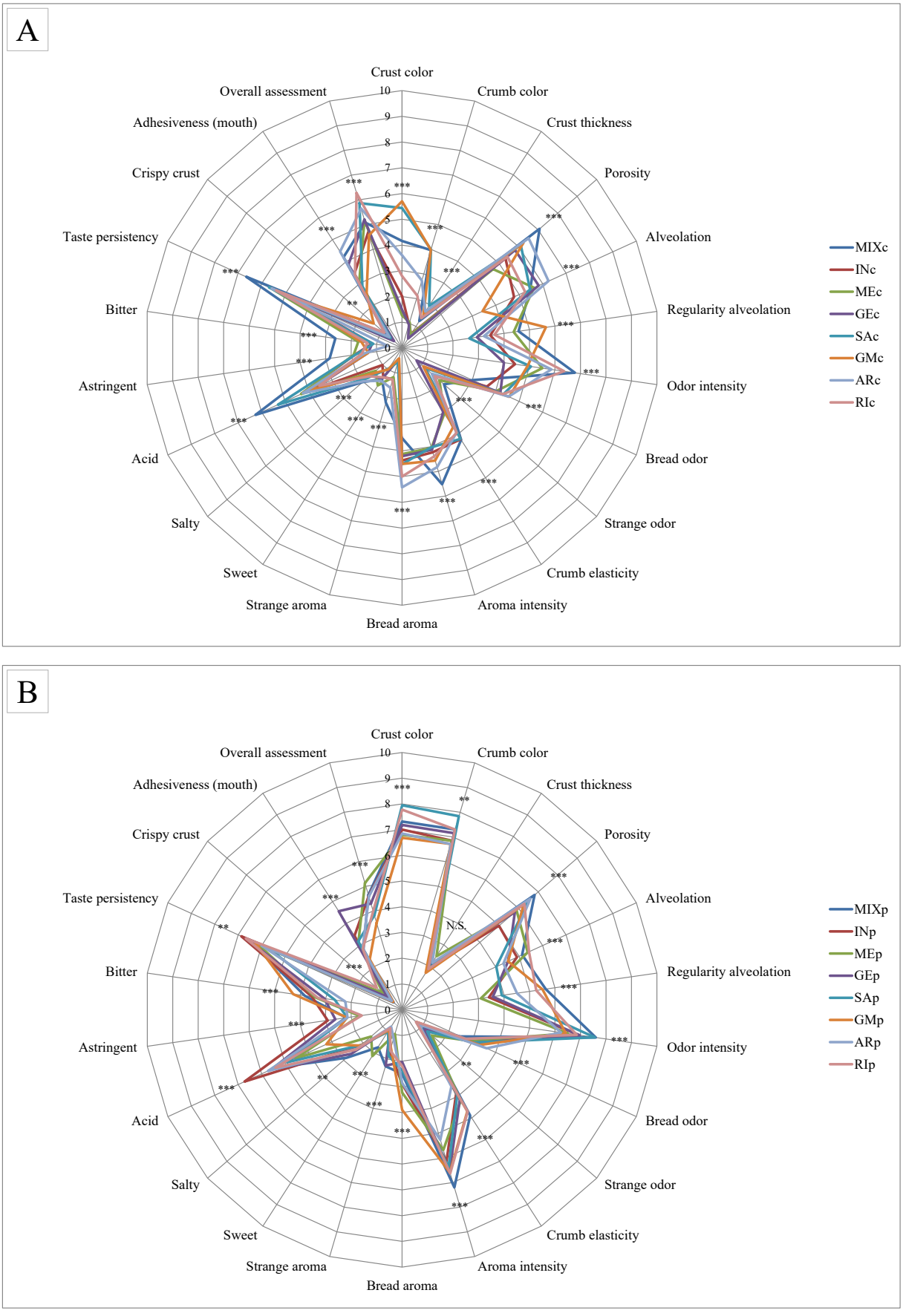

Fig. 1. Spider plots of descriptive sensorial analysis of breads. (A) C-Trial, breads made with flour. (B) P-Trial, breads made with flour + 10%(w/w) of *P. eryngii* powder.

the final color of the bread due to the chemical changes occurred during cooking (Ulziijargal et al., 2013).

On the contrary, as reported by Gaglio et al. (2019) bread odor and aroma decreased. Crust thickness, porosity, alveolation, regularity alveolation, strange odor, strange aroma, sweet, salty, astringent, crispy crust, and mouth adhesiveness were not affected by PP addition showing similar results of control breads. Results of the overall assessments showed that for the majority of *Pleurotus* breads the final scores were lower than those reached by control breads, but when Me flour was used, *Pleurotus* breads were preferred to control breads.

4.4.6 Proximate analysis, vitamin and microelements content of *Pleurotus* bread

Based on sensory tests, the breads obtained from Me flour and PP were further characterised for their chemical composition in order to investigate on their content in vitamins and microelements (Table 4). The results were compared with data of Type 1 flour bread analysis reported in online databanks of CREA "Centro di ricerca Alimenti e Nutrizione" of Italy (https://www.crea.gov.it/alimenti-e-nutrizione) and "Dipartimento Federale dell'Interno Ufficio federale della sicurezza alimentare e di veterinaria (USAV)" of Switzerland (https://valorinutritivi.ch). Type 1 flour bread is produced with flour obtained by grinding the whole grain without removing any part; thus, this flour contains also bran and wheat germ (Fiore et al., 2017). The levels of niacin (4.46 mg/100 g), thiamine (0.19 mg/100 g), and riboflavin (0.14 mg/100 g) found in *Pleurotus* breads were higher than those reported in databanks for type 1 flour bread (https://www.alimentinutrizione.it/tabelle-nutrizionali/000540). The addition of *P. eryngii*, being a source of B group vitamins (La Guardia et al., 2005) increased the final product with these substances. Gaglio et al. (2019) reported a similar result of B_2 vitamin (riboflavin), lower levels of niacin, and higher levels of thiamine (B_1). These vitamins are crucial for the enzymes involved in glucose metabolism, for the normal brain function, and for the nervous system (Ndung'u et al., 2015). Me *Pleurotus* breads contained 0.77 μg/100 g of D vitamin. This vitamin is generally absent in wheat breads Vitamin D, present in *P. eryngii* (La Guardia et al., 2005; Venturella et al., 2015), is fundamental for bone and muscles health and its supplementation may improve or prevent the occurrence of cardiovascular diseases, development of autoimmune diseases, and cancers in the colon, prostate and breast (Stroud et al., 2008). Regarding minerals, Me *Pleurotus* breads showed high contents of potassium (390 mg/100 g) and phosphorus (149 mg/100 g) followed by magnesium (36.50 mg/100 g) calcium (26.30 mg/100 g), iron (2.44 mg/100 g), zinc (1,62 mg/100 g), manganese (0.57 mg/100 g), copper (0.21 mg/100 g) and selenium (8.1 μg/100 g).

Table 4. Chemical composition of Type 1 flour bread and Mentana flour added with *P. eryngii* powder (10 % w/w) bread.

Substance	**Type1 flour bread**	**Mentana *Pleurotus* bread**	
Protein	8.3	7.89±0.48	g/100 g
Total Fats	0.3	1.19±0.079	g/100 g
Carbohydrates	52.0	42.86±0.845	g/100 g
Total Fiber	4.2	3.95±0.53	g/100 g
Insoluble Fiber	n.p.	3.16±0.47	g/100 g
Soluble Fiber	n.p.	0.79±0.34	g/100 g
Ashes	n.p.	3.86±0.23	g/100 g
Moisture	34.0	40.25±0.38	g/100 g
Dry Matter	66.0	59.75±0.38	g/100 g
Beta glucans	n.p.	220±73	mg/100 g
Total polyphenols (as catechin)	n.p.	106±11	mg/100 g
Calcium	13	26.3±2.7	mg/100 g
Iron	1.4	2.44±0.48	mg/100 g
Phosphorus	77	149±12	mg/100 g
Magnesium	38	36.5±4.3	mg/100 g
Manganese	n.p	0.57±0.13	mg/100 g
Potassium	150	390±38	mg/100 g
Copper	n.p.	0.213±0.045	mg/100 g
Sodium	680.0	615±30	mg/100 g
Zinc	1.3	1.62±0.32	mg/100 g
Chromium	n.p.	16.0±3.6	µg/100 g
Selenium	n.p.	8.1±1.8	µg/100 g
Iodine	n.p.	n.r:	mg/kg
Fluorides	n.p.	n.r.	mg/kg
Chlorides	n.p.	2.95±0.13	g/100g
Vitamin D	0	0.77±0,12	µg/100 g
Vitamin E (Alpha-Tocopherol)	0.28	0.107±0.069	mg/100 g
Vitamin B_1 (Thiamine)	0.08	0.187±0.035	mg/100 g
Vitamin B_2 (Riboflavin)	0.07	0.141±0.068	mg/100 g
Vitamin B_3 (Niacin)	0.79	44.62±11.01	mg/100 g
Vitamin B_5 (Pantothenic acid)	0.38	n.r.	mg/100 g
Vitamin B_6	0.19	n.r.	mg/100 g
Vitamin B_8 (Biotin)	n.p.	n.r.	mg/100 g
Vitamin B_9 (Folic acid and folates)	21	4.4±1,2	µg/100 g
Vitamin B_{12} (Cobalamin)	0	n.r.	mg/100 g
Vitamin A	0	n.r.	mg/100 g
Vitamin C (ascorbic acid)	0	n.r.	mg/100 g
Vitamin K_3	n.p.	n.r.	mg/100 g
Energy	239 1001	222±2 938±9	kcal/100 g KJ/100 g

Abbreviation: n.r., not releaved, value under detection limit; n.p. not present on databases. Data acquired from https://www.alimentinutrizione.it/tabelle-nutrizionali/000540 and https://valorinutritivi.ch/it/search/#/food/327856

Phosphorus and potassium are minerals present in *P. eryngii* fruiting bodies (Sardar et al., 2017). They have an essential role in bone health due to the effect on processes that support calcium regulation (Heaney, 2015). In particular, phosphorus is fundamental in bone growth and preservation of calcium loss (Haney, 2004).

Me *Pleurotus* breads showed a total polyphenol content of 106 mg/100 g (as catechin). An increase in bread polyphenols is performed also by fortification with legumes (Angioloni and Collar, 2012). These metabolites exert antioxidants effects absorbing free radicals, inhibiting peroxidase, and performing an oxygen scavenging activity (Keleș et al., 2011; Venturella et al., 2021). Betaglucans showed a concentration of 220 mg/100 g. These bioactive compounds play an important role in human health through the activation of different immune cells expression, the enhancement of resistance to different infections caused by bacteria, viruses, fungi and parasites, the modulation of cholesterol and glucose blood levels (Manzi and Pizzoferrato, 2000; Cheung, 2008; Venturella et al., 2021).

4.5 Conclusions

The focus of this survey was the production of functional breads by utilization of different types of flours, through addiction of 10% (w/w) of PP and fermentation were carried out with sourdough. The supplementation did not cause alteration of microbiological parameters but interferes with the chemical parameters acting as a buffer on pH, increasing TTA and consequently rising of quantity organic acids. Regarding bread attributes hardness and heigh values showed different results, while crust's redness and cell density of crumb rise. Sensory evaluation that the presence of PP in bread cause variation of few parameters as color, odor, and aroma intensity. As expected there has been an increase in certain vitamins (B_1, B_2, B_3, and D), total polyphenols, and beta-glucans content due to PP addition. Evaluated the beneficial effects on human health and the sought-after peculiar organoleptic characteristics of *P. eryngii*, bread produced with type 1 flour of the ancient wheat variety "Mentana" enriched with PP, given its technological and nutritional characteristics, is eligible among fortified products of commercial interest also in order to amplify the number of fortified products, targeted to different consumers with special nutritional requests like lactose-intolerant people or for ethical reasons like vegans.

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Chapter 5. Technological and Organoleptic Parameters of Craft Beer Fortified with Powder of the Culinary–Medicinal Mushroom *Pleurotus eryngii*

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5.1 Abstract

Beer is one of the oldest and most popular alcoholic beverages and is currently consumed worldwide. The various components used in the brewing process have a physiological impact on the consumer and current research aims to improve its technological and functional properties through the addition of natural compounds (plants or mushrooms). In this work, the addition of two different amounts (5 and 10 g/L) of *Pleurotus eryngii* var. *eryngii* in powder form added at different production stages (PRE and POST alcoholic fermentation) showed the improvement in yeast viability during the alcoholic fermentation, increased the alcoholic content, and improved the sensorial profile. Regarding the organoleptic profile in the experimental samples, cocoa/chocolate and mushroom aromas were found and the samples PRE10 and POST5 received the best ratings with respect to all evaluated parameters.

5.2 Introduction

Beer is one of the world's oldest and most popular alcoholic beverages and is currently consumed all over the world [1]. The Sumerians reported the first production of a beer-like drink around 6000 years ago [2]. The beer making process starts with the wort preparation which is made from raw materials which provide sugar as glucose and maltose to yeasts which perform the alcoholic fermentation (AF) [3]. Barley malt is mainly used for preparation but according to the recipe, other cereals can be added as wheat (raw or malted), corn, oat, and rice [4]. Beer can be considered a nutritionally valuable product because the various components used in the manufacturing process have physiological effects on consumption. It is characterized by a high carbohydrate content as well as the presence of protein, amino acids, vitamins, organic acids, microelements, and antioxidants [5]. However, despite being the most widely consumed alcoholic beverage in the world, beer is the subject of constant research focused on improving its technological aspects [6,7], raw material [8], and on the development of non-conventional beers [9].

Craft breweries have traditionally added fruits and spices to the brewing process to enhance the flavor and aroma of various beer styles [10]. Functional beers are unconventional beers that try to combine moderate alcoholic beverage consumption with health benefits [11]. This

aim is in line with the emerging trend in the functional food market which attracts customers based on well-established trends in today's society of disease prevention through a functional diet [12].

In recent years, the food industry's efforts have focused on adapting to consumer trends toward products with functional characteristics that can improve and prevent diet-related diseases [13] by adding naturally derived components or extracts to conventional products [14–16]. Recently, there has been much interest in the addition of food- and medicinal-interest mushroom powders and extracts into foods that are commonly consumed [16–19]. Mushrooms are known not only for their high nutritional value but also because they possess peculiar flavor, aroma, and aromatic compounds [20]. Various molecules contribute to the aroma composition of mushrooms, the most important among them being terpenoids that are used in food and cosmetics industries [21]. In particular, over the past decade, we have focused our attention toward the *Pleurotus eryngii* species complex which comprises high-quality edible mushrooms that grow on the roots of various Apiaceae plants [22]. Recent investigations based on the evaluation of morphological and ecological characters combined with molecular analysis have provided a new explanation for this critical taxonomic group. The largest cluster (*P. eryngii* s. str.) was subdivided into taxa at the variety level: *P. eryngii* (DC.) Quél var. *eryngii*, *P. eryngii* var. *ferulae* Lanzi, *P. eryngii* var. *thapsiae* Venturella, Zervakis and Saitta and *P. eryngii* var. *elaeoselini* Venturella, and Zervakis and La Rocca [23]. In this paper, we analyzed the influence that the addition of powdered *P. eryngii* var. *eryngii* (PEP), an edible and medicinal mushroom, brings on the physical, chemical, and sensory characteristics of craft beer. Two different amounts of powder were added (5 g/L and 10 g/L) in two different stages of production (before and at the end of AF) as reported in flow charts (Figure 1 and Figure 2). The main hypothesis of this research was to develop an innovative product with distinctive sensory characteristics. Based on our knowledge, this is the first work reporting the use of the mushroom *P. eryngii* var. *eryngii* in the brewing process of craft beer.

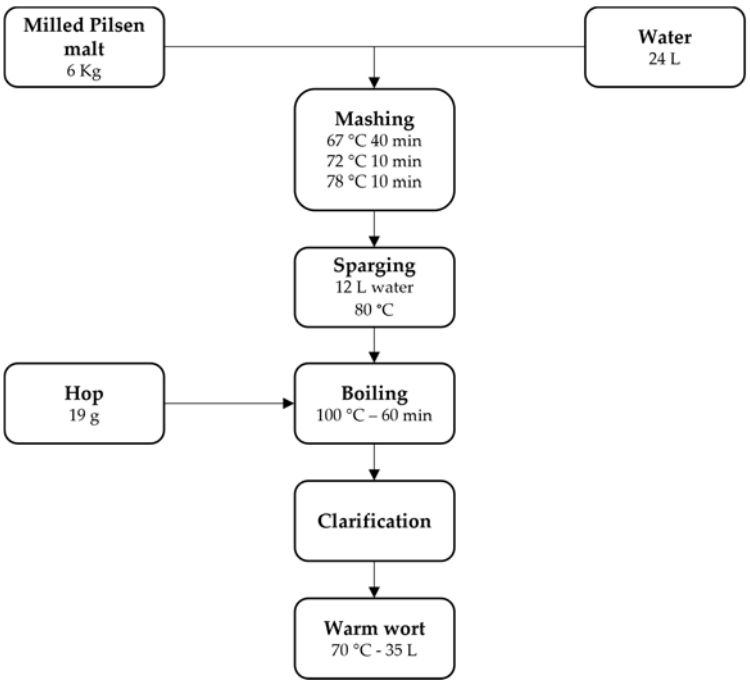

Figure 1. Wort production flow chart.

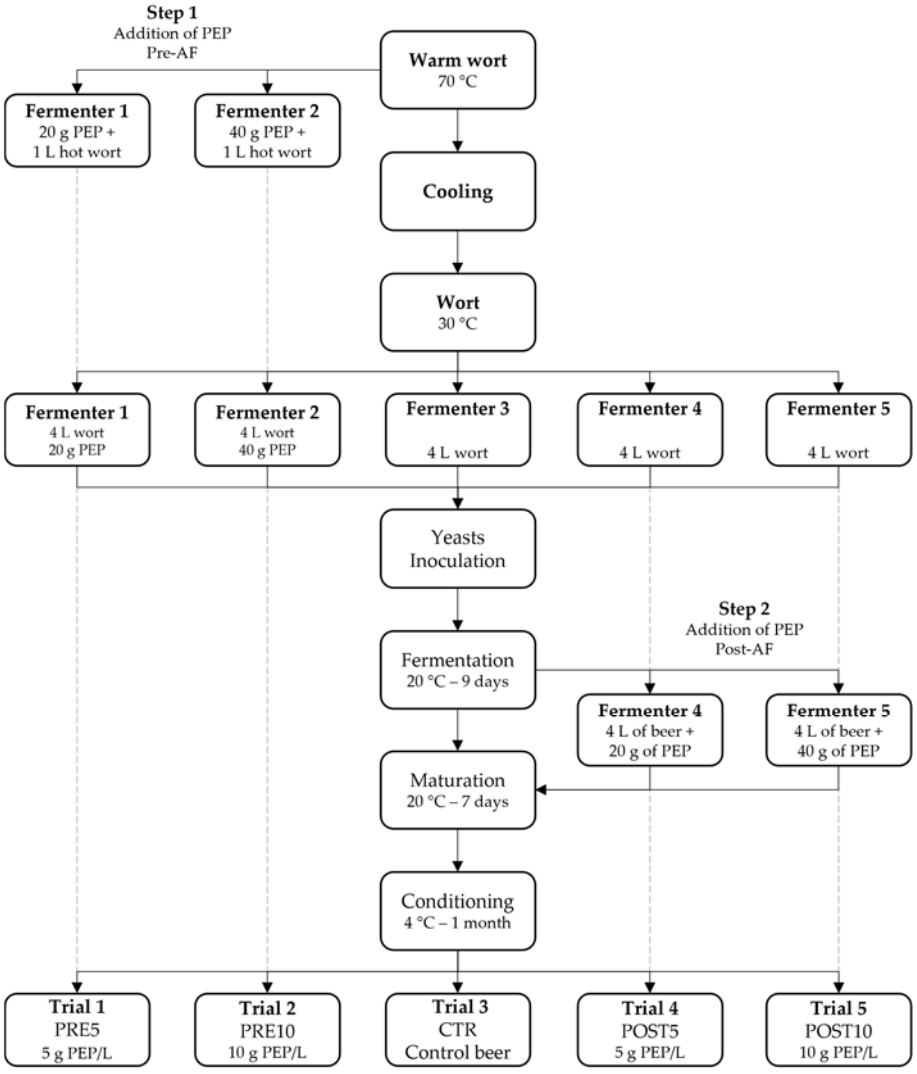

Figure 2. Experimental trial production flow chart.

5.3 Materials and Methods

5.3.1 Mushroom Material

P. eryngii var. *eryngii* basidiomes were collected in October 2022 in Basilicata (southern Italy) on grasslands via root residues of *Eryngium campestre* L. The collected samples were maintained in the laboratory under refrigerated conditions (4 °C) and they were subsequently identified and morphologically described. The herbarium samples are kept in the Herbarium of the Department of Agricultural and Forest Sciences of the University of Palermo (SAF). Under a laminar flow hood, a piece of flesh of basidioma was put, aseptically, in a Petri dish which contains potato dextrose agar (PDA) medium and incubated for 7 days at 26 ± 1 °C. After subsequent purification steps, the strain was stored in the Mycotheca of the Department (SAF). The strain number is C-143 and similar in productivity to the previously tested C-142 of similar geographical origin [24].

5.3.2 Substrate Preparation and Mushroom Cultivation

A piece of purified mycelium of *P. eryngii* var *eryngii* was used for spawn preparation by inoculation of wheat seeds that had been previously soaked in distilled water, placed in 1 L jars, and sterilized at 121 °C for 20 min. The substrate was made from wheat straw and wheat bran, moistened, mixed, and then transferred in heat-resistant polypropylene bags model XLS-T (Unicorn bags, Texas, USA) equipped with a filter (cut-off 0.2 µm). Each bag, weighing 4 kg, was sterilized at 121 °C for 1 h and, after cooling, was inoculated with spawn under aseptic conditions. The inoculated bags were sealed with manual impulse bag sealer (Tecnopack corporation, Sunrise, Florida, USA) and placed in an heratherm IGS60 incubator (Thermo Fisher Scientific, Waltham, Massachusetts, USA) at 26 ± 1 °C in dark conditions. After 60–80 days, the mycelium had completely colonized the substrate and was considered ready for basidiomata production that was carried out in a department greenhouse. After collection, the basidiomes were sliced and dried by using a stainless steel Valla air drier (Borgotaro, Parma, Italy), pulverized with Vorwerk Bimby® blender (Vorwerk and Co. KG, Wuppertal, Germany), and stored at 4 °C in vacuum-sealed bags until required.

5.3.3 Brewing Raw Materials and Beer Production

The traditional brewing process involves the use of water, barley malt, hops, and yeast. Brewing was performed in the pilot plant of the Agricultural and Forest Sciences of the University of Palermo (Italy) by using an “all-in-one” microbrewery plant Klarstein model 10,031,629 (Chal-Tec GmbH, Berlin, Germany), as reported in Figure 1. In total, 6 kilograms of Pilsen malt (BestMalz, Heidelberg, Germany) was ground using a two-roller mill (Brouwland, Beverlo, Belgium) and soaked in 24 L of water in which the pH had

previously been corrected by the addition of 6 g of $CaSO_4$ and 6 g of $CaCl_2$ as reported by Marconi et al. [25]. The mash was heated to 67 °C for 40 min to perform single-phase mash until complete sugar conversion, which was verified with an iodine solution test. Subsequently, the mixture was heated up for 10 min at 72 °C and 10 min at 78 °C. The cereal grains were washed (sparged) with 12 L H_2O (80 °C) for a total volume of 36 L. The wort was then boiled for 60 min during which time 19 g of Hallertau Magnum hop pellets (Mr. Malt®, Pasian di Prato, Italy) were added to obtain a final concentration of 25 IBU (international bitter units). The final volume after boiling was 35 L with 10 °Bx (Brix degree). The wort was then clarified in a whirlpool for 10 min of recirculation and 10 min of rest [25] until it reached 70 °C. The produced wort showed the following characteristics: 5.18 pH, 10 °Bx, 1048 SG (Specific Gravity), 8.756 g/L of D-glucose, 0.657 g/L of D-fructose, 19.231 g/L of sucrose, and 33.167 g/L of maltose.

An aliquot of 70 °C of warmed wort was put into 5 L fermenters (1 L for each) containing 20 g and 40 g of PEP, respectively, to break down the bacterial load present in the raw material and avoid undesired fermentation. After wort cooling, the fermenters were filled to 4 L to reach the PEP concentration of 5 g/L (PRE5) and 10 g/L (PRE10), respectively. A total of 5 fermenters filled with 4 L of wort were prepared and inoculated as reported in flow chart (Figure 2).

Each fermenter was inoculated with a commercial strain of *Saccharomyces cerevisiae* SafAle™ US-05 (Fermentis, Lesaffre, France) at approximately 2 * 10^6 CFU/mL [26] and incubated at 20 °C. At the end of the AF (day 9), the last 2 trials by addition of 20 g and 40 g of PEP corresponding to concentration of 5 g/L (POST5 sample) and 10 g/L (POST10 sample) were prepared. All trials were matured an additional 7 days after the end of AF (day 16) before being conditioned and bottled as described by Matraxia et al. [9]. All trials were produced in duplicate at two different times.

5.3.4 Sampling and Monitoring of Alcoholic Fermentation

The samples were taken at several points during the brewing process including wort, after the yeast strains inoculation (day 0), during the AF (day 2–day 9), during maturation (day 12–day 16), and after bottle conditioning. Each sample analysis was carried out in triplicate no later than 24 h after collection. The monitoring of yeast loads was carried out on Wallerstein Laboratory (WL) nutrient agar medium (Oxoid, Basingstoke, UK) and incubated at 25 °C for 48 h in aerobic conditions. All samples were serially diluted (1:10 ratio) in Ringer's solution before being spread onto plates.

5.3.5 Physico-Chemical Parameters of Worts and Beers

All samples were subjected to pH measurement which was analyzed by a pH70 vio FOOD (XS Instruments, Carpi, Italy) pH meter while a DBR salt (Zetalab srl, Padova, Italy) refractometer was used to determine the Brix degree value. The determination of sugars (D-glucose, D-fructose, sucrose, and maltose), acetic acid, and glycerol of wort were performed by enzymatic determination as described by Matraxia et al. [9]; all chemicals and standards were bought from R-Biopharm AG (Darmstadt, Germany) and to respect the calibration curve of analyzer iCubio iMagic M9 (Shenzhen iCubio Biomedical Technology Co. Ltd. Shenzhen, China), appropriate sample dilution was carried out.

Real extract, wort extract, apparent extract, alcohol, real degree of attenuation, energy, specific gravity, density, and pH of beers after conditioning were determined by BeerFoss™ FT Go (FOSS A/S, Hillerød, Denmark).

The beer's color was determined by spectrophotometry according to method 8.5 of the Analytica European Brewery Convention [27]. The beer samples were degassed in an ultrasonic bath at room temperature for 5 min and filtered through a syringe filter (0,45 μm, PVDF). The samples were diluted until the absorbance value was less than 0.8. The absorbance of beer sample was measured at wavelengths of 430 nm in a 10 mm cuvette. The value in EBC units was obtained by multiplying the absorbance by an appropriate factor.

5.3.6 Sensory Evaluation

The sensory evaluation of experimental beers was performed in a tasting room and in blind tasting conditions by twelve judges (from 27 to 46 years old) with backgrounds on sensory analysis, recruited at SAAF Department of the University of Palermo, and included a quantitative descriptive analysis to determine the color, odor, taste, and overall quality.

The sensory analysis of beers was conducted following the methodology reported by Matraxia et al. [9]. The panelists have evaluated 35 descriptive attributes related to aspect (color intensity and opacity), odor (intensity, complexity, fruity, floral, mushroom, cocoa, hoppy, malty/grainy, honey/caramel, acetic, oxidized/aged, sulphury, alcohol, DMS, and brine), taste (intensity, complexity, sweet, bitter, sour, astringent, fruity, mushroom, cocoa, spicy, hoppy, salty malty/grainy, roasted/burnt, body, oxidized/aged, DMS, and brine), and overall characteristics (visual, odor, taste, and overall satisfaction). Panelists also developed a consensus descriptive form for the evaluation of experimental beers, in which adjectives were paired with an unstructured 10-cm scale with the phrases "none/weak" and "strong" anchored at the left and right extremities, respectively [28].

The samples of beers (about 50 mL each) were provided at 16 °C in tasting glasses that respect the standard ISO type labelled with random codes. Between beers, water was

available for rinsing. Each examination was made in a separate booth between 10:00 and 12:00 a.m. [29]. The results were calculated using the appropriate statistical analysis as the mean of three evaluations.

5.3.7 Statistical Analyses

Microbiological, physicochemical, and sensorial data were tested for differences using the one-way analysis of variance (ANOVA; general linear model) followed by the *post hoc* Tukey's multiple range test applied for pairwise comparison. Statistical significance differences between samples was attributed for $p \leq 0.05$ using XLStat® add-in ver. 2014.5.03 (Addinsoft) for Microsoft Excel®.

5.4 Results and Discussion

5.4.1 Monitoring of Alcoholic Fermentation

The yeast loads evaluated during the different steps of the AF were reported in Figure 3. Before inoculums into wort, the yeasts were under the detection limit. Following the yeast addition, the loads ranged between 6.65 and 6.74 Log CFU/mL. After 2 days of fermentation, the yeast load increased by 0.55 and 0.54 log cycles in PRE5 and PRE10, respectively, (where PEP was already added) and between 0.34–0.39 log cycles in other trials but no statistical differences were found. In general, after 2 days of AF, yeast loads reached similar levels (7.08–7.24 Log CFU/mL) to those reported by Pirrone et al. [10]. Between day 5 and day 9 of AF, the differences between the samples with and without PEP were most striking and statistically significant with higher values of yeast loads in PRE5 and PRE10 trials. In particular, yeast loads were constant in PRE5 and PRE10 samples (between 6.95 and 6.81 Log CFU/mL) while they decreased (from approx. 6.42 to approx. 5.55 Log CFU/mL) in POST5, POST10, and CTR samples in which PEP was not present. After day 9 sampling (end of AF), PEP was added in the POST5 and POST10 samples, as reported in MM. The yeast loads in samples POST5 and POST10 reached values (6.78 and 6.88 Log CFU/mL, respectively) similar to those found in samples PRE5 and PRE10 (6.79 and 6.81 Log CFU/mL, respectively) on day 12. This enhanced growth may be attributed to carbohydrates present in the fruiting body of *P. eryngii* [21,30] that may be available to yeast. A similar trend was reported in papers in which the addition of fruit juice causes increased yeast growth during fermentation [10,31,32]. The number of yeast cells was stable at the following sampling times (day 14 and day 16) in both PRE and POST samples and gradually decreased from 5.56 Log CFU/mL (day 12) to a value of 5.18 Log CFU/mL (day 16) in CTR sample.

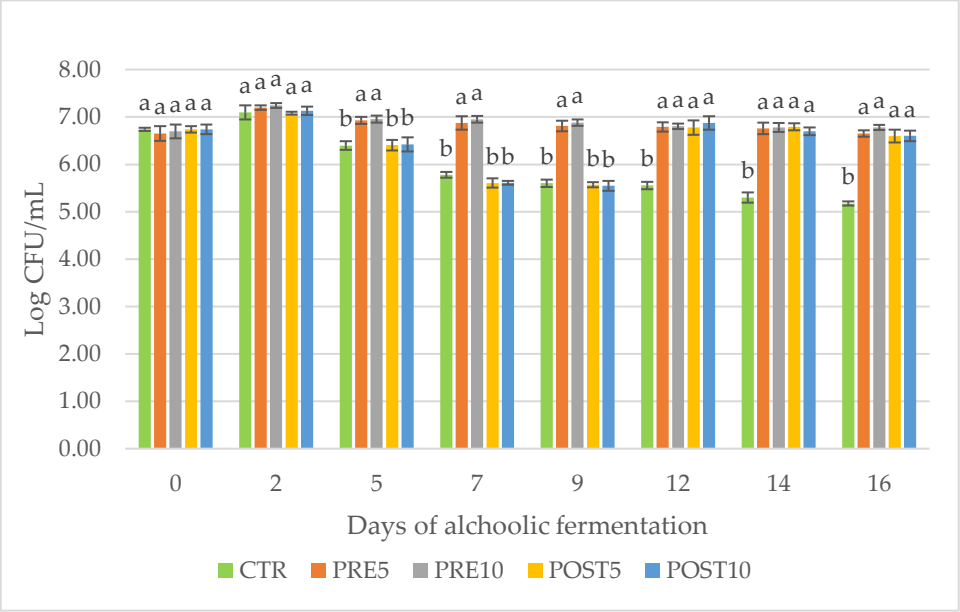

Figure 3. Monitoring of yeast loads during fermentation. CTR, wort without mushroom powder; PRE5, wort with 5 g/L of mushroom powder added before fermentation; PRE10, wort with 10 g/L of mushroom powder added before fermentation; POST5, wort with 5 g/L of mushroom powder added after fermentation; POST10, wort with 10 g/L of mushroom powder added after fermentation. Different superscript letters indicate that significant differences on yest load were displayed at each sampling time according to Tukey's test for $p < 0.05$.

5.4.2 Evolution of Chemical Parameters during Wort Alcoholic Fermentation

The chemical analysis of wort during AF showed differences between trials in D-fructose, D-sucrose, D-glucose, D-maltose, acetic acid, and glycerol contents (Table 1). At the beginning of AF (day 0), the samples that contained PEP (PRE5 and PRE10) showed a higher significant content of D-glucose, D-maltose, acetic acid, and glycerol than the samples without PEP. Regarding D-glucose, PRE5 and PRE10 were, respectively, 8.935 and 8.926 g/L significantly higher than that detected in samples without PEP, which ranged between 8.756 and 8.765 g/L. A progressive decrease in D-glucose content was recorded in the CTR, PRE5, and PRE10 samples from day 2 to the end of monitoring while in the POST5 and POST10 samples this occurred until day 9 when PEP was added. In the day 12 sample, the glucose content recorded was 0.270 g/L in POST5 and 0.492 g/L in POST10 samples. At the end of monitoring (day 16), the residual glucose content was 0.034 g/L in the CTR, 0.045 in PRE5, and 0.057 g/L in PRE10 while higher values were found in the POST5 and POST10 samples (0.224 g/L and 0.473 g/L, respectively). Similar behavior was observed in relation to the sucrose content which, at the end of monitoring, showed a content of 0.386 g/L in the POST5 sample and 0.830 g/L in the POST10 sample. Mushrooms are known to be a rich source of nutrients [33]; mushrooms belonging to the genus *Pleurotus* are

particularly characterized by a high content of sugars, which can be more than 65% of dry weight [34,35].

Glycerol was absent in the first two sampling days in the samples where PEP is not present (CTR and POST samples) while it was detected at a concentration of 0.04 g/L in both PRE samples at day 0 and at day 2 and this reached 0.870 and 0.886 g/L (PRE5 and PRE10, respectively). From day 5 to the end of sampling, no significant differences were found between all samples and the detected quantity ranged between 0.850–0.873 g/L. In beers, glycerol is generally between 1–3 g/L [4] and in fermented alcoholic beverages it is the main component of the body attribute [36] but it also enhances flavor intensity and has an influence on aroma volatility [37,38]. Acetic acid is one of the organic acids that yeast can produce during the brewing process and can affect the organoleptic characteristics of the products [39]. Although significant differences were found in the acetic acid content of different trials, the value is significantly lower than that found by other authors [10,40]. The low percentage of acetic acid is interesting from a sensory point of view as acetic acid is commonly blamed for an unpleasant taste in beers, especially a sour vinegary flavor [40].

Table 1. Evolution of chemical parameters of wort during alcoholic fermentation.

	CTR	PRE5	PRE10	POST5	POST10	S.S.
D-fructose (g/L)						
Day 0	0.657 ± 0.006 ^{a}	0.637 ± 0.038 ^{a}	0.644 ± 0.027 ^{a}	0.658 ± 0.006 ^{a}	0.656 ± 0.006 ^{a}	N.S.
Day 2	0.135 ± 0.004 ^{a}	0.087 ± 0.001 ^{b}	0.079 ± 0.003 ^{b}	0.137 ± 0.004 ^{a}	0.136 ± 0.004 ^{a}	***
Day 5	0.018 ± 0.005 ^{a}	0.023 ± 0.002 ^{a}	0.022 ± 0.002 ^{a}	0.018 ± 0.004 ^{a}	0.018 ± 0.003 ^{a}	N.S.
Day 7	0.032 ± 0.003 ^{a}	0.022 ± 0.001 ^{b}	0.023 ± 0.003 ^{b}	0.03 ± 0.003 ^{ab}	0.033 ± 0.003 ^{a}	*
Day 9	0.018 ± 0.003 ^{a}	0.007 ± 0.001 ^{b}	0.003 ± 0.001 ^{b}	0.018 ± 0.003 ^{a}	0.018 ± 0.003 ^{a}	***
Day 12	0.008 ± 0.001 ^{c}	0.022 ± 0.002 ^{a}	0.018 ± 0.004 ^{ab}	0.022 ± 0.003 ^{a}	0.011 ± 0.002 ^{bc}	**
Day 14	0.019 ± 0.004 ^{a}	0.015 ± 0.005 ^{ab}	0.011 ± 0.003 ^{abc}	0.009 ± 0.001 ^{bc}	0.005 ± 0.001 ^{c}	**
Day 16	0.022 ± 0.004 ^{a}	0.013 ± 0.004 ^{b}	0.010 ± 0.005 ^{bc}	0.006 ± 0.001 ^{bc}	0.002 ± 0.001 ^{c}	**
D-sucrose (g/L)						
Day 0	19.231 ± 0.149 ^{a}	19.066 ± 0.140 ^{a}	19.368 ± 0.198 ^{a}	19.380 ± 0.149 ^{a}	19.235 ± 0.149 ^{a}	N.S.
Day 2	0.250 ± 0.003 ^{b}	0.354 ± 0.027 ^{a}	0.225 ± 0.017 ^{b}	0.255 ± 0.003 ^{b}	0.258 ± 0.003 ^{b}	***
Day 5	0.067 ± 0.004 ^{c}	0.088 ± 0.005 ^{b}	0.128 ± 0.004 ^{a}	0.070 ± 0.004 ^{c}	0.066 ± 0.004 ^{c}	***
Day 7	0.058 ± 0.003 ^{b}	0.101 ± 0.002 ^{ab}	0.131 ± 0.037 ^{a}	0.060 ± 0.003 ^{b}	0.062 ± 0.003 ^{b}	***
Day 9	0.080 ± 0.001 ^{b}	0.116 ± 0.005 ^{a}	0.122 ± 0.028 ^{a}	0.080 ± 0.004 ^{b}	0.080 ± 0.004 ^{b}	**
Day 12	0.058 ± 0.003 ^{c}	0.086 ± 0.004 ^{c}	0.101 ± 0.005 ^{c}	0.386 ± 0.003 ^{b}	0.879 ± 0.042 ^{a}	***
Day 14	0.052 ± 0.003 ^{c}	0.070 ± 0.005 ^{c}	0.101 ± 0.004 ^{c}	0.479 ± 0.039 ^{b}	0.884 ± 0.034 ^{b}	***
Day 16	0.054 ± 0.004 ^{c}	0.075 ± 0.002 ^{c}	0.092 ± 0.004 ^{c}	0.458 ± 0.047 ^{b}	0.830 ± 0.020 ^{a}	***
D-glucose (g/L)						
Day 0	8.756 ± 0.028 ^{b}	8.935 ± 0.030 ^{a}	8.926 ± 0.027 ^{a}	8.760 ± 0.028 ^{b}	8.765 ± 0.027 ^{b}	***
Day 2	0.106 ± 0.002 ^{a}	0.120 ± 0.002 ^{a}	0.122 ± 0.016 ^{a}	0.106 ± 0.005 ^{a}	0.106 ± 0.004 ^{a}	N.S.
Day 5	0.043 ± 0.005 ^{c}	0.054 ± 0.003 ^{b}	0.076 ± 0.002 ^{a}	0.044 ± 0.005 ^{bc}	0.045 ± 0.005 ^{bc}	***
Day 7	0.034 ± 0.002 ^{c}	0.060 ± 0.003 ^{b}	0.077 ± 0.001 ^{a}	0.037 ± 0.002 ^{c}	0.036 ± 0.002 ^{c}	***

Day 9	0.069 ± 0.002 ^{b}	0.083 ± 0.001 ^{a}	0.089 ± 0.004 ^{a}	0.071 ± 0.002 ^{b}	0.067 ± 0.002 ^{b}	***
Day 12	0.037 ± 0.004 ^{c}	0.056 ± 0.001 ^{b}	0.063 ± 0.001 ^{a}	0.224 ± 0.017 ^{c}	0.493 ± 0.043 ^{c}	***
Day 14	0.033 ± 0.003 ^{c}	0.044 ± 0.003 ^{b}	0.059 ± 0.003 ^{a}	0.272 ± 0.022 ^{c}	0.505 ± 0.048 ^{c}	***
Day 16	0.034 ± 0.003 ^{c}	0.045 ± 0.002 ^{b}	0.057 ± 0.001 ^{a}	0.270 ± 0.017 ^{c}	0.473 ± 0.033 ^{c}	***
Maltose (g/L)						
Day 0	33.167 ± 0.123 ^{b}	34.478 ± 0.109 ^{a}	34.673 ± 0.153 ^{a}	33.27 ± 0.123 ^{b}	33.220 ± 0.123 ^{b}	***
Day 2	7.212 ± 0.169 ^{a}	6.402 ± 0.214 ^{b}	6.231 ± 0.226 ^{b}	7.120 ± 0.186 ^{a}	7.220 ± 0.269 ^{a}	**
Day 5	0.615 ± 0.047 ^{a}	0.672 ± 0.042 ^{a}	0.599 ± 0.002 ^{a}	0.615 ± 0.073 ^{a}	0.615 ± 0.080 ^{a}	N.S.
Day 7	0.516 ± 0.042 ^{b}	0.732 ± 0.027 ^{a}	0.659 ± 0.035 ^{a}	0.518 ± 0.042 ^{b}	0.516 ± 0.022 ^{b}	***
Day 9	0.526 ± 0.018 ^{ab}	0.435 ± 0.024 ^{b}	0.534 ± 0.047 ^{a}	0.526 ± 0.038 ^{ab}	0.536 ± 0.045 ^{a}	**
Day 12	0.349 ± 0.043 ^{b}	0.328 ± 0.045 ^{b}	0.510 ± 0.045 ^{a}	0.456 ± 0.015 ^{a}	0.415 ± 0.031 ^{ab}	**
Day 14	0.344 ± 0.016 ^{c}	0.325 ± 0.009 ^{c}	0.508 ± 0.020 ^{a}	0.438 ± 0.040 ^{b}	0.418 ± 0.023 ^{b}	***
Day 16	0.215 ± 0.036 ^{b}	0.224 ± 0.030 ^{b}	0.410 ± 0.035 ^{a}	0.401 ± 0.033 ^{a}	0.415 ± 0.031 ^{a}	***
Acetic acid (g/L)						
Day 0	0 ^{c}	0.006 ± 0.001 ^{b}	0.008 ± 0.001 ^{a}	0 ^{c}	0 ^{c}	***
Day 2	0 ^{c}	0.028 ± 0.005 ^{b}	0.101 ± 0.001 ^{a}	0 ^{c}	0 ^{c}	***
Day 5	0 ^{c}	0.005 ± 0.003 ^{b}	0.053 ± 0.002 ^{a}	0 ^{c}	0 ^{c}	***
Day 7	0 ^{c}	0.021 ± 0.005 ^{b}	0.035 ± 0.003 ^{a}	0 ^{c}	0 ^{c}	***
Day 9	0.010 ± 0.005 ^{b}	0.017 ± 0.004 ^{ab}	0.028 ± 0.001 ^{a}	0.011 ± 0.005 ^{b}	0.012 ± 0.005 ^{b}	**
Day 12	0.013 ± 0.003 ^{c}	0.035 ± 0.002 ^{b}	0.044 ± 0.003 ^{ab}	0.021 ± 0.005 ^{c}	0.049 ± 0.005 ^{a}	***
Day 14	0.022 ± 0.005 ^{c}	0.033 ± 0.002 ^{b}	0.063 ± 0.002 ^{a}	0.023 ± 0.004 ^{c}	0.030 ± 0.002 ^{bc}	***
Day 16	0.025 ± 0.005 ^{c}	0.036 ± 0.002 ^{b}	0.063 ± 0.002 ^{a}	0.023 ± 0.004 ^{c}	0.026 ± 0.001 ^{c}	***
Glycerol (g/L)						
Day 0	0 ^{b}	0.040 ± 0.002 ^{a}	0.041 ± 0.003 ^{a}	0 ^{b}	0 ^{b}	***
Day 2	0 ^{b}	0.870 ± 0.033 ^{a}	0.886 ± 0.013 ^{a}	0 ^{b}	0 ^{b}	***
Day 5	0.863 ± 0.044 ^{a}	0.857 ± 0.027 ^{a}	0.895 ± 0.026 ^{a}	0.865 ± 0.044 ^{a}	0.866 ± 0.044 ^{a}	N.S.
Day 7	0.856 ± 0.015 ^{a}	0.850 ± 0.009 ^{a}	0.865 ± 0.041 ^{a}	0.855 ± 0.015 ^{a}	0.858 ± 0.015 ^{a}	N.S.
Day 9	0.849 ± 0.024 ^{a}	0.862 ± 0.036 ^{a}	0.855 ± 0.005 ^{a}	0.852 ± 0.024 ^{a}	0.850 ± 0.024 ^{a}	N.S.
Day 12	0.858 ± 0.04 ^{a}	0.862 ± 0.027 ^{a}	0.860 ± 0.046 ^{a}	0.854 ± 0.028 ^{a}	0.884 ± 0.039 ^{a}	N.S.
Day 14	0.857 ± 0.022 ^{a}	0.859 ± 0.050 ^{a}	0.865 ± 0.040 ^{a}	0.867 ± 0.041 ^{a}	0.862 ± 0.011 ^{a}	N.S.
Day 16	0.856 ± 0.033 ^{a}	0.853 ± 0.005 ^{a}	0.867 ± 0.017 ^{a}	0.850 ± 0.032 ^{a}	0.873 ± 0.015 ^{a}	N.S.

Values are expressed as the average of three measurements. Abbreviations: S.S., statistical significance. wort samples: CTR, wort without mushroom powder; PRE5, wort with 5 g/L of mushroom powder added before fermentation; PRE10, wort with 10 g/L of mushroom powder added before fermentation; POST5, wort with 5 g/L of mushroom powder added after fermentation; POST10, wort with 10 g/L of mushroom powder added after fermentation. Data within a line followed by the same letter are not significantly different according to Tukey's test. Symbols: ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; N.S., not significant.

5.4.3 Physicochemical Properties of Beers

The main physicochemical properties of beers after bottling are reported in Table 2. All beers with the addition of PEP showed significantly higher alcohol content than the control beer. This is due to the sugars contained in PEP that could be used by the yeast during fermentation [41,42]. Similarly, the pH and color value in the beers containing PEP was significantly higher than in the CTR beer with the highest values in the samples containing 10 g/L PEP

(PRE10 and POST10). All the experimental samples showed higher wort extract values than control beer and, as reported by Carvalho et al. [31], consequently higher alcohol content. Other authors have noted similar characteristics following the addition of vegetal material in beers. Gasiński et al. [43] found an increased alcohol content following the addition of mangoes in beer while Xu et al. [44] observed an increment of alcohol content, pH, and color value following the addition of fresh or dried okra.

Table 2. Physicochemical properties of beers.

	CTR	PRE5	PRE10	POST5	POST10
Wort Extract (*w*/*w*)	11.42 ± 0.27 ^{b}	11.59 ± 0.22 ^{b}	11.81 ± 0.28 ^{ab}	12.09 ± 0.34 ^{ab}	12.47 ± 0.38 ^{a}
Real Extract (*w*/*w*)	5.15 ± 0.03 ^{b}	5.21 ± 0.03 ^{b}	5.34 ± 0.04 ^{a}	4.67 ± 0.04 ^{c}	4.33 ± 0.04 ^{d}
Apparent Extract (*w*/*w*)	3.69 ± 0.04 ^{b}	3.72 ± 0.07 ^{b}	3.84 ± 0.03 ^{a}	2.96 ± 0.02 ^{c}	2.46 ± 0.02 ^{d}
Alcohol (%)	4.14 ± 0.01 ^{d}	4.22 ± 0.04 ^{cd}	4.28 ± 0.01 ^{c}	4.90 ± 0.01 ^{b}	5.37 ± 0.01 ^{a}
Real Attenuation (%)	56.5 ± 0.01 ^{c}	56.6 ± 0.02 ^{c}	56.3 ± 0.03 ^{c}	62.9 ± 0.02 ^{b}	66.80 ± 0.03 ^{a}
Energy (kcal)	42 ± 0.9 ^{d}	43 ± 0.4 ^{cd}	44 ± 0.5 ^{bc}	45 ± 0.6 ^{ab}	46 ± 0.7 ^{a}
Specific Gravity	1.015 ± 0.002 ^{a}	1.015 ± 0.005 ^{a}	1.015 ± 0.003 ^{a}	1.012 ± 0.001 ^{a}	1.010 ± 0.001 ^{a}
Density (g/mL)	1.013 ± 0.003 ^{a}	1.013 ± 0.003 ^{a}	1.013 ± 0.003 ^{a}	1.010 ± 0.001 ^{a}	1.008 ± 0.002 ^{a}
pH	4.13 ± 0.04 ^{c}	4.26 ± 0.02 ^{b}	4.40 ± 0.04 ^{a}	4.37 ± 0.03 ^{a}	4.44 ± 0.04 ^{a}
Color EBC C.U.	3.82 ± 0.12 ^{d}	5.40 ± 0.63 ^{cd}	6.56 ± 0.45 ^{b}	5.63 ± 0.34 ^{bc}	11.85 ± 0.55 ^{a}

Values are expressed as the average of three measurements. Abbreviations: *w*/*w*, wight/weight; EBC C.U., European Brewing Convention Color Unit. Beer samples: CTR, beer without mushroom powder; PRE5, beer with 5 g/L of mushroom powder added before fermentation; PRE10, beer with 10 g/L of mushroom powder added before fermentation; POST5, beer with 5 g/L of mushroom powder added after fermentation; POST10, beer with 10 g/L of mushroom powder added after fermentation. Data within a line followed by the same letter are not significantly different according to Tukey's test. Mean values with different letters (a, b, c, d) within the same line are statistically different (p-value < 0.05).

5.4.4 Sensorial Evaluation

The sensorial evaluation of the experimental beers showed several significant differences between trials, as reported in Figure 4 A and 4 B. No differences were found in terms of complexity and cereal/grainy in aroma attributes and in sapidity, burt/cooked, and DMS in taste attributes. All samples that contained PEP showed cocoa and mushroom taste and aroma. In the PRE5 and PRE10 samples, the judges found an oxidized/aged taste and flavor

while in POST10 briny an acetic off flavor and briny and acid off taste were found. In general, the POST5 sample received the highest score in terms of visual, taste, aroma, and overall acceptance. PRE10 scored the same as POST5 in overall acceptance and taste but scored lower in aroma and visual perception. Similar results were reported by Leskosek-Cukalovic et al. [45] in beers fortified with *Ganoderma lucidum* extract where the experimental beers showed better body, taste, and overall impression than the control beers. As reported in previous paragraphs, mushrooms belonging to the *Pleurotus* genus are rich in sugars which were utilized by the yeasts, increasing the alcohol content in the experimental beers containing PEP, which, together with glycerol and residual sugars, is primarily responsible for the "body" attribute of the beer [46]. The overall ratings of the different attributes evaluated in the trials are shown in Figure 5.

5.5 Conclusions

In this work, we investigated the effects of powdered *P. eryngii* var. *eryngii* on the physical, chemical, and sensory properties of craft beer. The PEP addition was carried out in two phases of manufacturing (before and after AF) and two different quantities of powder (5 g/L and 10 g/L). PEP is rich in nutrients [21,30], particularly carbohydrates, that can be used by yeast for their metabolism, and this probably resulted in a statistically significant increase in yeast loads in all trials in which it was added. This hypothesis is confirmed by the higher amount of D-glucose and maltose detected in the trials in which PEP was added at the beginning of AF (PRE5 and PRE10). PEP-containing samples, both PRE and POST, showed a higher alcohol content, EBC color unit, and pH value than the control. The addition of PEP had a positive impact on the taste–olfactory characteristics of the beer evidenced by sensory analysis. The highest aroma production compared to the control occurred in the POST5 trial, which was the most highly rated in the sensory analysis in all parameters evaluated. In conclusion, the initial hypothesis of this research, i.e., to develop an innovative product with unique sensory characteristics was proven. Further analysis could be carried out by testing different wort conditions and by trying to assess the possible application to different beer styles.

Figure 4. Spider plot of the sensory analysis performed on beers. A) Odor; B) Taste. Beer samples: CTR, beer without mushroom powder; PRE5, beer with 5 g/L of mushroom powder added before fermentation; PRE10, beer with 10 g/L of mushroom powder added before fermentation; POST5, beer with 5 g/L of mushroom powder added after fermentation; POST10, beer with 10 g/L of mushroom powder added after fermentation. *p* value: *, $p < 0.05$; **, $p <$ 0,01; ***, $p < 0.001$; n.s., not significant.

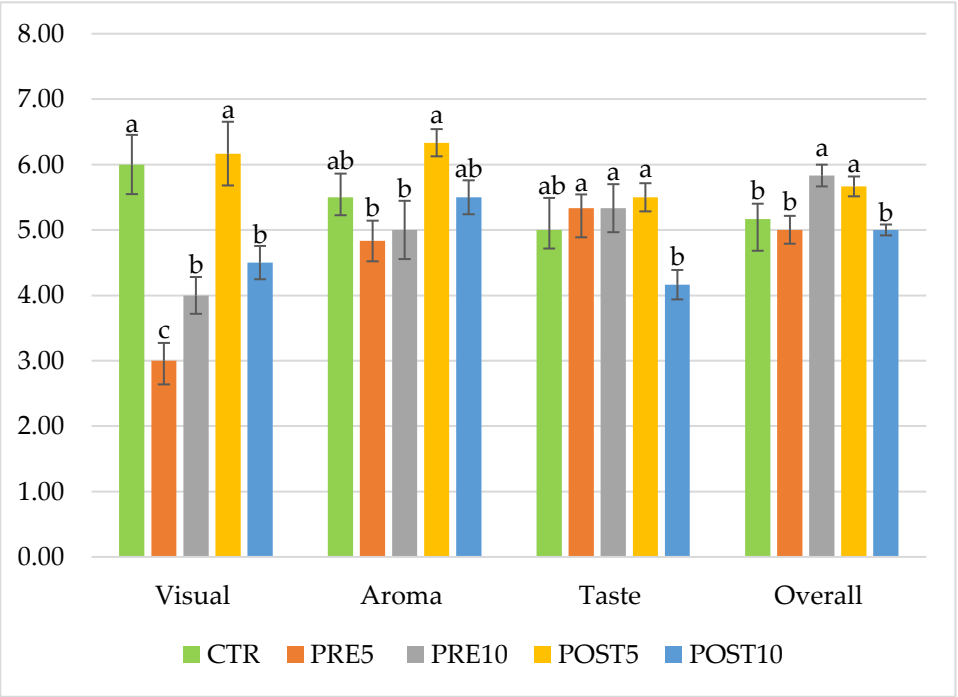

Figure 5. Visual, aroma, taste, and overall scores of beers. CTR, beer without mushroom powder; PRE5, beer with 5 g/L of mushroom powder added before fermentation; PRE10, beer with 10 g/L of mushroom powder added before fermentation; POST5, beer with 5 g/L of mushroom powder added after fermentation; POST10, beer with 10 g/L of mushroom powder added after fermentation. Different superscript letters indicate significant differences in scores performed at each attribute according to Tukey's test for $p < 0.05$.

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Chapter 6. General conclusion

This doctoral thesis highlighted the biological properties that medicinal mushrooms play within the human body due to the bioactive molecules they contain. Medicinal mushrooms have important health benefits and exhibit a broad spectrum of pharmacological activities, including antiallergic, antibacterial, antifungal, anti-inflammatory, antioxidative, antiviral, cytotoxic, immunomodulating, antidepressive, antihyperlipidemic, antidiabetic, digestive, hepatoprotective, neuroprotective, nephroprotective, osteoprotective, and hypotensive activities. The growing interest in mycotherapy requires a strong commitment from the scientific community to expand clinical trials and to propose supplements of safe origin and genetic purity. Bioactive compounds of selected medicinal mushrooms and their effects and mechanisms in *in vitro* and *in vivo* clinical studies was reported in this dissertation and at the same time we analyzed the mushrooms therapeutic use and pharmacological activities.

Mushrooms have contributed to the development of key active ingredients in medicinal chemistry and important tools for human and animal health, nutrition and functional foods. Studies on the beneficial effects of medicinal mushrooms on nutrition and health of humans and farm animals were considered in this thesis. an overview of the chemical structure and composition of substances found in mushrooms was presented, with particular reference to phenolic compounds, triterpenoids and sterols, fatty acids and lipids, polysaccharides, proteins, peptides and lectins.

The nutritional value and chemical composition of wild and cultivated mushrooms in Italy are analyzed in the thesis, which at the same time discusses mushrooms as nutraceuticals and the use of mushrooms in functional foods. The nutraceutical benefits of UV irradiation of cultivated species of basidiomycetes to synthesize high amounts of vitamin D2 were also highlighted. Finally, attention was paid to studies on the biological activities of some Italian wild and cultivated mushrooms, with special reference to species belonging to the genus Pleurotus. and the potential of medicinal mushrooms as a source of drugs, nutraceuticals and functional foods was highlighted.

During the thesis work, functional breads were produced with the addition of *P. eryngii* powder, applying traditional sourdough technology. *P. eryngii* powder was added to flour of different wheat varieties and durum wheat semolina. The sourdough starter was produced with selected strains of lactic acid bacteria belonging to the species *Levilactobacillus brevis*, *Weissella cibaria* and *Leuconostoc citreum*. The addition of 10% (w/w) Pleurotus powder did not affect the fermentation process, but the pH, TTA and organic acid values of the doughs prepared with *Pleurotus* powder were higher than the control. Sensory evaluation

indicated that the bread made with Mentana flour supplemented with *Pleurotus* powder was more highly valued by the judges and, therefore, was analyzed for vitamins and trace elements, showing an increase in vitamins B_1, B_2, B_3 and D, total polyphenols and beta-glucans, clearly showing that the inclusion of *P. eryngii* var. *eryngii* can be used to increase the functional aspects of bread. Beer is one of the oldest and most popular alcoholic beverages and is currently consumed worldwide. The various components used in the brewing process have a physiological impact on the consumer, and current research aims to improve its technological and functional properties through the addition of natural compounds (plants or mushrooms). In this thesis, the addition of two different amounts of *P. eryngii* var. *eryngii* powder added at different stages of production (PRE and POST alcoholic fermentation) was evaluated and showed improved yeast viability during alcoholic fermentation, increased alcohol content and improved sensory profile compared to the control. Regarding the organoleptic profile of the experimental samples, cocoa/chocolate and mushroom aromas were found, and the samples in which 10 g/L of mushroom were added pre-fermentation those in which 5 g/L of mushroom were added post-fermentation received the best ratings on all parameters evaluated. In conclusion, the adaptive plasticity of using *P. eryngii* powder for the development of foods and beverages with high sensory and functional qualities was demonstrated.

Publication list

- Cateni, F., Gargano, M. L., Procida, G., Venturella, G., **Cirlincione, F.**, & Ferraro, V. (**2021**). Mycochemicals in wild and cultivated mushrooms: nutrition and health. *Phytochem. Rev.*, 1-45.
- Venturella, G., Ferraro, V., **Cirlincione, F.**, & Gargano, M. L. (**2021**). Medicinal mushrooms: bioactive compounds, use, and clinical trials. *Int. J. Molecul. Sci.*, 22(2), 634.
- Gargano, M. L., De Mastro, G., D'Amico, F. S., **Cirlincione, F.**, Di Gristina, E. & Barone, G. (**2021**). Acclimatization, distribution and potential economic use of *Argania spinosa* (Sapotaceae) in southern Italy. *Flora Mediterr.*, 31: 173-181.
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