The Pharma Innovation Journal 2023; 12(5): 3918-3929

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(5): 3918-3929

www.thepharmajournal.com
Received: 22-03-2023
Accepted: 28-04-2023

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Bioactive peptides from mushroom proteins

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DOI: https://doi.org/10.22271/tpi.2023.v12.i5at.20427

Abstract
Bioactive peptides (BPs) are multifunctional proteins that have a variety of activities and strong pharmacological effects. Due to the food-derived BPs' extensive spectrum of health benefits and numerous bioactivities, including antioxidant, immunological, anti-ageing, and anti-inflammatory activities, research on these compounds has not been interrupted throughout the past few decades. To balance the daily rise in the intake of functional foods and nutraceuticals, it is important to adopt healthy lifestyle choices. As a result, these peptides are crucial in the creation of numerous functional meals. Additionally, studies have demonstrated that peptide treatments are safer from a toxicological standpoint and have fewer adverse effects than small molecule medications. Long recognized for their culinary qualities, mushrooms have been a favourite food of the most important civilizations in history. They are currently the subject of renewed investigation due to their medicinal properties. Mushrooms provide substantial amounts of necessary proteins, dietary nondigestible carbohydrates, unsaturated fats, minerals, and different vitamins. These nutritional advantages have increased mushroom consumption and led to the creation of a variety of processed mushroom products. Bioactive peptides that have been directly or indirectly isolated from mushrooms or their mycelia are known as mushroom bioactive peptides (MBAPs). The advantages of MBAPs for promoting health and preventing disease, including their antihypertensive, antioxidant, and antibacterial actions, are becoming more widely acknowledged. However, this area is still underdeveloped, and there aren't many pertinent reviews. Therefore, the purpose of this study was to outline the current state of MBAP research with a focus on preparatory and biological operations.

Keywords: Bioactive peptides, mushroom, antioxidant, antibacterial, antihypertensive

Introduction
The mushroom is a natural source of bioactive compounds, which provide a wide range of medicinal properties. Traditionally used in traditional Chinese medicine for centuries, mushrooms contain natural antioxidative, antitumor, antiviral, antimicrobial, and immunomodulating agents (Zaidman *et al*., 2005) [99]. It is an edible fungus having a lot of protein, which makes up between 10 and 40 percent of its dry weight (Uzun *et al*., 2009) [91]. This value varies between species as well as within the same species, though. Due to their great digestibility and nine necessary amino acids, mushroom proteins are regarded as a superior alternative to muscle protein (Gupta *et al*., 2019) [32]. They have been found to contain a variety of bioactive phytochemicals, including proteins, terpenoids, polysaccharide-peptide complexes, and polysaccharides. They have also been reported to have potential medical benefits, including the ability to lower cholesterol and have immunomodulatory, antiviral, and antitumor properties (Sheu *et al*., 2004) [83]. Although mushrooms that are peculiar to Basidiomycetes have been discovered to contain a good amount of bioactive proteins, they may also contain more bioactive peptides than those reported. Mushrooms typically have a high protein content, with an average of 23.80 g 9.82 g/100 g dry weight (J. Zhou *et al*., 2020) [103]. These proteins have undergone extensive research as starting points for isolating bioactive peptides from various studies. There has been evidence that mushrooms manufacture several 'potential drugs', including polysaccharides, phenolics, sterols, proteins, and peptides, which are responsible for their therapeutic effects (R. Zhou *et al*., 2018) [104].
A bioactive peptide (BP) is a peptide that can exert physiological effects on humans, and isolated small protein fragments can also be considered BPs (Chakrabarti *et al*., 2018) [12]. Bioactive peptides, protein fragments encoded within the parent protein with favourable effects on body processes and/or human health beyond their nutritional value, carry out the physiological features of dietary proteins (Korhonen & Pihlanto, 2006) [46]. Antihypertensive,

antioxidant, anti-inflammatory, anti-atherogenic, opioid, antibacterial, antithrombotic, immunomodulatory, and mineral binding capabilities are among the physiological effects of bioactive peptides (Guha and Majumder 2019; Nagaoka 2019) [31, 69]. Enzymatically, through food processing, or by microbial fermentation, these peptides can be released (Korhonen and Pihlanto 2006; Wu *et al*. 2015) [46, 95].

Enzymatic hydrolysis, fermentation, or digestive process could be used to produce bioactive peptides (Chalamaiah *et al*., 2018) [14]. When liberated from the precursor protein from which they are encoded, bioactive peptides display varying biological activity depending on the amino acid content, sequence, and chemical structure (Lemes *et al*., 2016) [56]. They are promising possibilities for innovative healthcare products and functional foods. However, due to several bioprocess problems, they haven't been thoroughly investigated (Korhonen 2009; Agyei *et al*. 2016) [45, 3]. Industrial scale-up for large-scale manufacturing, as well as ineffective purification, approaches specific to bioactive peptides, appear to be significant challenges (Agyei *et al*., 2016) [3].

In addition to their structural variations, BPs also have some common structural characteristics, including their length, which ranges from 2 to 20 amino acid residues, and a high frequency of hydrophobic amino acid residues present within them. (Kitts & Weiler, 2005) [44]. The properties of amino acids related to acidity and basicity are inherited by the peptides they constitute. The acid-base conduct of the peptides is decided by the free amino group of the N-terminal residue (with a slightly lower pKa than the corresponding free amino acid), the free carboxylic group of the C-terminal residue (with a slightly higher pKa than the corresponding free amino acid), and the ionizable groups of side chains of the residues in the chain. The chemical reactions involving amino and carboxyl-terminal groups of a peptide are comparatively similar to those of free amino acids, and they include acylation and esterification reactions. Notably, the reactivity of the amino group with specific reagents, including ninhydrin and o-phtaldialdehyde, is commonly utilized for detecting and measuring the quantity of the peptides (Landi *et al*., 2022) [51].

BAPs, unlike proteins, may be entirely absorbed by the colon, they can either generate local effects in the digestive tract or reach the circulatory system intact, allowing them to perform their physiological effects (Erdmann *et al*., 2008) [23]. They also showed a stronger affinity for tissues and are more stable and less poisonous. Because of their properties, using BAPs as functional foods or in medication is thought to be beneficial in avoiding the adverse effects associated with synthetic substances (J. Zhou *et al*., 2020) [103].

The main drawbacks of using bioactive peptides are their potential toxicity and the bitter taste that results from their high hydrophobic amino acid content in the finished product. Different strategies have been used to address these problems, such as separating the six hydrophobic amino acids or the particular peptide sequences using exopeptidases or covering up the bitter taste with fragrance chemicals (He *et al*., 2019) [33]. Furthermore, most bioactive proteins' biological functions have been attributed to encoded BAPs that can be released without losing their bioactivities. (Daliri *et al*., 2017; Montesano *et al*. 2020) [19, 65]

When certain peptides are subjected to heating treatment and combined with carbonyl compounds, like sugars, they may undergo the Maillard reaction, leading to the creation of various melanoidin pigments. These pigments can impact the development of flavors, aromas, and colors in food, whether intentionally desired or not (Fu *et al*., 2019) [26]. Ultimately, the process of breaking down proteins into smaller peptides during food production (such as cooking or fermentation) and storage plays a significant role in determining the rheological properties of the food. (Karami & Akbari-adergani, 2019) [40].

Table 1: Marketable bioactive peptides

Company name	The source	The sequence of amino acids	Benefits	In the marketplace	Production method	References
Lacitum®	Milk	YLGYLEQLLR	De-stressing	Supplements and drinks	Trypsin-enzymatic hydrolysis	(Nagai *et al*., 2006) [68]
Calpis	Sour milk	VPP y IPP	An antihypertensive effect	Drinks	Anaerobic fermentation with *S. cerevisiae* and L. helveticus CP790	(Siltari *et al*., 2012) [85]
Lacprodan® Whey	Whey	N/A	Maintains normal blood sugar levels	The powder product	Alcalse-enzymatic hydrolysis	(Chalamaiah *et al*., 2019) [13]
Capolac®	Milk	CPP	Absorption of calcium	Ingredient	The isolation process	(Chalamaiah *et al*., 2019) [13]
Cholesterol block	Soy	CSPHP	Reduction of cholesterol	Drink	Enzymatic hydrolysis	(Chalamaiah *et al*., 2019) [13]

Current techniques for the isolation of peptides

Microbial fermentation and enzyme hydrolysis are the most common methods used to produce bioactive peptides. As a result of these methods, a variety of bioactive peptides are released, which are screened and purified based on their requirements (Kumari B *et al*., 2020) [49]. BAPs are still being researched for new sources and extraction methods, as well as the health benefits they provide. There has been a lot of interest in finding and characterizing bioactive proteins and peptides in recent decades. Given the difficulty in identifying bioactive proteins and peptides, quantifying them has been a difficult undertaking that has necessitated ongoing analytical advancement (Alves *et al*., 2019) [5]. Any protein, regardless of its source, becomes a reservoir of peptides when hydrolyzed, but first, the proteins must be isolated from other matrix components and purified. Pure proteins can be measured and described to determine their nature and amino acid sequences, as well as their bioactivity. Following that enzymolysis, or microbial fermentation can be used to produce protein hydrolysates, from which the peptide contents can be isolated using chromatographic methods (Alves *et al*., 2019) [5].

Enzymatic Hydrolysis

Complex proteins can be hydrolyzed by enzymes at appropriate temperatures and pH values (Norris & J., 2013) [70]. Enzymatic hydrolysis is not as time-consuming as microbial fermentation, and it is more predictable and scalable as well. Proteolytic enzymes are added sequentially or simultaneously according to the optimal temperature and pH to produce bioactive peptides. However, sustained hydrolysis would result in low molecular-weight proteins (H. Zhang *et al*., 2012) [100]. To date, the most efficient and prevalent method of producing BAPs is the enzymatic hydrolysis of food proteins (A. Sánchez & Vázquez, 2017) [77]. It has been reported that MBAPs with antioxidative, antimicrobial, antidiabetic, and tyrosinase inhibitory properties have been synthesized by enzymatic hydrolysis. It is more common among MBAPs to produce antioxidant peptides, since peptides exhibit superior antioxidant properties (Mishra *et al*., 2018) [63]. Hydrolysis can be improved by pre-processing, such as ultra-high-pressure treatment, which increases the soluble protein content and enhances hydrolysis efficiency (Zhao *et al*., 2017) [102]. The choice of the most appropriate enzyme is also important. In addition to the specificity of the protease, the characteristic AA sequences of released peptides are determined by the parent protein. By using the appropriate enzymes or serially combining them, the number or type of BAPs that can be obtained from food proteins is limitless (Agyei & Danquah, 2011) [1]. Therefore, choosing the right proteases is crucial. In most cases, GI enzymes such as trypsin, pepsin, chymotrypsin and pancreatin are used to generate MBAPs, which may be due to their greater stability in the GI tract after digestion, and higher bioavailability upon absorption (Xu *et al*., 2019) [97].

The majority of reports describing MBAPs produced by enzymatic hydrolysis of mushroom-derived proteins have not characterized the AA sequence, but have only conducted a preliminary purification using ultrafiltration or GFC to obtain different peptide fractions, or a mixture of peptides without purification. In contrast, endogenous MBAPs are usually purified, identified, or even synthesized. The preparation methods and properties of produced and endogenous MBAPs make them distinct in several ways (J. Zhou *et al*., 2020) [103].

By contrast, the enzymatic hydrolysis of mushroom-derived proteins to obtain BAPs involves quite a different process. As proteins are usually extracted first and then hydrolyzed by enzymes, a hydrolysate consisting of peptides is produced; therefore, purification is unnecessary. Moreover, as previously mentioned, numerous peptides can be produced from one single mushroom species by optimizing factors, such as the type of enzymes used, the degree of hydrolysis, and the modification methods used (e.g., glycosylation reactions and selenization), which poses a great challenge in purifying and identifying peptides. Additionally, different peptides, especially small peptides (5AAs), are difficult to identify when using tandem-MS because a small number of MS fragments are obtained (Lahrichi *et al*., 2013) [50].

Microbial Fermentation

This method involves cultivating bacteria or yeast on protein substrates to hydrolyze the proteins with their enzymes. Proteins are broken down by the proteolytic enzymes secreted by bacteria or yeast when they grow, releasing peptides from the parent proteins in the process. In most instances, the bacteria of choice are grown in broth at the right temperature to reach their exponential phase (Rizzello *et al*., 2017) [74]. The amount of hydrolysis depends on the type of microbe, the duration of fermentation, and the concentration of substrate, as does the number of peptides generated. Lactobacillus species are most commonly used for this process. Thus, it is clear that protein hydrolysates may function differently in different cultures due to the presence of different proteolytic systems in microorganisms (Daliri *et al*., 2017) [19].

Yeast and filamentous fungi have also been employed to produce bioactive peptides in addition to bacteria starters (Giri, 2012; Lima *et al*., 2015) [29, 57]. To speed up the proteolytic process, proteins can be co-cultured utilising a variety of bacteria, or even yeast and bacteria (Chaves-López *et al*., 2014) [16]. The mixture is centrifuged after fermentation to recover the supernatant. To produce shorter peptide sequences, the supernatant may subsequently undergo further hydrolysis using proteolytic enzymes. The low molecular weight peptides in the supernatant can also be isolated using solvent extraction or other techniques, purified, and their amino acid sequences identified using mass spectrometry (Babini *et al*., 2017) [7].

Microbial fermentation consists of several systems. Submerged fermentation and solid-state fermentation are, however, the most common methods of fermentation (Nadu, 2012) [67]. The submerged fermentation process involves the culture of microorganisms in a liquid medium that contains nutrients. Meanwhile, solid-state fermentation involves microbial growth on solid substrates rich in nutrients. Besides releasing nutrients in a controlled manner, it is suitable for fungi and microorganisms that require less moisture (Cruz-Casas *et al*., 2021) [17].

The advantages of microbial proteases over commercial enzymes in hydrolysis reactions are numerous. Due to their minimal nutritional requirements and rapid maturation, microorganisms are much cheaper to cultivate (Marciniak *et al*., 2018) [59]. Also, microbial fermentation releases bioactive peptides obtained from edible food proteins, and GRAS microorganisms are also involved, that's why bioactive peptides are considered safer and healthier (Fan *et al*., 2019) [23].

An incorrect application of factors such as sugar type, oxygen availability, competition with microbes, and time can negatively influence the outcome. Therefore, microbial fermentation should be carried out after investigating the appropriate parameters (Melini *et al*., 2019) [61]. Exopolysaccharides, bacteriocins, and other compounds that are produced by microbial fermentation in addition to bioactive peptides have biological properties of their own and raise questions about whether the bioactivities observed are a result of the peptides or these other compounds (Martínez-Medina *et al*., 2018) [60].

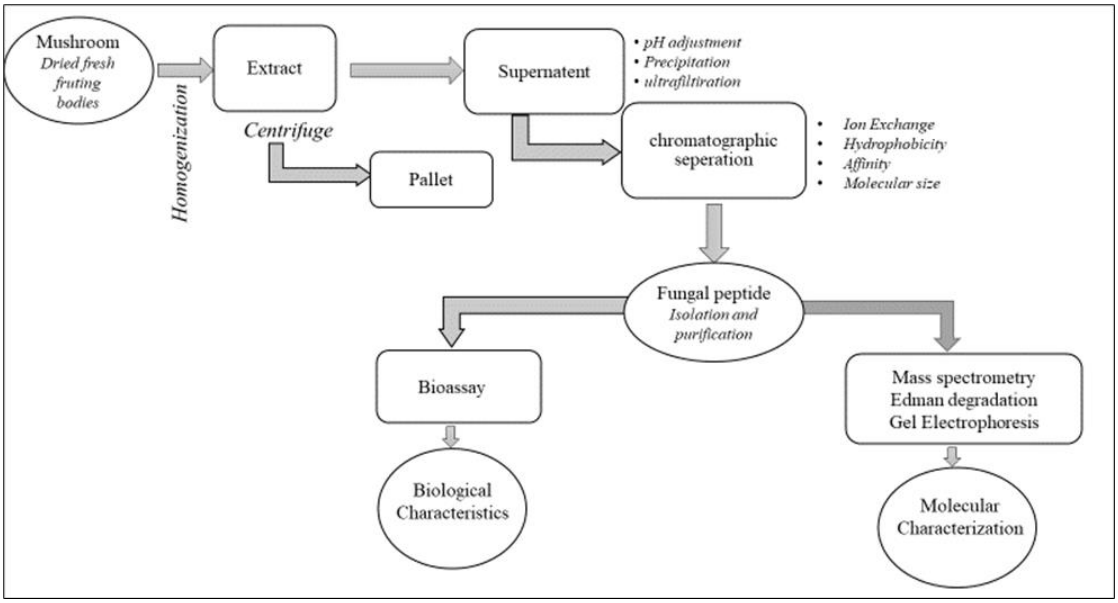

Fig 1: Process flow diagram for the extraction, isolation, purification, and molecular characterisation of peptides.

Biological Properties of MBAPs

Many BPs formed from proteins are inactive until they are released (enzymatic hydrolysis) from parental-derived proteins, at which point they take on their specialised role. BPs can have favourable impacts on human health and are implicated in the reduction/prevention of chronic diseases, for which these compounds have acquired prominence as functional foods and nutraceuticals (Séverin & Wenshui, 2005) [80]. The different role activities attributed to the BPs can be summarized as mineral building, Antioxidant, antithrombotic, anticancer, antibiotic and antimicrobial, antifungal, and antihypertensive for instance, dairy products contain peptides exerting pharmacological effects similar to opium, also known as opioid peptides (Aluko, 2015) [4]. Mushrooms are one of the most important natural sources of bioactive compounds with numerous therapeutic properties. Commonly used in traditional Chinese medicine for centuries, mushrooms are rich in natural antioxidative, antitumor, antiviral, antimicrobial, and immunomodulatory agents, with medicinal effects proven by researchers. Researchers in the food and pharmaceutical industries are increasingly interested in mushrooms (Landi *et al*., 2022) [51]. In Japan and other Asian countries, many species have long been employed in traditional Chinese remedies or functional foods. The use of secondary metabolites from mushrooms to develop new medications or lead chemicals is gaining popularity these days. Small molecule chemicals, polysaccharides, proteins, polysaccharide-protein complexes, and other bioactive ingredients have been extracted from mushrooms (Ferreira *et al*., 2010).

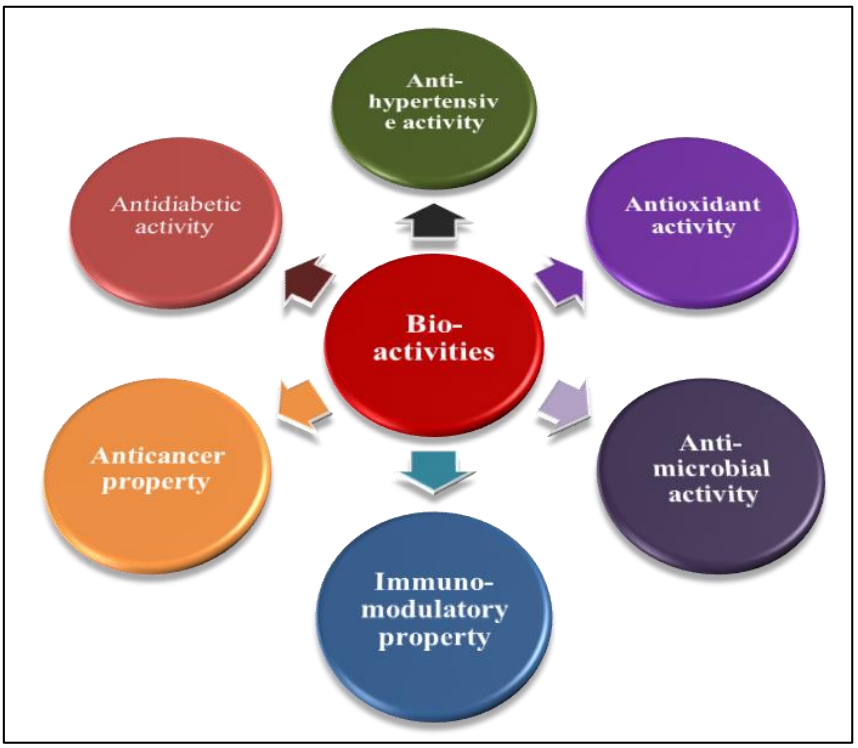

Fig 2: Biological activities of Mushroom bioactive peptides

Antihypertensive activity of MBAPs

Hypertension (also known as high blood pressure) has several negative health consequences and is dubbed the "silent killer" since it causes several organ dysfunctions such as cardiovascular disease, strokes, and renal difficulties (Mohamed Yahaya *et al*., 2014) [64]. As a result, preventing or controlling hypertension is critical in preventing the onset of a variety of diseases, including cardiovascular disease, which has been identified as the leading cause of death worldwide (Singh *et al*., 2014) [86]. The renin-angiotensin system (RAS), the kinin-nitric oxide system (KNOS), the neutral endopeptidase system (NEPS), and the renin-chymase system (RCS) are among the biochemical systems that regulate blood pressure (Shahidi & Li, 2014) [81]. RAS is thought to be the most important control mechanism for blood pressure regulation, with renin and ACE as crucial regulators. Among these ACE has a twofold effect on blood pressure, making it the key target in antihypertensive treatment (Bechaux *et al*., 2019; Shahidi & Li, 2014) [8, 81]. According to Bechaux *et al*., (2019) [8] and Ryan *et al*., (2011) [75] ACE inhibitory peptides, in contrast to synthetic ACE inhibitors such as captopril, which have a variety of side effects (e.g., coughing and skin rashes), are intriguing alternatives due to their high bioavailability and absence of adverse effects. As a result, they are the most thoroughly examined BAPs. Ryan *et al*., (2011) [75] further added it's worth noting that there's a disconnect between ACE inhibitory peptide *in vitro* and *in vivo* test results, which might be attributable to *in vivo* intestine changes.

Because mushrooms are high in protein, they might be a useful source of antihypertensive peptides (Lau *et al*., 2012) [54]. All aqueous extracts of fruiting bodies from nine edible mushroom species suppressed more than 70% of ACE *in vitro* at 10 mg/mL. Hu *et al*., (2016) [36] found an ACE inhibitory peptide purified from a water extract of Tricholoma matsutakei (a highly prized mushroom) fruiting bodies with an IC50 value of 0.40 M, which was lower than the IC50 values of all whey- and casein-derived ACE inhibitory peptides and most meat- and byproducts-derived ACE inhibitory peptides listed in two reviews by Hernández-Ledesma *et al*., (2011) [34] and Bechaux *et al*. (2019) [8] respectively. WALKGYK was found in its AA sequence. It was then chemically synthesized and given the name TMP. TMP was orally provided to SHRs to validate it's *in vivo* ACE inhibitory effect, and findings showed that TMP could significantly (p 0.05) lower SBP in SHRs at doses of 25 mg/kg and 50 mg/kg of BW, with a maximal drop of 18 mmHg and 36 mmHg, respectively, 2 h post-administration.

During or after the trial, no negative effects such as allergic responses or coughing were seen. TMP was also shown to be a non-competitive ACE inhibitor with excellent thermostability (40–90 °C) and pH stability (2–11), as well as antioxidant action. As a result, it has been proposed by (K. Hu *et al*., 2016) [36] that T. matsutake be utilised as a functional food to aid in the prevention of hypertension-related disorders.

Two putative ACE inhibitory peptides from the mushroom Pleurotus cystidiosus were discovered, with the following AA sequences and IC50 values: AHEPVK (IC50: 62.8 M) and GPSMR (IC50: 277.5 M). After *in vitro* GI digestion, their ACE inhibitory effects were maintained or even improved. The peptide with the AA sequence of GPSMR was hydrolyzed after GI digestion, and it was expected that it would release a genuine ACE inhibitor, GP, from its precursor (Lau, Abdullah, Aminudin, *et al*., 2013) [52, 53].

Table 1: Mushroom-derived ACE inhibitory peptides.

Mushroom species	AA sequence	IC50	Mechanism	References
Hypsizygus marmoreus	LSMGSASLSP	190 µg/mL	Non-competitive	(Kang *et al*., 2013) [39]
Pleurotus cystidiosus	AHEPVK GPSMR	62.8 µM 277.5 µM	Competitive ND	(Lau, Abdullah, & Shuib, 2013) [52, 53]
Agaricus bisporus	AHEPVK RIGLF PSSNK	63 µM 116 µM 129 µM	Competitive Competitive Non-competitive	(Lau *et al*., 2014) [55]
Tricholoma matsutake	WALKGYK LLVTLKK IISKIK ILSKLK LIDKVVK	0.40 µM 0.95 µM 1.19 µM 4.02 µM 0.62 µM	Non-competitive ND ND ND ND	(Geng *et al*., 2016) [28]
Ganoderma Lucidum	QLVP QLDL QDVL	127.9 µM 151.5 µM 155.8 µM	mixed-type Non-competitive Competitive	(Q. Wu *et al*., 2019) [96]

Antioxidant activity of MBAPs

During normal cellular metabolism, free radicals and reactive species, such as reactive oxygen species (ROS), are constantly created in the human body (Liu *et al*., 2016) [58]. Endogenous antioxidant systems are responsible for the timely removal of free radicals and reactive species; however, in some cases, such as inflammation, smoking, air pollution, drug use, and irradiation, endogenous antioxidant systems can become overwhelmed, resulting in oxidative stress, which can lead to cancer, ageing, and atherosclerosis (Bechaux *et al*., 2019; Singh *et al*., 2014) [8, 86]. Antioxidants can remove free radicals directly by providing protons and/or electrons, or indirectly by inhibiting endogenous oxidases, increasing the activity of antioxidant enzymes (e.g., via activation of the Keap1-Nrf2 signalling pathway), and chelating metal ions involved in radical production (Jin *et al*., 2013; Perron NR & Brumaghim JL, 2009; Szeto, 2006; Yang *et al*., 2018) [38, 72, 90, 98]. There is an inverse association between dietary antioxidant consumption and the likelihood of acquiring oxidative stress-related illnesses (B. Hu *et al*., 2013) [35]. Because food-derived antioxidants have little or no negative effects, there has been a surge in interest in identifying antioxidants from diverse food sources across the world (Samaranayaka & Li-Chan, 2011) [76]. Several studies have found that mushrooms are high in antioxidants such as polysaccharides, phenols, proteins, peptides, carotenoids, ergosterol, vitamins C and E, and others

(Islam *et al*., 2019; Kozarski *et al*., 2015; C. Sánchez, 2017) [37, 48, 78]. Mushrooms can also be cultivated more quickly than plants, making them a reasonably plentiful source of natural bioactive chemicals for commercial purposes (Chandra *et al*., 2020) [15]. Antioxidant polysaccharides and phenols have received a lot of attention over the years, whereas antioxidant peptides generated from mushrooms have gotten less attention.

G. lucidum is a medicinal mushroom that has been used for centuries to treat a variety of ailments (Cao *et al*., 2018) [11]. It's also a good source of natural bioactive chemicals, including antioxidants (B.S. *et al*., 2016) [6]. Sun *et al*., (2004) [89] isolated an antioxidant peptide fraction from a water extract of fermented G. lucidum powder. With an IC50 of 27.1 g/mL, G. lucidum peptides (GLP) inhibited soybean lipoxygenase activity. GLP with an IC50 value of 25 g/mL suppressed hydroxyl radicals generated in a deoxyribose test. *In vitro*, GLP has a considerable (p 0.05) antioxidant activity, which was determined by looking at peroxidation in the mitochondrial membrane and rat liver tissue homogenates. As a result, it was determined that GLP was the predominant antioxidant agent in G. lucidum, notably in decreasing lipid peroxidation in biological systems, as shown by an *in vivo* experiment. GLP at a dose of 180 mg/kg BW exerted significant (p 0.01) hepatoprotective effects in D-galactosamine-induced liver injury in mice via its antioxidant activity, as evidenced by a significant (p 0.01) decrease in the activities of serum aspartate transaminase (AST) and alanine transaminase (ALT), and malondialdehyde (MDA) levels in the liver, as well as activity of superoxide dismutase (SOD) and GSH levels in the liver also show some inflation (Shi *et al*., 2008) [84]. Girjal *et al*., (2012) [30] identified a peptide with a molecular weight of 3.35 kDa from a water extract of G. lucidum fruiting bodies in another investigation. This peptide (0.2 mg) was shown to scavenge 74.21 percent of DPPH radicals, 72.16 percent of superoxide anion radicals, and 72.87 percent of hydroxyl radicals, which was similar to what ascorbic acid did (5 mg). This peptide has a lot of Phe, Asp, Pro, His, and Ile, according to AA analysis. In comparison to other studies, the authors argued that a low MW and unique AA composition were responsible for enhanced antioxidant activity (Girjal *et al*., 2012) [30], which agrees with Zou *et al*., (2016) [105] findings's.

Table 2: Antioxidant peptides derived from mushrooms.

Mushroom species	Molecular Characterstics	Values and methods of evaluation	References
Grifola frondosa	<3 kDa fraction (2385 and 1138 Da)	DPPH radical scavenging activity, 89.6% at 2.5 mg/mL Ferric reducing power, 2.71 at 2.5 mg/mL Ferrous ion chelating activity Lipid peroxidation inhibition	(Dong *et al*., 2015) [21]
Tricholoma matsutake	WALKGYK	DPPH radical scavenging activity, 50% at 10 mg/mL	(Geng *et al*., 2016)
Agaricus bisporus	1–3 kDa Rich in hydrophobic and negatively charged AAs	DPPH radical scavenging activity, IC50 = 0.13 mg/mL Ferrous ion chelating activity Ferric reducing power Lipid peroxidation inhibition	(Kimatu *et al*., 2017) [43]
Agaricus bisporus	Peptide mixtures	DPPH radical scavenging activity, 73.68% Ferrous ion chelating activity, 11.75% Ferric reducing power, 0.282 Lipid peroxidation inhibition, 79.71% (hydrolysate at 0.25 mg/mL)	(Farzaneh *et al*., 2018) [24]
Terfezia claveryi	Peptide mixtures	DPPH radical scavenging activity, 51.50% Ferrous ion chelating activity, 21.36% Ferric reducing power, 0.271 Lipid peroxidation inhibition, 85.85% (hydrolysate at 0.25 mg/mL)	(Farzaneh *et al*., 2018) [24]
Morchella esculenta	Peptide mixtures	DPPH radical scavenging activity, IC50 = 6.03 mg/mL ABTS radical scavenging activity, IC50 = 0.071 mg/mL H2O2 scavenging activity, IC50 = 5.28 mg/mL Ferric reducing power, 4.76 µg Vc/mg sample Nitrite and superoxide anion radical scavenging activity Total antioxidant activity	(Q. Zhang *et al*., 2018) [101]
Cordyceps sinensis	Peptide mixtures	DPPH radical scavenging activity, IC50 = 4.79–18.7 mg/mL ABTS radical scavenging activity, IC50 = 4.51–14.05 mg/mL Heavy metal ions chelating effect	(Mishra *et al*., 2019) [62]

Antimicrobial activity of MBAPs

To protect themselves from environmental infections, mushrooms produce low MW antimicrobial compounds like terpenes and steroids, as well as high MW molecules like polysaccharides, proteins, and peptides (Stajic *et al*., 2018) [88]. Mushrooms contain a lot of natural antibiotics (Sivanandhan *et al*., 2017) [87]. Antimicrobial peptides have gained popularity in recent years due to their excellent effectiveness and specificity, low drug interaction and toxicity, and lack of resistance development by bacteria (Kaur-Boparai, 2022) [41].

With IC50 values of 10 M, 50 M, 80 M, and 0.75 mM, cordymin, an antifungal peptide derived from the medicinal mushroom C. militaris, suppressed mycelial development in Mycosphaerella arachidicola, Bipolaris maydis, Rhizoctonia solani, and Candida albicans, respectively. Cordymin also exhibited excellent thermostability (100 °C), pH stability (6–13), and mental ion stability (unaffected by 10 mM Mg2+ and

10 mM Zn2+) (Wong *et al*., 2011) [94].

Poompouang & Suksomtip (2016) [73], isolated another unique antifungal peptide with a molecular weight of 17 kDa from the fruiting bodies of the edible mushroom Lentinus squarrosulus yielded At a dose of 30 g/disc, it showed high antifungal activity against a variety of human fungal pathogens, particularly two clinical isolates, Trichophyton mentagrophytes and Trichophyton rubrum, with inhibition zone diameters of 25.7 mm and 22.8 mm, respectively. However, it lacked antimicrobial properties.

Mygind *et al*., (2005) [66] isolated plectasin from seudoplectania nigrella, an antibacterial peptide that was the first defensin (endogenous peptide antibiotics) to be identified from a fungus.

Antiviral peptides generated from mushrooms are rare compared to antifungal and antibacterial peptides. The human immunodeficiency virus (HIV) is the cause of acquired immunodeficiency syndrome (AIDS), a virus that is one of the most difficult to cure diseases in the world (Cui *et al*., 2006) [17]. Wang *et al*., (2007) [93] used mushrooms to identify anti-HIV peptides that target the HIV-1 reverse transcriptase (RT) and found that at 1 mg/mL, hot water extracts of Trametes suaveolens, Lactarius camphoratus, Pleurotus pulmonarius, Sparassis crispa, Russula paludosa, and P. sajor-caju all inhibited HIV-1 RT activity by more than 50%, with the R. paludosa extract having the greatest inhibitory effect (97.6%).

Unlike antimicrobial proteins, mushroom-derived antimicrobial peptides lack specific bioactivity. These peptides can bind easily to negatively-charged membranes of microbes because they exhibit amphipathic and cationic properties, which make them effective antimicrobials (Kim & Wijesekara, 2010) [42]. It is important to note that most of them do not demonstrate specific mechanisms of their inhibitory actions or the flexibility of their application. Using different mechanisms, antimicrobial peptides may reduce the likelihood of microbial resistance in the body (J. Zhou *et al*., 2020) [103].

The majority of antimicrobial peptides formed from mushrooms are endogenous, and a study of their characteristics revealed that some of them are unique peptides, indicating that there may be additional or different ways of inhibitory activity; however, this has to be confirmed.

Immunomodulatory property

According to neoteric research, a contemporary sedentary lifestyle increases the risk of cancer, coronary heart disease, and strokes, as well as having negative effects on the immune system (Agyei & Danquah, 2012) [2]. Agyei & Danquah, (2012) [2] stated the mechanism of action of immunomodulatory peptides is unclear and neglectful. Peptides can also be employed to produce novel types of medications, despite these limitations. Immunomodulatory peptides have immunostimulant as well as immunosuppressive properties. Immunostimulant peptides boost immunological strength, whereas immunosuppressive peptides are utilized to treat autoimmune diseases and organ rejection (Gauthier *et al*., 2006) [27].

Immunostimulant peptides boost immunological strength, whereas immunosuppressive peptides are utilized to treat autoimmune diseases and organ rejection (Gauthier *et al*., 2006) [27].

Maestri *et al*., (2016) stated that because these peptides do not accumulate in the body over time, there is less risk of toxicity. Immunomodulatory peptides are thought to be a safe alternative to pharmaceutical drugs such as chemokines, antibiotics, and cytokines They also trigger the activity of natural killer cells and macrophage phagocytosis. They are considered curative agents since they have neuronal and hormonal mechanisms.

Sheu *et al*., (2004) [83] purified APP, a novel immunomodulatory protein (APP) from edible Jew's ear mushroom using DE-52 and MonoQ anion-exchange chromatography, ammonium sulphate fractionation, and 5% cold acetic acid in the presence of 0.1% 2-mercaptoethanol. APP is a simple non-carbohydrate protein that can agglutinate red blood cells in mice.

Anticancer property

Cancer has been on the increase throughout the world in recent years. Despite advances in cancer research and treatment procedures, it continues to be a major source of illness and mortality. Chemotherapy and radiation, two common cancer treatments, are frequently accompanied by significant side effects (Rodrigues *et al*., 2009). Peptides have gained interest as a possible alternative to conventional cancer medicines due to the need for innovative treatments that are more biocompatible and have fewer adverse effects. One of the most important features is its tiny size, which allows it to penetrate tissue more easily (Ortiz-Martinez *et al*., 2014) [71]. By fighting signals of growth inhibition, cancer cells can avoid cell death and reproduce. As a result, triggering apoptosis would be a good way to deal with them. Studies have shown that peptides can stimulate apoptosis and angiogenesis (De Mejia & Dia, 2010) [20], both of which are important factors in tumor metastasis, and that bioactive peptides are readily available in fruits and vegetables, allowing for the development of anti-cancer therapeutics in the future (Sarmadi & Ismail, 2010) [79]. Induction of apoptosis by activating the apoptotic pathway by modulating caspases, the revival of p53 (Burz *et al*., 2009) [9] activity, inhibition of Bcl-2 proteins, IAP, and proteasome (Call *et al*., 2008) [10], necrosis initiation, bodily immune boost, and angiogenesis inhibition are some of the mechanisms by which bioactive peptides exert anti-proliferative effects (Sharma *et al*., 2019) [82].

Challenges

Despite having a range of advantages, bioactive peptides suffer some difficulties, including technological, economic, and regulatory concerns, which prevents their full potential from being realized. Production is the main concern. There is a lack of industrial-scale production from an economic standpoint. Laboratory-scale preparations are ineffective for large-scale production and are low-key. Major difficulties preventing their effective commercialization as medicinal medicines include limitations of industrial scaleup (Pihlanto-Leppala, 2002).

Continuous reactor and membrane ultrafiltration have been used to produce peptides continuously in recent years (Korhonen & Pihlanto, 2003) [47]. Fouling, however, is a significant drawback and a barrier to industrial scaleup. These issues raise the price of producing peptides, which is one of its downsides. According to a review by Brady *et al*. (2008), up to 70% of the capital expenditures for industrial production are thought to be spent on the separation and purification

processes. To produce a high output of bioactive peptides, efficient and affordable technologies for peptide fraction optimization, separation, and enrichment must be developed (Korhonen & Pihlanto, 2006) [46].

In their investigation of immunomodulatory peptides, Korhonen & Pihlanto (2007) [47] discovered that because of their small size, these peptides react with other dietary ingredients during processing, creating byproducts that potentially pose serious health risks.

Companies have chosen *in vitro* research over *in vivo* and clinical trials because they are less expensive, despite Foltz *et al*., (2010)'s [25] review opposition. Only after research on a peptide's absorption, distribution, metabolism, and excretion (ADME) profile can the efficacy of that peptide be accepted as real (Foltz *et al*., 2010) [25]. It is crucial to research their fate in the GI tract even before looking at their kinetics. An antihypertensive peptide called blactoglobulin failed to exhibit its action after being broken down by gastrointestinal and serum proteases, as shown by (Walsh *et al*., 2004) [92]. The bioactive peptides must therefore undergo the necessary testing before they may be used therapeutically.

In addition to these technological, safety, and efficacy problems, bioactive peptide regulations present another difficulty. For the protection of customers, there must be strict rules governing the creation, distribution, and use of bioactive peptide products.

Conclusions and future perspectives

Mushrooms are a good source of BAPs since they contain a lot of high-quality proteins. Many BAPs, the majority of which are endogenous peptides, including antihypertensive, antioxidant, antibacterial, anticancer, and other BAPs, have currently been found in various mushrooms. Only a small number of BAPs retrieved from mushrooms are now known, in contrast to the enormous number of BAPs isolated from animals and plants. A total of 140,000 species have been identified as fungal species, of which roughly 2,000 are edible and 200 have historically been collected for use as food or medicinal.

A. bisporus, C. sinensis, and G. lucidum are three of the most frequently studied among them since they are three of the most widely consumed and cultivated species. Many other foods have been found to have bioactive properties, such as one that lowers cholesterol, but MBAPs have not yet been confirmed to have any of these properties. Therefore, mushrooms represent a rich and vast, but mostly unexploited, source of BAPs. The relationship between peptides' chemical structures and their functions and mechanisms of action is well known. Consequently, MBAPs have achieved many achievements, especially regarding antihypertensive peptides. In spite of this, most mushrooms are still consumed in their natural form and MBAPs are rarely used in commercial products. Research in this field should focus on more in-depth studies, such as *in vivo* testing and investigation of the underlying mechanism of action, since it is relatively undeveloped.

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