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Mushroom Biomass: Some Clinical Implications of β-Glucans and Enzymes

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ABSTRACT

Mushrooms have attracted market attention because they are a potential source of bioactive compounds able to perform several functions in organisms with benefits for the health of the consumer. Cultivation processes vary according a) industrial fermentation - in large vats to produce extracted form of mushrooms or b) closed cultivation system - individually grown in jars on an aseptic "substrate" with controlled lighting and irrigation to produce a biomass form of mushrooms. Biomass is the mycelium with primordia (young fruiting body - before the mushroom blooms) containing all the nutrients and active compounds, including β-glucans, enzymes and secondary metabolites. The classification of mushroom biomass varies according to the presentation; the biomass can be classified as a "food" if in powder form or, classified as a "dietary supplement" in tablet form. While tablet mushroom biomass is considered a dietary supplement, mushroom extracts are designated pharmaceutical compounds, pharmanutrients or nutraceuticals. Here we illustrate the difference between mushrooms in the biomass and extract forms, the similarities and differences on its content on enzymes, secondary metabolites and on β-glucans, as a soluble and fermentable fibre. Of particular note is the rich enzyme activity in the biomass form of mushrooms. Such activity includes enzymes that prevent oxidative stress (superoxide dismutase), enzymes that prevent cellular growth (protease, glucoamylase) and enzymes that promote detoxification (cytochrome P-450, peroxidase, glucose-2-oxidase). β-glucans have been proposed to act as "biological response modifiers" based on their effects on the immune system, and its role in the prevention and treatment of various metabolic syndrome-linked diseases. This review focuses also on some described health-promoting potential of mushroom biomass, all through immunomodulation. The role of intestinal microbiota is enhanced.

Keywords : Mushroom nutrition; β-Glucans; dietary supplements; biomass.

INTRODUCTION

Mushrooms are widely appreciated all over the world for their nutritional value and medicinal properties. They have low fat, high protein and vitamin contents. Mushrooms contain several minerals and trace elements, as well as substantial amount of dietary fibres and they are also producers of bioactive molecules and valuable enzymes with different therapeutic effects[1,2]. Therefore, they are considered as perspective organisms to develop different healthcare biotech-products. The main groups of bioactive molecules produced by different mushrooms are polysaccharides, terpenoids,

phenolics compounds, polypeptides, terpenes, steroids and lectins. More than 126 therapeutic effects of these molecules were revealed[3].

Infectious diseases remain one of the major threats to human health. Although numerous antibiotics have been used against pathogens, antimicrobial resistance is an increasing public health problem. The antibiotics in mushrooms are less well documented in the discovery of new antimicrobial agents with different structural types. Mushrooms need antibacterial and antifungal compounds to survive in their natural environments[4]. Therefore, antimicrobial compounds could be isolated from many mushroom species and could be of beneficial for humans[2,4]. Most of the medicinal extracts and biomass from mushrooms are different forms of polysaccharides, and all of them are strengthens of the immune system with few or no side effects[5]. The degenerative diseases associated with aging include cancer, cardiovascular disease, immune system decline, brain dysfunction, and cataracts[6]. They are also associated with free radicals because oxidative damage to DNA, proteins, and other macromolecules accumulates with age and has been postulated to be a major type of endogenous damage leading to aging[7]. The consumption of plant foods, such as fruits, vegetables, red wines, and juices, provides protection against various diseases, including cancer and cardio- and cerebrovascular diseases[8]. This protection can be explained by the capacity of antioxidants in the plant foods and mushrooms to scavenge free radicals, which are responsible for the oxidative damage of lipids, proteins, and nucleic acids[8,9].

This review focuses on the health-promoting potential of mushroom biomass with especial emphasis on the physiologically active polysaccharides, enzymes and secondary metabolites that have been demonstrated to possess anti-tumour, immunomodulating, anti-oxidant, radical scavenging, cardiovascular, anti-hypercholesterolemia, anti-viral, anti-bacterial, anti-parasitic, anti-fungal, detoxification, hepatoprotective, and anti-diabetic properties[3,10].

Mushroom Composition

Fungi (yeasts, molds and mushrooms) are major decomposers in certain ecosystems and essential associates of many organisms. Recent estimates based on high-throughput sequencing methods suggest that as many as 5.1 million fungal species exist. Some 14000 species of fungi can be considered as mushrooms, and at least 2000 species are identified as edible. Fungal biologists debated for more than 200 years about which organisms should be reckoned as Fungi. In less than 5 years, DNA sequencing provided a multitude of new characters for analysis and identified about 10 phyla as members of the monophyletic kingdom Fungi[11]. Mushroom is the fleshly, spore bearing fruiting body of a fungus, typically produced above ground on soil or on its food source.

Besides the nutritional properties, mushrooms have attracted market attention because they are a potential source of bioactive compounds able to perform several functions in organisms with benefits for the health of the consumer[2]. Two main families of mushrooms are: 1) Ascomycota – spores produced inside ascospores (e.g. Penicillin, Cordyceps, Truffles); 2) Basidiomycota – spores produced on cells called basidia, include most of the so-called "Medicinal Fungi" (e.g. Shiitake, Maitake, Reishi, Coriolus). Modern systematics, based on morphological characters and analysis of rDNA sequences, divides the Fungi Kingdom into four major phyla or divisions: Chytridiomycota, Zygomycota, Ascomycota, and Basidiomycota. The two fungal phyla that produce large, visible fruit bodies are the Ascomycota and Basidiomycota[12].

Although in the past fungi were considered plants, some of the characteristics that distinguish them from plants are that they possess cell walls containing chitin rather than cellulose and lack chloroplasts[13]. Only 35 are grown on a commercial scale, 20 are cultivated on an industrial scale and only 12 have the greatest concentration of β-glucans. Cultivation processes vary according to a) industrial fermentation - in large vats to produce extracted form of mushrooms or b) closed cultivation system - individually grown in jars on an aseptic "substrate" with controlled lighting and irrigation to produce a biomass form of mushrooms. The chemical constituents of mushroom include water, polysaccharides, proteins, nucleosides, fatty acids, sterols, polyphenols, selenium, cerebrosides and triterpenes[14]. Mushrooms contain

dietary fibres belonging to β-glucans, chitin and heteropolysaccharides (e.g. pectinous substances, hemicellulose, polyuronides), making up as much as 10-50% in the dry matter. Much of the active polysaccharides, water soluble or insoluble, isolated from mushrooms, can be classified as dietary fibres (i.e. β-glucan, xyloglucan, heteroglucan, chitinous substance) and their protein complexes[2]. Hydrolytic enzymes, closely associated with the cell wall, have been implicated in the maintenance of wall plasticity and may have roles during branching and cross-linking of polymers. Most fungal cell wall hydrolases identified to date have chitinase or glucanase activity[15].

Mycelium is the vegetative part of a fungus, consisting of a mass of branching, thread-like hyphae. Fruiting Body is the portion of a mushroom above ground, such as the stem and cap. During its life, the mycelium has one goal: to preserve and promote the existence of the species. Mushroom or mycelium cells struggle to survive against invaders. In order to survive, mycelia have developed highly efficient and proactive immune systems. Many scientists now believe that the highly developed immune system, the mycelia, has evolved over the years, along with its ability to break down organic matter in nature, are exactly what makes mushrooms so valuable to humans and other mammals.

Mushroom derivates

There are various methods of extraction of mushrooms, although with a similarity among them, developed mainly to extract the anti-cancer polysaccharides from mushroom fruit-bodies, mycelium and liquid media[16]. Extraction, fractionation, purification and chemical modification are some of the stages involved, while the concentration of polysaccharides in certain medicinal mushroom species can be related to the stage of development of the mushroom fruit body and also to the time after harvest and subsequent storage conditions[17].

It has been demonstrated the impact of digestive juices on the β-1,3 and β-1,6 glucan content in a mushroom extract compared to a biomass equivalent[18]. A comparison of the impact of digestive enzymes *(in vitro*) on the constituents of two Reishi compounds, one an extract of fruiting bodies *versus* a biomass equivalent: a) in the absence of proteolytic enzymes; b) in the presence of pepsin; c) in the presence of trypsin. Active β-1,3-1,6 glucan content in both samples was equivalent and significant “before”. However the situation changes when samples were in the presence of proteolytic enzymes (simulation of digestion). Biomass sample showed higher level of active β-glucans in a human digestive system simulation. Biomass sample was more resistant than the extract sample to digestive enzymes[18].

Both mycelium and primordia (young fruit body) cultivated into a biomass is grown on a sterilized (autoclaved) substrate. This cultivation process ensures the powder is free from contamination by other fungi and that pesticides and heavy metals are absent. The cultivation system is proprietary, allowing for standardized production of biomass from several mushrooms.

Biomass versus Extract Form

While mushroom biomass is considered a “dietary or food supplement”, mushroom extracts are pharmaceutical compounds or “nutraceuticals”. Biomass is the mycelium with primordia (young fruiting body - before the mushroom blooms). It contains all the nutrients and active compounds, including enzymes, secondary metabolites and β-glucans. In the view of the FDA, the isolation, concentration, and purification targeting a single active ingredient from mushrooms extracts can designate them as pharmaceuticals or “pharmanutrients”. Using the mushrooms and mycelium in their natural forms as biomass makes them “functional foods” or “dietary supplements”. This has strong implications on the type of evaluation and approval by the respective food or medicine authorities. However, both, extracts or biomass forms, can be considered as acting as “prebiotics” on their mode of action, as probable mediators on the microbiome effects.

The extracted form is more exposed to the action of the proteolytic enzymes since there are no physio-chemical barriers to prevent such exposure, compared to biomass equivalent.

In extract form, proteins (i.e. enzymes), are denatured by the hot water extraction process which is conducted at temperatures above 40°C and by substances used in the process, such as alcohol

and sodium hydroxide. There is a clear correlation between the changes in protein expression that occur during different developmental stages. Enzymes related to cell wall synthesis are most highly expressed during fruiting body formation compared to the mycelium and primordial stages[19].

Some species of mushrooms synthesize enzymes that may play important functions in the organism. The biomass form of mushrooms contain, not only protein-bound polysaccharides (PSK; PSP), typical on extracts, but also active enzymes responsible for: 1) Preventing oxidative stress; e.g. Laccase, Superoxide dismutase (SOD); 2) Inhibiting cell growth; e.g. Proteases, Glucoamylases; 3) Promoting detoxification; e.g. Peroxidases, Cytochrome P450, Glucose-2-oxidase.

When exposed to proteolytic enzymes, such as pepsin and trypsin the differences in enzyme content between both samples (biomass and extract), is due to their differences in their biological forms. Mushroom biomass is more resistant to proteolytic enzymes (gastric juices) than the extracted form. Concentrated mushroom extracts are more exposed to the action of the proteolytic enzymes since there are no physio-chemical barriers (unlike in mushroom biomass) to prevent such exposure.

Extraction methods

Historically, polysaccharides (PSK) were first successfully extracted from *Coriolus versicolor* but nowadays are extracted from several mushroom species. When used in traditional Chinese medicine, *Coriolus versicolor* mushroom, for example, was picked, dried and steeped in boiling water and drunk as a tonic to gain access to its properties. In 1970s, Japanese scientists were the first who did research on *Coriolus versicolor* employing modern technology, and successfully extracted a PSK from the CM-101 strain[20]. PSK is patented and proven that it can effectively enhance immunity of human body. The approximate molecular weight of PSK is 100,000 Da, and the protein component is reported at the β-1,6 side chain[21].

There are two primary *Coriolus versicolor* extraction products – PSK and PSP. Just the same as Japanese PSK (Krestin), PSP is a protein bound polysaccharide extracted from the deep layer cultivated mycelia of Yun Zhi strains and different fermentation and extraction methods (PSP uses Cov-1 strain and is extracted with alcoholic precipitation while PSK uses CM-101 strain, and is extracted with salting out with ammonium sulphate), their polysaccharide components and hence their curative effects are not entirely the same[22].

Both of these extraction processes starts with *Coriolus versicolor* mushroom mycelia then it is extracted with hot water. After hot water extraction, PSP under goes an ethanol refining process. Finally, the refined product is capsulated and it can be taken orally with ease.

Both PSK and PSP are extracts from the mycelia of *Coriolus versicolor*. The quantity of PSK and PSP in *Coriolus* mycelium is quite small. However, since most of the effects come from cellular signalling, the amount needed should be quite small.

As a new type of "Biological Response Modifiers" (BRM) different from the Japanese PSK, the production technology of PSP is unique. The fermentation process takes only 64 hours, 3 times quicker than that reported in Japanese PSK patent. PSK patent literature reports that PSK takes 10 days for fermentation. PSP has more medicinal ingredients and higher pharmacological activities. Research publications to date have not been consistent enough to determine specific daily requirements of polysaccharide peptide or also β-glucan for optimal health. Focusing on a recommendation for overall health is complicated by the fact that not all β-glucans are created equal[23,24].

Thousands of studies have been reported in vast number of journals concerning the use of extracts of mushrooms on health, with significant positive results[25-29]. Their use as medicaments in China and Japan is a routine and approved by the authorities[30]. Not so much evidence exist concerning the use of biomass of mushrooms, used in the European market since 1994.

β-glucans

β-glucans are a group of β-D-glucose polysaccharides that are found in the cell walls of bacteria, fungi (mushrooms), yeasts (e.g.

Saccharomyces cerevisae), algae, lichens and plants (e.g. oats and barley). β-glucans are used in various nutraceutical and cosmetic products, as texturing agents, and as soluble fibre supplements[31,32].

A limited number of sugar monomers can create thousands of complex glucans. Post-translational glucan modifications are generally though to be important for protein folding, steric protection from proteolytic degradation, and regulation of protein-protein interactions. It is estimated that up to 70% of mammalian proteins are glucosylated. The glucans are attached to proteins via "N" or "O" linkages[33].

The polysaccharide peptides can be found in the mycelium, while the fruiting body mainly contains polysaccharides. Where there is polysaccharide there is polypeptide and the polysaccharide and peptide of PSP are closely bound and not separated. β-glucans are healthy fibres that humans cannot digest, but that can be digested by some species of our gut bacteria[34] . β-glucans form a linear backbone with 1-3 β-glucosidic_bonds but vary with respect to molecular mass, solubility, viscosity, branching structure, and gelation properties, causing diverse physiological effects in animals[35].

Not all β-glucans are able to modulate immune functions. These properties mainly depend on the primary chemical structure of the β-glucans. Cellulose for example, a 1,4-β-linked glucan, does not exhibit immunomodulatory effects[36]. In contrast, β-glucans derived from fungi and yeast, which consist of a (1,3)-β-linked backbone with small numbers of (1,6)-β-linked side chains, are essentially known for their immune-modulating effects[37].

Some Clinical Uses of Mushroom Biomass

The determination and reported β-glucan values from several independent laboratories vary greatly even though identical assays were performed. The most commonly employed assays for determination of beta-glucan content can only detect soluble β-glucans; the insoluble β-glucans remain undetected. β-glucans differ in their solubilities depending on their size, functionality, and interaction with other molecules. So does the variability concerning secondary metabolites in mushroom. The connection between our gut, brain, and immune function explains why our intestinal health, the food and dietary supplements we ingest, can affect our mental and neurological health[38].

Mushroom β-glucans enhances the body's own use of macrophages and T-lymphocytes, rather than directly attacking any tumours[39]. In their natural states, yeast and mushrooms contain a mixture of β-1,3-glucan and β-1,6-glucan. Oats and barley contain a mixture of β-1,3-glucan and β-1,4-glucan. Among these sources, yeast typically has the highest β-glucan content and oats and barley the second highest[28]. Enterocytes facilitate the transportation of β-1,3-glucans and similar compounds across the intestinal cell wall into the lymph, where they begin to interact with macrophages to activate immune function[40].

There are multiple recognised clinical uses of β-glucans. β-glucans are used for: high cholesterol[41] and to balance fasting blood glycaemia level[42], diabetes[43], cancer[44,45], and HIV/AIDS[46]. β-glucans are also used to boost the immune system in people whose body defences have been weakened by conditions such as Chronic Fatigue Syndrome[47,48], physical and emotional stress[49]; or by treatments such as radiation[50] or chemotherapy[27]. β-glucans are also used for colds (common cold), flu (influenza), H1N1 (swine) flu, allergies, hepatitis, Lyme disease, asthma, ear infections, rheumatoid arthritis and multiple_sclerosis[9,51-55]. Positive results were also shown in alleviating the symptoms of: Hay fever[56], Alcohol Induced Liver Steatosis (Fatty Liver)[57], Fibromyalgia[58,59], Ulcerative Colitis[60], Crohn´s Disease[61], LSIL HPV Infection[62], Gout[63], Leaky Gut Syndrome[64], Alzheimer's Disease[10].

Mushroom Enzymes and their role in health

Proteases are involved in digesting long protein chains into shorter fragments by splitting the peptide bonds that link amino acid residues. Some detach the terminal amino acids from the protein chain (exopeptidases, such as aminopeptidases, carboxypeptidase A); others attack internal peptide bonds of a protein (endopeptidases, such as trypsin, chymotrypsin, pepsin, papain, elastase)[65] .

There is strong evidence suggesting that important antioxidant and cytoprotective enzymes are present in various edible fungi[66]. This point out

the importance of a therapeutic strategy based on nutritional interventions with biomass mushroom supplementation to prevent and limit the deleterious consequences in many disorders (e.g., associated with high oxidation damage such as coronary heart disease, asthma, inflammatory conditions, chronic and neurodegenerative disorders). Cell damage is induced by Reactive Oxygen Species (ROS) which can be either free radicals or molecules containing reactive oxygen atoms, i.e., hydrogen peroxide, superoxides, hydroxyl anions[65]. Oxidant species produce free radicals or are activated chemically by them. Natural Killer cells (NK) are susceptible to ROS and lose their activity. High oxidation promotes cell and tissue damage, aging, inflammation, degeneration[67].

In general, oral administration of certain enzymes contribute to the reduction of chronic toxic overload in the organism and the optimisation of the following[68]: Balance pH levels: blood and extracellular matrix; Removal of toxic substances; Recovery of intestinal bacterial balance (biota balance); Enhancement and balance of the immune system; Improvement in cell metabolism.

Enzymes that prevent oxidative stress

Oxidative stress is a condition characterized by elevated levels of intracellular reactive oxygen species (ROS). ROS either are, or break down to form, free radicals. ROS include superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH^-) that are capable of reacting with, and damaging DNA, proteins, and lipids. There is a range of enzymes involved in generating deleterious free radical species while others have antioxidant properties[69]. Superoxide dismutase (SOD): SOD (and SOD mimicking substances) can restore the NK cell activity by protecting them from oxidative damage caused by ROS[70].

Enzymes that inhibit cellular growth

Digestive enzymes extracted from fungal sources generally have the ability to operate over a broad range of acidity conditions (pH 3.5 to 8.5). This means that they can survive the digestive process, the acidity of the stomach and the alkalinity of the small intestine. Fungal-based digestive enzymes can therefore assist in digesting food in the stomach and then continue to assist in the process after the food enters the small intestine. This helps to ensure that food is completely digested[71]. Protease enzymes have been shown to conduct the process of proteolysis, degrade cancer cells as well as toxins. Proteases can assist in removing excess protein from the circulatory system[72]. Glucoamylase enzyme breaks down large starch molecules in the human body into glucose, the useful main energy compound[73].

Enzymes that promote detoxification

Peroxidase: Peroxidase enzymes catalyse phenolic and related compounds with various benefits and actions widely distributed in the body, improving detoxification processes and also decreasing high oxidation[74].

Cytochrome P450: A very diverse group of naturally occurring and synthetic compounds are metabolized in the liver by the Cytochrome P450 (CYPs). These CYP substrates range from endogenous compounds such as steroids and cholesterol, to drugs and carcinogens. The oxidized products are more polar, and the result is generally an easier detoxification[75].

Glucose-2-oxidase: Catalyses the oxidation of several allopyranoses (carbohydrates) producing hydrogen peroxide and 2-keto-D-glucose. This activity has been found to exhibit anti-tumour activity (*in vitro*) against Ehrlich ascites tumour cells by inhibiting cell proliferation[18].

Concluding remarks

The objective of the current review was to illustrate the role of β-glucan and enzymes, comparing mushroom extracts with mushroom biomass, as a soluble and fermentable fibre, in the prevention and treatment of various metabolic syndrome-linked diseases. An overview of the health benefits associated with β-glucan from mushroom biomass, its possible mechanisms of action, and its potential food applications were given.

Scientists have been studying the effect of β-1,3/1,6-glucans on the immune system for more than 100 years, however we have only recently begun to develop a true understanding of the effects of this polysaccharide. β-1,3/1,6-glucans is known as a biological response modifier as it is able to bond

with the surface of certain innate immune cells and improve the immune system's ability to fight off viral, bacterial, fungal and parasitic infections.

This review showed that mushroom biomass is orally effective, promoting better ingestion and absorption of polysaccharides into the immune system at intestinal level. In this form the product is absorbed to a high percentage into the immune system, not just passed through and expelled by the body as large particle globular glucans. These β-1,3/1,6-glucans are the nutritional fuel for the immune system thus enabling a normalized immune response, fighting back against health invaders maintaining good health.

However, many over-the-counter mushroom products are not standardized, making it difficult to compare potency between brands. It is also still unclear if PSK, PSP, and other mushroom extracts and biomass have comparable effects. Probing studies in humans are needed to understand the implications of the observed effects on immune function, gut microbiota, cognition, periodontitis, cancer mechanisms, body composition and body weight. Studies are needed to define how much, how often, and perhaps in what pattern specific mushroom species may be consumed to bring about substantial biologic and health responses, as well as to understand the specificity of mushroom impacts on health.

Both Extract and Biomass forms of mushrooms act as Prebiotics. Both affect positively the immune function thus having clinical impact. Extract forms are much better documented but are considered as Nutraceuticals, i.e. as Medicines; Biomass forms are richer in enzymes + secondary metabolites and considered as Food Supplements, the former in need of medical prescription. More studies are needed to compare both forms in different mushrooms. The way forward must include research on the influence of different mushrooms and forms on the gut flora. The gut-brain axis must be taken into consideration.

Promising evidence, still in its infancy, suggests a positive role for mushrooms and their bioactive components, particularly ergothioneine, on well-being and general health. While β-glucans are the new frontier in health management, much research still remains to be done.

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