Bioactive peptides from mushroom proteins

Adheeti Jain, Rahul V Rathod and Rushikesh Subhash Pharate

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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) [121]. 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) [122]. 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) [106]. 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; Nagaoa 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) [40]. 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-phthaldialdehyde, 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) [13]. 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) [20]. 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

<table>
<thead>
<tr>
<th>Company name</th>
<th>The source</th>
<th>The sequence of amino acids</th>
<th>Benefits</th>
<th>In the marketplace</th>
<th>Production method</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactium®</td>
<td>Milk</td>
<td>YLGYLEQLLR</td>
<td>De-stressing</td>
<td>Supplements and drinks</td>
<td>Trypsin-enzymatic hydrolysis</td>
<td>(Nagai et al., 2006) [66]</td>
</tr>
<tr>
<td>Calpis</td>
<td>Sour milk</td>
<td>VPP y IPP</td>
<td>An antihypertensive effect</td>
<td>Drinks</td>
<td>Anaerobic fermentation with S. cerevisiae and L. helveticus CP790</td>
<td>(Siltari et al., 2012) [85]</td>
</tr>
<tr>
<td>Lacprodan® Whey</td>
<td>Whey</td>
<td>N/A</td>
<td>Maintains normal blood sugar levels</td>
<td>The powder product</td>
<td>Alcalse-enzymatic hydrolysis</td>
<td>(Chalamaiah et al., 2019) [13]</td>
</tr>
<tr>
<td>Capolac®</td>
<td>Milk</td>
<td>CPP</td>
<td>Absorption of calcium</td>
<td>Ingredient</td>
<td>The isolation process</td>
<td>(Chalamaiah et al., 2019) [13]</td>
</tr>
<tr>
<td>Cholesterol block</td>
<td>Soy</td>
<td>CSPHP</td>
<td>Reduction of cholesterol</td>
<td>Drink</td>
<td>Enzymatic hydrolysis</td>
<td>(Chalamaiah et al., 2019) [13]</td>
</tr>
</tbody>
</table>

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). 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). To date, the most efficient and prevalent method of producing BAPs is the enzymatic hydrolysis of food proteins (A. Sánchez & Vázquez, 2017). It has been reported that MBAPs with antioxidative, antimicrobial, anti-diabetic, 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). 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). 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 (Ageyi & Danquah, 2011). 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). 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 factions, 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). 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 (5AAAs), are difficult to identify when using tandem-MS because a small number of MS fragments are obtained (Lahriri et al., 2013).

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). 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). Yeast and filamentous fungi have also been employed to produce bioactive peptides in addition to bacteria starters (Giri, 2012; Lima et al., 2015). 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). 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).

Microbial fermentation consists of several systems. Submerged fermentation and solid-state fermentation are, however, the most common methods of fermentation (Nadu, 2012). 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). 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). 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).

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). 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).
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]. 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. WALGKYK was found in its AA sequence. It was then chemically synthesized and given the name TMP. TMP was orally provided to SHR to validate it's in vivo ACE inhibitory effect, and findings showed that TMP could significantly (p 0.05) lower SBP in SHR's 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) [96] 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 competitive and non-competitive ACE inhibitory peptides. Some studies have shown that mushrooms are high in antioxidants such as polysaccharides, phenols, proteins, peptides, carotenoids, ergosterol, vitamins C and E, and others

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]. 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 antigens 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

<table>
<thead>
<tr>
<th>Mushroom species</th>
<th>AA sequence</th>
<th>IC50</th>
<th>Mechanism</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypsizygus marmoreus</td>
<td>LSGASLSP</td>
<td>190 μg/mL</td>
<td>Competitive</td>
<td>(Kang et al., 2013) [39]</td>
</tr>
<tr>
<td>Pleurotus cystidiosus</td>
<td>AHEPVK</td>
<td>62.8 μM</td>
<td>Competitive</td>
<td>(Lau, Abdullah, &amp; Shuib, 2013) [52, 53]</td>
</tr>
<tr>
<td></td>
<td>GPSMR</td>
<td>277.5 μM</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Agaricus bisporus</td>
<td>AHEPVK</td>
<td>63 μM</td>
<td>Competitive</td>
<td></td>
</tr>
<tr>
<td></td>
<td>RIGLF</td>
<td>116 μM</td>
<td>Competitive</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PSSNK</td>
<td>129 μM</td>
<td>Non-competitive</td>
<td></td>
</tr>
<tr>
<td>Tricholoma matsutake</td>
<td>WALKGYK</td>
<td>0.40 μM</td>
<td>Non-competitive</td>
<td>(Geng et al., 2016) [28]</td>
</tr>
<tr>
<td></td>
<td>LLVTLKK</td>
<td>0.95 μM</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ILSKIK</td>
<td>1.19 μM</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ILSKLK</td>
<td>4.02 μM</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td></td>
<td>LDKVVK</td>
<td>0.62 μM</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Ganoderma Lucidum</td>
<td>QLVP</td>
<td>127.9 μM</td>
<td>mixed-type</td>
<td>(Q. Wu et al., 2019) [96]</td>
</tr>
<tr>
<td></td>
<td>QDL</td>
<td>151.5 μM</td>
<td>Non-competitive</td>
<td></td>
</tr>
<tr>
<td></td>
<td>QDV</td>
<td>155.8 μM</td>
<td>Competitive</td>
<td></td>
</tr>
</tbody>
</table>
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) [38]. 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) [18]. 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 activity (Girjal et al., 2012) [30]. It's also a good source of natural bioactive chemicals, making them a reasonably plentiful source of natural antioxidants for commercial purposes (Chandra et al., 2019) [11]; Kozarski et al., 2015; C. Sánchez, 2017) [37, 48, 78].

Table 2: Antioxidant peptides derived from mushrooms.

<table>
<thead>
<tr>
<th>Mushroom species</th>
<th>Molecular Characteristics</th>
<th>Values and methods of evaluation</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grifola frondosa</td>
<td>&lt;3 kDa fraction (2385 and 1138 Da)</td>
<td>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</td>
<td>(Dong et al., 2015) [21]</td>
</tr>
<tr>
<td>Tricholoma matsutake</td>
<td>WALKGYK</td>
<td>DPPH radical scavenging activity, 50% at 10 mg/mL, Ferric reducing power, Lipid peroxidation inhibition</td>
<td>(Geng et al., 2016)</td>
</tr>
<tr>
<td>Agaricus bisporus</td>
<td>Rich in hydrophobic and negatively charged AAs</td>
<td>DPPH radical scavenging activity, IC50 = 0.13 mg/mL, Ferrous ion chelating activity, Ferric reducing power</td>
<td>(Kimatu et al., 2017) [43]</td>
</tr>
<tr>
<td>Agaricus bisporus</td>
<td>Peptide mixtures</td>
<td>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)</td>
<td>(Farzaneh et al., 2018) [24]</td>
</tr>
<tr>
<td>Terfezia claveryi</td>
<td>Peptide mixtures</td>
<td>DPPH radical scavenging activity, 31.50%, Ferrous ion chelating activity, 21.36%, Ferric reducing power, 0.271, Lipid peroxidation inhibition, 85.85% (hydrolysate at 0.25 mg/mL)</td>
<td>(Farzaneh et al., 2018) [24]</td>
</tr>
<tr>
<td>Morchella esculenta</td>
<td>Peptide mixtures</td>
<td>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</td>
<td>(Q. Zhang et al., 2018) [101]</td>
</tr>
<tr>
<td>Cordyceps sinensis</td>
<td>Peptide mixtures</td>
<td>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</td>
<td>(Mishra et al., 2019) [62]</td>
</tr>
</tbody>
</table>

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 seudolpectania 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 (Cai et al., 2006) [11]. 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) [45]. 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) [7], 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) [62].

### 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) [93]. 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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