8-1-2024

Research trends in production, separation, and identification of bioactive peptides from fungi: A critical review

Tanvir Ahmed
Edith Cowan University

Angéla Juhász
Edith Cowan University

Utpal Bose
Edith Cowan University

Netsanet Shiferaw Terefe

Michelle L. Colgrave
Edith Cowan University

10.1016/j.jff.2024.106343
Ahmed, T., Juhász, A., Bose, U., Terefe, N. S., & Colgrave, M. L. (2024). Research trends in production, separation, and identification of bioactive peptides from fungi–A critical review. *Journal of Functional Foods, 119*, Article 106343. https://doi.org/10.1016/j.jff.2024.106343

Journal of Functional Foods 119 (2024) 106343

Contents lists available at ScienceDirect

Journal of Functional Foods

journal homepage: www.elsevier.com/locate/jff

Research trends in production, separation, and identification of bioactive peptides from fungi – A critical review

Tanvir Ahmed [a], Angéla Juhász [a], Utpal Bose [a,b], Netsanet Shiferaw Terefe [c], Michelle L. Colgrave [a,b,*]

[a] Australian Research Council Centre of Excellence for Innovations in Peptide and Protein Science, Edith Cowan University, School of Science, Perth, WA 6027, Australia
[b] CSIRO Agriculture and Food, St Lucia, QLD 4067, Australia
[c] CSIRO Agriculture and Food, Werribee, VIC 3030, Australia

ARTICLE INFO

Keywords:
Fungal proteins
Bioactive peptides
Novel technologies
Innovative methods
Bioactive properties

ABSTRACT

Fungal-derived bioactive peptides (BPs) are increasingly attracting significant academic and industry research interest due to their high-quality protein content and potential environmentally friendly credentials. Fungi encompass a diverse range of species, some of which remain underexplored with demonstrated the ability to synthesise complex peptides with unique and potent bioactivities. While some recent reviews have focused on fungal protein sources and BPs, there remains a knowledge gap in the technologies and methods used to produce, isolate, and identify BPs from various fungal species. This article critically evaluates the strengths, challenges, and future directions of the recent methods and technologies employed in fungal BP research, addressing the limitations of traditional peptide production, separation, and identification methods. This review discusses multiple studies on using fungal strains to release BP from various food protein substrates through fermentation methods. Additionally, it highlights the production of BP from different fungal strains using endogenous and exogenous enzymes. The challenges associated with fungal cell wall disruption for BP production are addressed and several recent studies employing a combination of enzymes and innovative processing technologies to overcome these challenges are discussed. Moreover, the review covers various separation and purification techniques used in fungal BP studies, outlining their advantages and limitations. The role of genomics, advanced mass spectrometry, multi-omics approaches, *in-silico* resources, and bioinformatics tools for discovering BP from fungi are covered along with the application of fungal BPs in the food, pharmaceutical, and cosmeceutical industries.

1. Introduction

In recent years, the physiological role of individual food-derived proteins within the human diet and animal feed has gained recognition. Dietary proteins can exert physiological effects directly or when released as bioactive peptides (BPs) through enzymatic hydrolysis. They can serve as a reservoir for BPs, which are inactive within the parent protein sequence but are rendered active after proteolysis by endogenous or exogenous enzymes or as a result of various processes such as gastrointestinal digestion and food processing (including cooking and fermentation).

BPs typically have amino acid sequences of two to thirty residues, with a low molecular weight (MW) of <5,000 Da compared to a complete protein sequence. Research has revealed that BPs from various food sources have a broad spectrum of biological effects, such as memory and cognitive function, appetite regulation, opiate-like actions, hepatoprotective, anti-diabetic, antimicrobial, antioxidant, anti-inflammatory, immunomodulatory, hypocholesterolemia, anticancer, antibacterial, antifungal, antiviral, and antihypertensive activities (Martinez-Medina et al., 2019).

Due to the balanced essential amino acids, animal peptides have been considered major contributors to human diets and the subject of extensive research. However, recent years have seen a growing interest in plant-, algae-, and edible insect-based peptides driven by the

* Corresponding author at: Australian Research Council Centre of Excellence for Innovations in Peptide and Protein Science, Edith Cowan University, School of Science, Perth, WA 6027, Australia.
E-mail addresses: tanvir.ahmed@ecu.edu.au (T. Ahmed), a.juhasz@ecu.edu.au (A. Juhász), u.bose@ecu.edu.au (U. Bose), netsanet.shiferawterefe@csiro.au (N. Shiferaw Terefe), m.colgrave@ecu.edu.au (M.L. Colgrave).

https://doi.org/10.1016/j.jff.2024.106343
Received 14 March 2024; Received in revised form 12 June 2024; Accepted 2 July 2024
Available online 10 July 2024

increased protein demands of a growing population, as well as environmental, ethical and health considerations. Plant-based peptides are emerging as a complement to animal sources due to their cost-effectiveness, widespread availability, and diverse biological activities. A significant advantage of plant-derived BPs is their general acceptance across different cultures and belief systems, making them a morally and ethically uncontroversial choice. Unlike animal sources, plants are globally recognised as suitable raw materials for various industries, simplifying the sourcing and utilisation of plant-derived BPs. However, the enzymatic hydrolysis of plant-derived proteins frequently results in an undesirable bitterness (Wei et al., 2018). Despite showcasing promising bioactivity, algal peptides remain less understood than those from other sources. Notably, the considerable variation in algal protein content, influenced by factors like season, temperature, and harvest location, poses challenges for consistent BP production (Fan et al., 2014). On the other hand, edible insects could be a sustainable source of peptides due to their low footprints and environmental production costs. Overcoming potential allergen concerns, consumer acceptance, establishing efficient rearing facilities, and developing optimal processing technologies are crucial challenges toward unlocking their full potential as a peptide source.

There has been a rising interest in exploring fungi as a potential source of BPs, driven by several compelling factors (Fig. 1). Fungi encompass a vast and diverse range of species, with a substantial portion remaining underexplored, particularly those within the *Basidiomycetes* group, which holds promise as a valuable reservoir of uncharted bioactive proteins (Kehinde and Sharma, 2020). Furthermore, fungi have demonstrated their capacity to synthesise peptides featuring complex and distinct structures, often challenging to replicate using traditional chemical methods, providing them with specialised and potent bioactivities. Additionally, fungi exhibit exceptional adaptability to various environments, even those characterised by harsh conditions, enabling the generation of peptides with remarkable functional properties. Consequently, there is a growing recognition of the potential of BPs derived from fungi for pioneering and environmentally friendly advancements in nutrition, biotechnology, and pharmaceuticals. For example, the commercially produced fungal (*Pseudoplectania nigrella*) antimicrobial peptide Plectasin (Novozyme®, Denmark) holds promise against *Staphylococcus aureus*, achieving 100 % growth inhibition at 100 μg/mL (Li et al., 2017). Caspofungin (derived from a fermentation product of *Glarea lozoyensis*), a unique lipopeptide composed of a cyclic hexapeptide attached to a fatty acid side chain, was the first echinocandin antifungal agent approved for treating *Candida* infections in the USA (Song & Stevens, 2016).

Until now, limited reviews have summarised information on fungal proteins and the evolving technologies for preparing BPs from fungal proteins. A recent review paper by Wang et al. (2023a) presents a comprehensive overview of the most recent research concerning fungal protein sources, characteristics, preparation methods, and applications published over the past five years, to provide a more in-depth understanding of fungal proteins. Furthermore, Landi et al. (2022) categorised BPs derived from mushrooms based on their MW to address inconsistencies, particularly the misclassification of polypeptides with 100 or more amino acid residues as peptides. Additionally, Mirzaei et al. (2021) presented a comparative review that synthesised existing research exclusively centered on peptides derived from yeast. The paper covered topics such as production methods, structure–function relationships, stability, diverse bioactivities, and underlying action mechanisms of the identified methods. A common thread in these reviews is the recognition that the stability, transepithelial transport, and biological activities of protein hydrolysates and their peptides are influenced by the protein source and its physicochemical properties, which, in turn, are determined by the methods used for peptide preparation, identification, and quantification. Given the limitations of

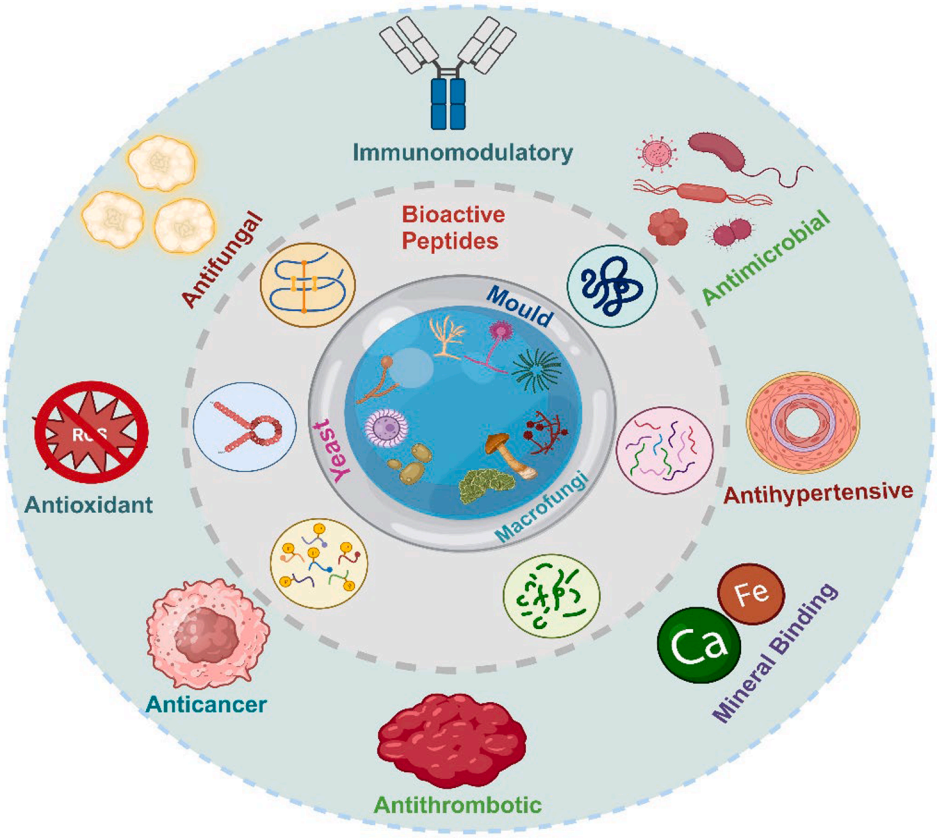

Fig. 1. Health-promoting potential of fungal-derived bioactive peptides. Created with Biorender.com.

traditional peptide isolation, identification, and quantification, researchers are actively exploring innovative approaches to improve peptide preparation from limited protein sources. Therefore, this review paper critically evaluates the strengths, potential challenges, and future directions for developing various technologies and methods for predicting, identifying, extracting, isolating, quantifying, and producing BPs from various fungal species.

2. Bioactive peptides released via fermentation of different protein substrates using fungal strains

Using fungal strains to release BPs through microbial fermentation is a captivating area in biotechnology with promising applications in various industries. This method, involving the cultivation of microfungi, bacteria, or yeasts on protein substrates, facilitates the hydrolysis of substrate proteins into BPs (Fig. 2B). Various fungal strains such as *Aspergillus oryzae*, *Aspergillus sojae*, *Flammulina velutipes*, *Rhizopus*

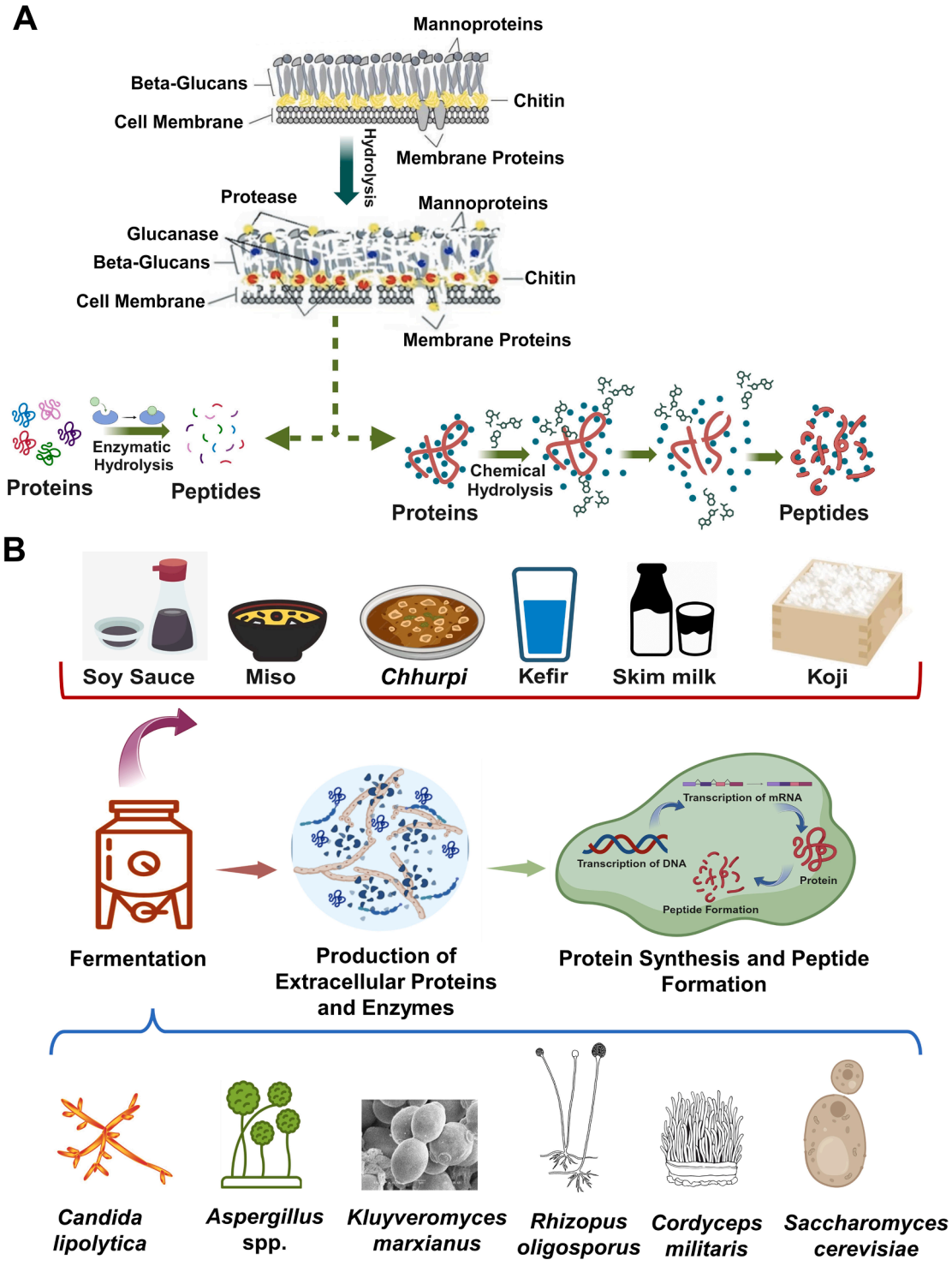

Fig. 2. Graphic representation of enzymatic hydrolysis, chemical hydrolysis (A), and fermentation of different protein substrates using fungal strains (B), resulting in the release of bioactive peptides. Created with Biorender.com.

oligosporus, *Actinomucor elegans*, *Cordyceps militaris*, *Candida lipolytica*, *Kluyveromyces marxianus*, *Neolentinus lepideus*, *Kluyveromyces marxianus*, *Saccharomyces cerevisiae*, *Stropharia rugosoannulata*, *Ganoderma lucidum* have been successfully employed for peptide production through fermentation from diverse protein sources due to their enzymatic activities, substrate preferences, and distinct metabolic capabilities (Table 1). Fungi exhibit a broad range of metabolic diversity, enabling them to efficiently utilise different carbon and nitrogen sources found in various protein substrates. The specificity of fungal strains for certain protein sources allows for selecting strains that are particularly adept at breaking down specific types of proteins, whether derived from plants or animals. Enzyme production is a critical factor, and different fungi strains produce a variety of enzymes, including proteases, essential for breaking down proteins into peptides.

Several studies have highlighted the use of yeast in the synthesis of BPs. These studies have demonstrated that yeast cells play a significant role in generating BPs through fermentation, primarily attributed to their proteolytic activity. This enzymatic activity contributes to enhancing the functional attributes of fermented foods, including okara, soy sauce, koji, miso, red bean flour, kefir, fermented milk, and soft chhurpi. Building on the long tradition of using filamentous fungi like *Aspergillus oryzae*, *Aspergillus sojae*, *Rhizopus oligosporus*, *Actinomucor elegans*, and *Peniophora* sp. in fermented foods, recent research has identified several BPs with promising antioxidant and angiotensin-converting enzyme (ACE) inhibitory activities (Table 1). In one study, fermenting bovine colostrum with *Candida lipolytica* MIUG D67, MIUG D99 (10^6 CFU/100 mL), and 2.5 % kefir grains for 24 and 48 h resulted in the production of peptides with high antioxidant (ABTS: 63–92 %) and ACE inhibitory activity (88.6–94.4 %) (Gaspar-Pintiliescu et al., 2020). These findings underscore yeast's positive role in increasing metabolite content with biological activities during fermentation. Although lactic acid bacteria (LAB) are well-known producers of BPs, proteolytic yeasts offer a promising alternative avenue for generating BPs. These yeasts naturally reside in fermented milk products and possess potent enzymes, including carboxypeptidases and aminopeptidases, which efficiently break down milk proteins. The study of Rai et al. (2016) indicates that the utilisation of *Kluyveromyces marxianus* YMP45 and *Saccharomyces cerevisiae* YAM14 as adjunct starters, together with LAB, results in an improvement in the antioxidant activities (44–60 μg ascorbic acid equivalent (AAE)/mg) of chhurpi, a traditional cheese consumed in Bhutan, Tibet and Nepal. Moreover, the concentration of lower MW peptides (<1 kDa) significantly increased at the fermentation's end, suggesting improved digestibility and absorption of skim milk peptides in yeast-mixed fermentation (Zhang et al., 2017).

Researchers worldwide have also reported the mycelial secretion of proteases from various fungal strains and their potential application for peptide release from different protein sources. For instance, protease extract from brewer's spent yeast (0.725 U/mL) was used for hydrolysis of proteins in brewers' spent grain (Amorim et al., 2019b) and sardine sarcoplasmic protein (Vieira et al., 2017) to generate ACE inhibitory and antioxidant peptides. Similarly, a study by Pokora et al. (2017) showed that a serine protease from *Yarrowia lipolytica* could produce antioxidant peptides from egg white protein hydrolysate, indicating the potential of yeast enzymes for the release of BPs. In another study, a novel approach combined 30 h lactic acid bacteria fermentation with *Aspergillus mellius*-derived Prozyme 6 protease hydrolysis, accelerating BP production from 39.6 to 463.3 mg/g. The fermented whey product exhibited *in vitro* ACE inhibition, primarily attributed to peptides in the 800–900 Da (Tsai et al., 2006). In two different studies, *Cordyceps militaris* has been shown to produce proteases that are able to hydrolyse chickpea proteins into BPs. The peptides that formed due to the catalytic action of the proteases produced by the starter organism led to significant ACE inhibitory activity of fermented red bean flour (IC_{50}: 0.63 mg protein/mL) (Xiao et al., 2018). Similarly, the production of carboxypeptidase by *Neolentinus lepideus* enabled the production of 30–40 times more dipeptide Tyr-Pro (YP) (450 μg/mL) when compared to LAB in fermented skim milk. This was associated with twofold higher ACE inhibitory activity (Okamoto et al., 2020).

Although peptides released through microbial fermentation by fungal strains offer advantages in terms of specificity, diversity, and cost-effectiveness, certain challenges still need to be addressed. These include the potential generation of undesirable compounds, and/or the consistency and reproducibility of desirable BP liberation alongside optimisation of refining processes. Research findings suggest that

Table 1
Fungal strains release peptides through fermentation methods using various protein sources[1].

Protein source	Fungal strains	Fermentation conditions	Peptides or peptide fraction	Bio-functional activities	References
Casein (mixed with Miso paste)	*Aspergillus oryzae*	40 h, 30 °C	VPP and IPP	ACE inhibitory	(Inoue et al., 2009)
Okara	*Actinomucor elegans*	60 h, 25 °C, 95 % humidity	Peptide content: 117.00 ± 0.36 mg l-glutathione/g dw	Antioxidant and ACE inhibitory	(Guan et al., 2017)
Soy sauce	*Aspergillus sojae*	192 h, 20–45 °C	GT, AP, VP, AI, VG	ACE inhibitory	(Nakahara et al., 2010)
Koji and Miso	*Aspergillus oryzae*	Koji: 31 °C, 36 h, 95 % humidity; Miso: 28 °C, 12 weeks	γ-ECG (oxidized), γ-EVG, γ-EV, γ-EE, γ-EF, and γ-EL	Kokumi γ-glutamyl peptides	(Rodriguez Valerón et al., 2023)
Red bean flour	*Cordyceps militaris*	25 °C, 7 days	Peptide fraction with MW < 15 kDa	ACE inhibitory	(Xiao et al., 2018)
Bovine colostrum	*Candida lipolytica*	30 °C, 48 h and 72 h, 150–18 rpm	Peptide fraction with MW ≤ 10 kDa	Antioxidant, ACE inhibitory	(Gaspar-Pintiliescu et al., 2020)
Skim milk	*Kluyveromyces marxianus*	35 °C, pH: 4.6	Peptide fraction with MW 0–10 kDa	Flavour enhancement	(Zhang et al., 2017)
Skim milk	*Neolentinus lepideus*	28 °C, 7 days	YP	Anti-hypertensive	(Okamoto et al., 2020)
Cow and low-fat milk	*Peniophora* sp.	30 °C, 200 rpm.	YP	Anti-hypertensive	(Okamoto et al., 2021)
Soft chhurpi	*Kluyveromyces marxianus*, *Saccharomyces cerevisiae*	30 °C, 48 h		Antioxidant	(Rai et al., 2016)
Edible fungus	*Stropharia rugosoannulata*	26 °C, 7 days, 100 r/min.	Peptide fraction with MW ≤ 3 kDa	Taste characteristics (salty and umami)	(Li et al., 2022c)
Edible fungus (*Lentinus edodes*)	*Saccharomyces cerevisiae*, *Aspergillus oryzae*, *Aspergillus niger*	40 °C, 4 days	AG, SGTAP, HAVMILP, CYL	Taste characteristics (umami)	(Chen et al., 2021)
Edible fungus	*Ganoderma lucidum*	25.6 °C, 6 days, 146 rpm	QLVP, QDVL, QLDL	ACE inhibitory	(Wu et al., 2019)

[1] MW: Molecular weight, ACE: Angiotensin-converting enzyme.

employing physical techniques such as ultrasound for pre-treating the substrate or during fermentation can enhance mass transfer, reduce fermentation durations, and elevate productivity and quality. For instance, low-frequency ultrasound has demonstrated its potential to influence fermentation dynamics by improving mass transfer and cell permeability, leading to heightened process efficiency and production rates (Li et al., 2021). Similarly, low-intensity magnetic field-assisted fermentation can enhance mycelial biomass, polysaccharide, and triterpenoid yields in *Antrodia camphorate* and shorten the fermentation period (Li et al., 2017). Overall, in the pursuit of harnessing BPs from fungal strains, a multifaceted approach is warranted. Upon discovery, chemical synthesis offers precise control over peptide production, while molecular modelling can aid in sequence design, allowing researchers to customise peptides for desired functionalities. Likewise, chemical hydrolysis, using agents to break down proteins into BPs, presents an alternative avenue for generating diverse peptide profiles from fungal sources, although specificity could be a limiting factor. Using endogenous or exogenous enzymes to break down complex fungal proteins, on the other hand, could be a more viable strategy. This approach mimics the natural biological processes, allowing for the extraction of BPs that may not be accessible through other methods.

3. Release of fungal bioactive peptides by endogenous and exogenous enzymes

The release of BPs from fungal strains involves a combination of spontaneous biochemical reactions and those dependent on specific catalysts (Fig. 2A). These catalysts play a crucial role in minimising the energy barrier necessary for the effective transformation of substrates into products. Enzymes, among these catalysts, emerge as highly specialised entities that not only accelerate reaction rates but also contribute to the precision of metabolic processes, spanning from simple to complex biological reactions. Enzymes involved in biological reaction could be either endogenous (found in the substrate) or exogenous (sourced from other organisms and added to the substrate) hydrolysing enzymes. The main enzymes involved in the release of BPs from fungi are peptidases/proteases, which include both exopeptidases and endopeptidase. While exopeptidases cleave the terminal residues, endoproteases target internal bonds within polypeptides. Consequently, exopeptidases release a single amino acid residue, dipeptide, or tripeptide based on their type (Gurumallesh et al., 2019). On the other hand, endopeptidases break down proteins into peptides of various sizes. Crucially, both endogenous and exogenous enzymes display a sequence specificity by favouring specific amino acids adjacent to the targeted peptide bond for hydrolysis. This preference aligns with the amino acid sequence found in the proximity of the enzyme's catalytic site. The amino acid residues at the protease's catalytic site correspond to specific amino acids present in the protein substrate. In each case, these residues are labelled based on their proximity to the targeted peptide bond for hydrolysis and their orientation toward the C- or N-terminus (Tavano, 2013). As a result, diverse peptides can be produced with various enzyme and substrate combinations. While an endogenous or exogenous enzyme has the potential to catalyse the hydrolysis of multiple peptide bonds, the cleavage rate can vary significantly based on the specific bond.

3.1. Use of fungal proteases for enriching food products with bioactive peptides

Based on the amino acid residues present at the active sites and catalytic mechanisms, proteases can be grouped as glutamic acid proteases, metalloproteases, aspartic proteases, cysteine proteases, threonine proteases, and serine proteases. To date, several fungal strains have been used as a source of endogenous proteolytic enzymes to improve physicochemical, nutritional, and sensory profiles of different food products. In a study by Wang et al. (2022b), the impact of endogenous enzymes extracted from straw mushrooms (*Volvariella volvacea*) (EESM) on the flavour profile of Cantonese sausages was investigated. The results demonstrated that EESM enzymes enhanced the production of savoury amino acids and associated volatile compounds. This improvement was attributed to their ability to promote proteolysis, lipolysis, and lipid oxidation moderately and concurrently within the sausages. While EESM proved effective in influencing flavour, the research team further investigated the broader impact of incorporating straw mushrooms. Their inclusion not only enriched the sausages nutritionally with increased amino acids but also resulted in a fascinating textural transformation. The increased amino acid content, attributed to straw mushrooms' inherent abundance and proteolytic enzymes, significantly boosted the nutritional profile. Interestingly, the porous, sponge-like structure observed within the sausages suggested protein network relaxation. This, in turn, led to a subtle decrease in hardness, cohesiveness, and chewiness, hinting at the formation of a unique, "compact-spongy" texture due to interactions between mushroom enzymes and meat proteins during processing (Wang et al., 2018a). Another study reported that incorporating 2.5 % fresh *Flammulina velutipes* (FFV) resulted in softer textures compared to using the same amount of dried FFV in Cantonese sausages. This suggests that the fresh mushrooms had a higher concentration of bioactive enzymes, likely proteases, which could break down muscle proteins into smaller amino acids and peptides within the sausages (Wang et al., 2019). This observation aligns with findings from other studies using fungal enzymes from *Lentinula edodes*, *Agaricus bisporus*, and *Sarcodon aspratus* to tenderise bovine muscle, supporting the hypothesis that mushroom proteases effectively degrade meat proteins (Lee et al., 2017).

3.2. Fungal bioactive peptide production using exogenous proteases

Also, various exogenous proteolytic enzymes such as alkaline protease, neutral protease, glutaminase, corolase, alcalase, Flavourzyme, trypsin, chymotrypsin, neutrase, papain, pancreatin, and bromelain have been investigated for the release of fungal BPs due to their short generation time and ease of genetic manipulation (Table 2). For instance, alcalase (derived from *Bacillus lichenformis*) was investigated to produce antioxidant protein hydrolysates (Wongaem et al., 2021). This study showed that protein extract from the split gill mushroom (*Schizophyllum commune*), subjected to hydrolysis with alcalase, yields antioxidant peptides (e.g., MYSEKHGSGGT, PGTRGAIAASSPQV, MVSTLAVLGIREP, EKEAAELGKGSF, and MSVTLLLLFISLVWVTISGLN). Additionally, the fraction with a MW $<$ 0.65 kDa demonstrated cellular antioxidant activity (azinobis ethyl benzthiazoline sulfonic acid, ABTS: IC_{50} − 0.0258 ± 0.0004 μg/mL) in a human intestinal cancer cell line (HT-29). Similarly, the application of neutral protease (derived from *Bacillus amyloliquefaciens*) effectively yielded a set of peptides with high antioxidant activity (PAQPPPPPNR, SPSKPPPPSS, VAPEEHPVLLTEAPLNPK, LGGLGNPHFI, VTYPSRLSASPAY, PRPPPPPPP, APPPPPPPPKQP, PPPPPPPPPPPPPP, PQYGAPPPP) from *Agrocybe aegerita* proteins (Song et al., 2020). Paisansak et al. (2021) identified an ACE inhibitory peptide (KIGSRSRFDVT) derived from shiitake mushroom (*Lentinula edodes*) hydrolysed utilising alcalase. Furthermore, trypsin and chymotrypsin were applied to *Kluyveromyces marxianus* and *Saccharomyces cerevisiae* for the production of ACE inhibitory peptides (LPGSVHLAL, VLSTSPPPL) (Mirzaei et al., 2018), and antioxidant peptides (MW = 0–3 kDa) (Mirzaei et al., 2016). Other exogenous enzymes investigated include papain and bromelain, both cysteine endoproteases derived from papaya latex and pineapple stem, respectively, have been studied previously. The enzymatic hydrolysate of *Boletus edulis* Bull.: Fr. protein, produced using bromelain, demonstrated high antioxidant activity (2,2-Diphenyl-1-picrylhydrazyl, DPPH: 12.08 ± 1.98 mmol Trolox/mg protein, ABTS: 94.14 ± 3.96 mmol Trolox/mg protein) at a concentration of 1 mg protein/mL. Additionally, it exhibited moderate ACE inhibitory activity with an IC_{50} value of 0.12 mg/mL (Kaprasob et al., 2022). On the other hand, pepsin enhanced the production of anti-

Table 2
Use of endogenous and exogenous enzymes to obtain bioactive peptides from fungi strains[1].

Fungi Strains	Enzyme used	Peptides or peptide fraction	Functional Activities	References
Agrocybe aegerita	Neutral protease	PAQPPPPPNR, SPSKPPPPPSS, VAPEEHPVLLTEAPLNPK, LGGLGNPHFI, VTYPSRLSASPAY, PRPPPPPPP, APPPPPPPPKQP, PPPPPPPPPPPPPPP, PQYGAPPPP	Antioxidant	(Song et al., 2020)
Selenium-rich brewer's yeast	Alkaline protease	Peptide fraction with MW < 1 kDa	Antioxidant	(Amorim et al., 2019b)
Agaricus bisporus scraps	Alkaline protease, complex protease	YPWT, VYPW, LVYP	ACE inhibitory	(Wang et al., 2022a)
Mushroom foot	Alkaline protease	Peptide fraction with MW 0–3 kDa	Alcohol intoxication	(Zhao et al., 2017)
Spent brewer's yeast	Protease	PWW, SQPW, RYW	ACE inhibitory	(Amorim et al., 2019a)
Saccharomyces cerevisiae	Protease	Peptide fraction with MW < 3 kDa	Antiulcer and antiproliferative properties against tumor cells	(Amorim et al., 2016)
Boletus edulis Bull.: Fr.	Bromelain	LIYAQGFSK	Antioxidant, ACE inhibitory	(Kaprasob et al., 2022)
Schizophyllum commune	Alcalase	MYSEKHGSGGT, PGTRGAIAASSPQV, MVSTLAVLGIREP, EKEAAELGKGSF, MSVTLLLLFISLVWVTISGLN	Antioxidant	(Wongaem et al., 2021)
Lentinula edodes	Alcalase	KIGSRSRFDVT	ACE inhibitory	(Paisansak et al., 2021)
Stropharia rugosoannulata	Trypsin	Octapeptides, nonapeptides, and decapeptides were predominant.	ACE inhibitory	(Li et al., 2022b)
Agaricus blazei Murrill (ABM)	Pepsin	The ABM peptide yield increased from 14 % to 25 %.	Anti-inflammatory activity	(Zou et al., 2023)
Aspergillus terreus	Pepsin	Peptide yield increased by 60 %.	Anticoagulant activity	(Costa-Junior et al., 2020)
Agaricus bisporus	Pancreatin	Peptide fraction with MW 0–3 kDa	Antioxidant	(Izanloo & Mahoonak, 2023)
Morchella sextelata	Neutrase-Flavourzyme	Peptide fraction with MW ≤ 3 kDa	Taste characteristics (umami)	(Gao et al., 2021)
Hericium erinaceus	Alcalase	Peptide fraction with MW ≤ 0.65 kDa	Antioxidant	(Sangtitanu et al., 2020)
Ganoderma lucidum	Pepsin-pancreatin	DRVSIYGWG, ALLSISSF	Antioxidant	(Aursuwanna et al., 2022)
Saccharomyces cerevisiae	Glutaminase, corolase, alcalase, Flavourzyme, trypsin	Glycopeptide-rich yeast extracts	Anti-inflammatory activity	(Williams et al., 2016)
Saccharomyces cerevisiae	Trypsin, chymotrypsin	Peptide fraction with MW 0–3 kDa	Antioxidant, ACE inhibitory	(Mirzaei et al., 2016)
Kluyveromyces marxianus	Trypsin, chymotrypsin	LPGSVHLAL, VLSTSPPPL	ACE inhibitory	(Mirzaei et al., 2018)
Cordyceps militaris	Papain-alkaline protease	The highest yield of the peptide was 16.73 %.	Anti-bacterial activity against *Bacillus subtilis*, *Staphylococcus aureus*, and *Escherichia coli*.	(Xu et al., 2023)

[1] MW: Molecular weight, ACE: Angiotensin-converting enzyme.

inflammatory peptides from *Agaricus blazei* Murrill, resulting in a yield ranging from 14 % to 25 % (Zou et al., 2023). Moreover, it increased the yield of anticoagulant peptides (60 %) from *Aspergillus terreus* (Costa-Junior et al., 2020).

Recent research has highlighted the potential of combining different exogenous proteolytic enzymes for protein hydrolysate generation. For instance, the hydrolysate derived from morel mushroom (*Morchella sextelata*) using a combination of neutrase-Flavourzyme exhibited the highest concentrations of flavor 5-nucleotides (4.84 ± 0.32 mg/g), free amino acids (224.83 ± 0.87 mg/g), and small peptides (<3 kDa), resulting in superior overall flavour characteristics (Gao et al., 2021). Moreover, pepsin, the primary digestive enzyme in the stomach, is a broad-spectrum protease that preferentially hydrolyses peptide bonds containing the aromatic amino acids Phe, Trp, and Tyr. This process leads to the formation of oligopeptides with enhanced biological properties. The peptides (DRVSIYGWG, ALLSISSF) formed through pepsin-pancreatin digestion of *Ganoderma lucidum* protein showed a strong antioxidant property (ABTS: 59.27 ± 4.05 μg/mL) and DPPH: 119.77 ± 4.43 μg/mL) due to amino acid sequences with hydrophobic residues (Aursuwanna et al., 2022). In another study, an effective enzymatic hydrolysis process was identified, utilising an alkaline protease to papain ratio of 4:3 for hydrolysing *Cordyceps militaris* polypeptide solution (<3,000 Da). The hydrolysate produced using this optimised process exhibited notable antibacterial efficacy against *Staphylococcus aureus*, *Bacillus subtilis*, and *Escherichia coli*, resulting in inhibitory zones measuring (10.32 ± 0.23) mm, (6.67 ± 0.12) mm, and (12.08 ± 0.22) mm, respectively (Xu et al., 2023).

3.3. Combination of protease and processing technologies to produce fungal bioactive peptides

Gautério et al. (2023) stated that fungi possess rigid cell walls that must be disrupted or permeabilised to obtain BPs. Cell disruption is a critical step in releasing BPs, where the integration of endogenous and exogenous enzymes plays a vital role. However, challenges exist including suboptimal activity of endogenous enzymes within the cellular milieu and potential inhibitory effects from endogenous protease inhibitors. Besides, exogenous enzymes may encounter issues related to stability, specificity, and compatibility with the complex cellular environment. Such issues highlight the need for optimised processing parameters (i.e., enzyme-substrate ratio, hydrolysis time, pH, and reaction temperature) to mitigate these challenges. Consequently, there is a contemporary shift towards using a combination of endogenous and exogenous enzymes, along with innovative processing technologies such as ultrasound, microwave, high-pressure processing, and pulsed electric field (PEF) to enhance the release of BPs from fungal cells (Fig. 3).

The combined use of ultrasound and enzymes has been shown effective for improving the accessibility of enzymes to peptide bonds,

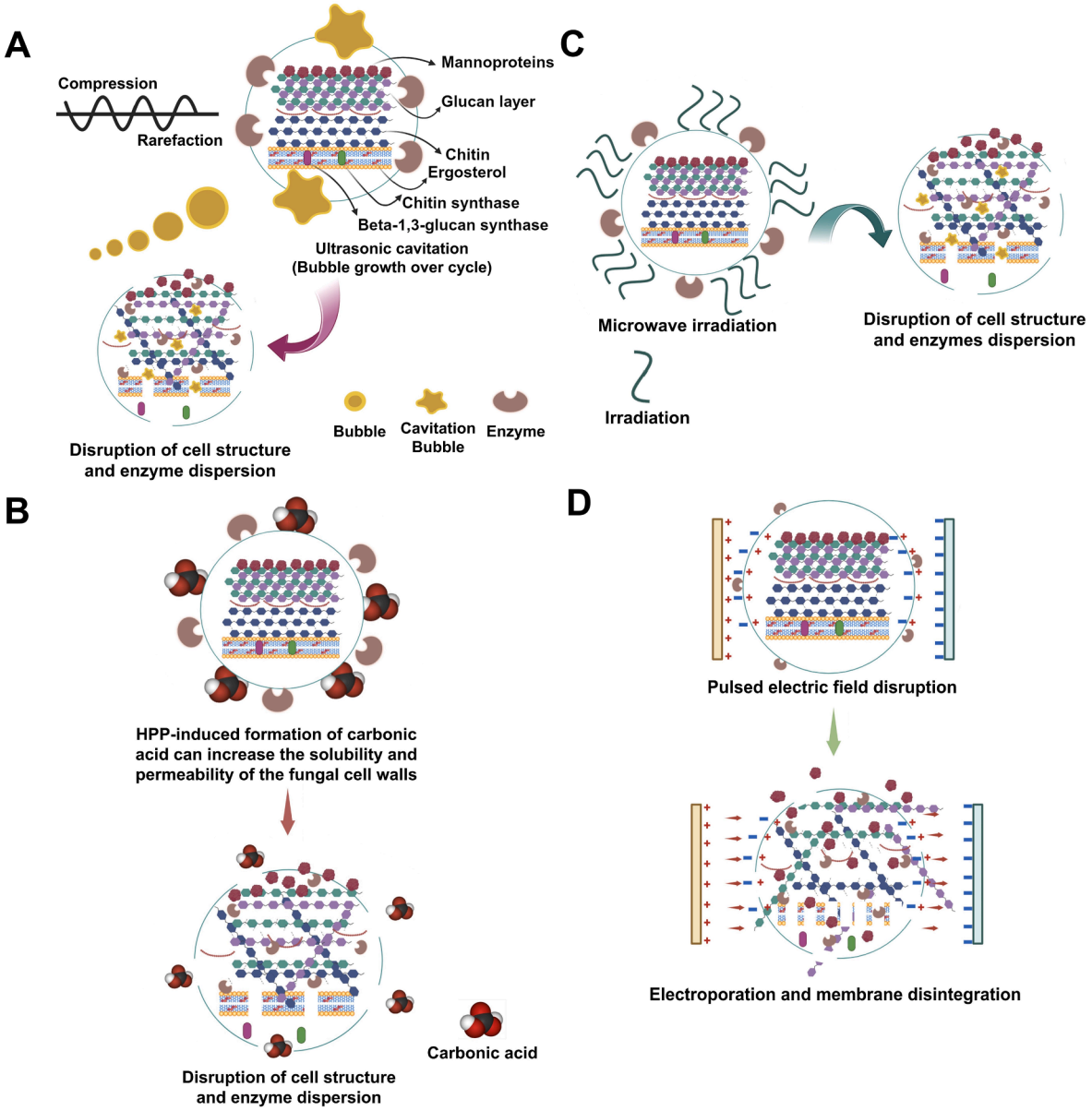

Fig. 3. Possible fungal cell disruption mechanisms of ultrasound (A), high hydrostatic pressure (HPP) (B), microwave (C), and pulsed electric field (D)-assisted enzymatic hydrolysis process to produce fungi-derived bioactive peptides. Created with Biorender.com.

ultimately enhancing BP release. The study of Izanloo and Mahoonak (2023) observed that *Agaricus bisporus* samples treated with 100 % ultrasound power and a reduced 60-min pancreatin enzymatic hydrolysis exhibited superior antioxidant properties (DPPH, 80.3 %) compared to untreated samples or those with 40 % or 70 % ultrasonic power pretreatment. Zou et al. (2023) showed that the use of ultrasonic extraction (time: 30 min) and pepsin enzymatic hydrolysis for *Agaricus blazei* Murrill peptides resulted in a 14 % to 25 % increase in yield when ultrasonic power was increased from 180 W to 360 W. Izannloo et al. (2023) conducted a study examining the influence of four enzymes—alcalase, trypsin, pepsin, and pancreatin—combined with microwave and ultrasound pretreatment under optimal hydrolysis conditions on the antioxidant capacity of *Agaricus bisporus* hydrolysed protein. Their findings demonstrated that all samples subjected to microwave and ultrasound pretreatment exhibited the highest DPPH free radical inhibition activity of approximately 80 % (ultrasound) and 75 % (microwave) when pepsin was used for hydrolysis.

Another approach, high hydrostatic pressure, can exert various effects that may potentially boost enzymatic hydrolysis. These effects include alterations in enzyme structure, leading to the exposure of additional active sites and enzyme activation. High pressure can also induce changes in the structure of the substrate protein, improving enzyme accessibility and enhancing interaction with the substrate. Tissue or cell decompartmentalisation can also occur, resulting in the release of endogenous enzymes and increased interaction with substrates. It is worth noting that high hydrostatic pressure can lead to enzyme inactivation when the pressure surpasses 400 MPa, and the specific threshold for inactivation depends on the enzyme (Terefe et al., 2014). Zhao et al. (2017) demonstrated that ultra-high-pressure processing with alkaline proteases enhances proteolysis and the release of BPs with activation effects on alcohol metabolic enzymes *in vitro* derived from mushroom foot protein.

Other novel processing technologies such as high-pressure homogenisation, microwave, and PEF could also facilitate the enzymatic hydrolysis process for releasing BPs from fungal strains. For instance, a recent study explored the impact of high-pressure homogenisation (flow rate: 10 dm/h; power: 1500 bar) as a pre-treatment on enzymatic hydrolysis and antioxidant properties of yeast protein hydrolysate from

Kluyveromyces marxianus (Moosavi et al., 2020). The authors concluded that combining high-pressure homogenisation pretreatment with trypsin hydrolysis is an optimal approach for producing yeast protein hydrolysate with high DPPH (297μMTE/mg protein) and ABTS (1,189 μMTE/mg protein) radicals scavenging activities. The high-frequency (300 MHz to 300 GHz) electromagnetic fields of microwaves induce a periodic fluctuation, organising polar molecules like water and proteins into an ordered pattern throughout the material. This organised arrangement, coupled with molecular friction and the movement of polar molecules, results in the efficient heating of the solution. It has been shown that under the intense heat of microwave irradiation, a plant powder sample underwent rapid moisture evaporation, resulting in cell wall breakdown releasing proteins into the solvent (Duarte et al., 2014). Subsequently, the addition of enzymes enabled hydrolysis of the protein-peptide chains, breaking them down into their amino acid components and facilitating the extraction of BPs. Nevertheless, PEF could enhance the production of BPs by facilitating processes like unfolding, denaturation, and gelation of cellular components. Additionally, PEF can induce polarisation of protein molecules, breaking noncovalent bonds like hydrogen bonding and hydrophobic interactions and releasing sulfhydryl groups. The duration of PEF application determines its effectiveness in breaking covalent bonds, such as disulfide bonds. Utilising PEF technology, Liu et al. (2012) increased the extraction yield of spent brewer's yeast protein from 2.17 ± 0.021 % to 3.34 ± 0.050 %. In another study, post-PEF treatment of *Saccharomyces cerevisiae* biomass affected 90 % of cell viability when incubated for 24 h, resulting in an extract with 187.82 ± 3.75 and 114.91 ± 2.86 mg/g dry weight of protein and amino acids, respectively (Berzosa et al., 2023).

It is important to note that the cost of enzymes is the major component of the overall cost in enzymatic BPs production, requiring specialised methods (i.e. immobilisation techniques, optimisation of reaction conditions, genetic engineering for enhanced stability, cofactor regeneration systems, enzyme recycling, process intensification, and exploration of native microorganisms) to retain their activity. The emphasis on single enzyme studies and the limited exploration of multiple exogenous enzymes for fungal BPs production underscore a critical gap. Future research should pivot towards a more comprehensive understanding of the catalytic properties of both endogenous and exogenous enzymes. This knowledge is crucial for manipulating and optimising the hydrolytic process to yield fungal BPs with specific functional characteristics. The specificity of endogenous and exogenous enzymes, particularly in relation to protein substrates, plays a vital role in defining the bioactivity of the released peptide sequences. Enzymes with broad substrate specificity generally result in unpredictable degradation of products during the hydrolytic process. Despite this unpredictability, their use remains attractive for producing hydrolysates with a high degree of hydrolysis, where small fragments may exhibit activity (Mazorra-Manzano et al., 2018). Conversely, limited hydrolysis with endogenous and exogenous enzymes possessing high specificity ensures an efficient release of peptides with expected sizes and properties. Achieving total protein hydrolysis through the action of a single enzyme is not always feasible and typically necessitates a combination of different enzymes and conditions for the efficient release of small fragments and free amino acids. Consequently, a complementary or sequential approach involving a mixture of endo- and/or *exo*-proteases with diverse specificity is essential for achieving a complete release of bioactive fragments. Finally, even with an optimised release, the crude hydrolysate presents a complex obstacle. The diverse charges, sizes, and physicochemical properties of various peptide fragments within the mixture make separation and purification highly challenging. To address these challenges, recent trends are leaning towards membrane filtration, i.e., ultrafiltration and chromatographic separation methods for separating and purifying fungal BPs.

4. Separation and purification of bioactive peptides from fungi

Fungal protein hydrolysed products, produced through enzymatic and microbial fermentation methods, typically contain components like BPs, large protein molecules, soluble impurities, and insoluble substances. These need separation from the active peptides. Research indicates that small peptide molecules have higher biological activity than large ones. Therefore, separation and purification techniques can be used to prepare small oligopeptides and active peptides with higher purity. Peptide separation and purification are the foundation for peptide structure–activity relationship determination. Suitable methods are chosen based on peptide physicochemical properties such as isoelectric point, polarity, and molecular weight.

4.1. Ammonium sulfate precipitation

To isolate peptides, a common technique is ammonium sulfate precipitation. This technique leverages the principle of salting out, where increasing the concentration of ammonium sulfate reduces the solubility of proteins, causing them to precipitate out of the solution. The process begins by gradually adding ammonium sulfate to the peptide solution while continuously stirring to ensure uniform distribution. The addition is typically done in stages, allowing for the selective precipitation of different peptide fractions. Each stage involves the removal of precipitated proteins by centrifugation and then the collection of the supernatant for further analysis or subsequent precipitation steps (Naseem et al., 2024). This stepwise approach enhances the purity of the target peptides by separating them from other proteins and contaminants. In a study, different ammonium sulfate saturations (0–100 %) were tested to precipitate lectin from *Laetiporus sulphureus*. The relative agglutinating activity significantly increased with saturations up to 40 % ($p < 0.05$), peaking at 30 UH/mg protein. The activity values stabilised from 40 % to 60 % saturation ($p > 0.05$). Beyond 60 %, the activity declined, reaching 10 UH/mg protein at 80 % saturation ($p < 0.05$) (Wang et al., 2018b). In another study, the γ-glutamyl transpeptidase (GGT) from shiitake mushroom (*Lentinus edodes*) was purified using ammonium sulfate. At 30 % saturation, most low MW proteins were precipitated and removed, while at 70 % saturation, most GGT was precipitated (Li et al., 2012).

One key advantage of ammonium sulfate precipitation is its ability to preserve peptides' biological activity. The mild conditions under which precipitation occurs minimise the risk of denaturing bioactive molecules, maintaining their functional integrity. Moreover, ammonium sulfate can act as a stabilising agent, protecting peptides from degradation during purification. This makes it particularly suitable for purifying sensitive BP that may lose activity under harsh conditions. Optimisation of the precipitation process is crucial for achieving high purity and yield. Factors such as pH, temperature, and the ionic strength of the solution must be carefully controlled. Additionally, the specific ammonium sulfate concentration required for adequate precipitation can vary depending on the properties of the target peptides. Empirical determination of these parameters is often necessary to fine-tune the purification process for each specific peptide. Following precipitation, the collected peptide precipitates can be further purified using additional techniques such as dialysis, chromatography, or ultrafiltration. These methods help to remove residual ammonium sulfate and other impurities, resulting in highly purified BP suitable for downstream applications. For instance, Ravikumar et al. (2012) combined ammonium sulphate precipitation with anion exchange (DEAE-cellulose) chromatography to produce protease from *Pleurotus sajor-caju* using solid-state fermentation. The first purification step involved precipitating proteins with 70 % ammonium sulfate, resulting in a 1-fold increase with an 88 % yield of the original activity. The second step used anion exchange column chromatography, where the protease activity was concentrated in a single peak, achieving a 3-fold purification. Another study used ammonium sulfate precipitation, anion-exchange chromatography, and

ultrafiltration to produce laccase from *Pleurotus ostreatus* HK35 (Hungary strain) (Isanapong et al., 2024). The initial purification step reduced total (68 U) and specific (3 U/mg) laccase activity, respectively. Anion exchange chromatography concentrated the eluted fractions with high laccase activity, resulting in a specific activity of 3 U/mg, 36 % recovery, and two-fold purification. The final ultrafiltration step desalted and concentrated the sample, increasing the total (32 U/mL) and specific (15 U/mg) laccase activity, yielding a 30 % recovery and 7-fold purification.

4.2. Membrane filtration

Membrane filtration is commonly used to clarify and reduce microbial count, macromolecules like non-hydrolysed proteins, and other aggregates. Additionally, it retains suspended colloidal particles generated during fermentation and processing. The application of membrane filtration extends to clarifying solutions before the fractionation steps. Various membrane separation methods, such as dialysis and ultrafiltration, have been used to concentrate and enrich fungal peptides.

4.2.1. Dialysis

Dialysis is a commonly used purification technique for BPs, using a semi-permeable membrane to separate molecules based on size. This method exploits the concentration gradient as the driving force so that small molecules (permeate) like salts and solvents pass through the membrane and larger biomacromolecules (retentate) such as proteins and peptides are retained. This selective permeability is crucial for removing unwanted small contaminants from peptide solutions. Although effective, dialysis alone is often insufficient for achieving high purity of BPs. It is typically employed as part of a multi-step purification process, such as ammonium sulfate precipitation, ion exchange chromatography, immobilised metal affinity chromatography, size exclusion chromatography, and centrifugal separation. For example, Yao et al. (2024) purified acid protease from *Aspergillus tubingensis* using a combination of ammonium sulfate precipitation and dialysis, followed by ion-exchange chromatography to identify aspergillopepsin I (ATAP). This enzyme is a potential candidate for hydrolysing soybean proteins. After fermenting the *Aspergillus tubingensis* in cereal powder medium for 48 h, the acid protease activity reached 54,000 U/L. Following ammonium sulfate precipitation, dialysis, and ultrafiltration, the specific activity of the crude enzyme solution increased to 5, 6, and 8 U/mg, respectively.

4.2.2. Ultrafiltration

Ultrafiltration molecular weight cut-off (MWCO) is particularly effective for separating macro-peptides and non-hydrolysed proteins (Fig. 4A). Membranes with an MWCO at 1–10 kDa are suitable for the fractionation of BPs with desired MW. Furthermore, membranes with an MWCO below 1 kDa are employed for peptide concentration (Liang et al., 2023). The choice between permeate or retentate depends on the target bioactivity for further processing/analysis. For example, Marson et al. (2022) separated brewer's spent yeast hydrolysate peptides from RNA and sugars using a 3-step process with nanofiltration and ceramic ultrafiltration. Two sequences of MWCO membranes (15, 8, 1 kDa and 50, 8, 1 kDa) were employed, and the 15 kDa membrane in the first step retained more components, enhancing subsequent steps' performance. The permeation process enriched the solution with smaller peptides (90 %, <1 kg mol^{-1}), leading to an improved purity profile with reduced

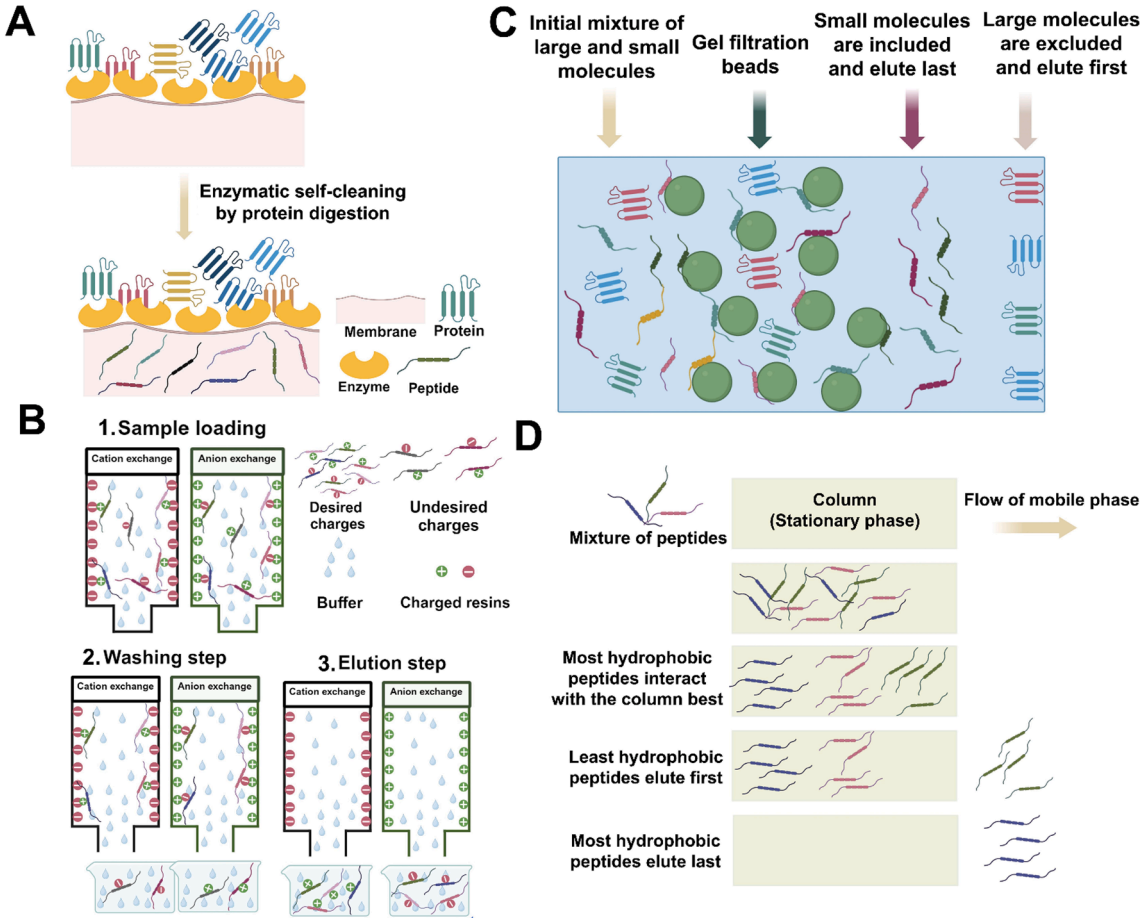

Fig. 4. Schematic representation of ultrafiltration (A), ion exchange chromatography (B), gel filtration chromatography (C), and reversed-phase high-performance liquid chromatography (D) separation process. Created with Biorender.com.

total sugar and RNA. An investigation of peptides from *Agaricus blazei* Murrill (ABMP) involved utilising various pore sizes in ultrafiltration to yield four distinct ABMP fractions and compare their antioxidant and immune-regulatory abilities (Zou et al., 2023). Specifically, the ABMP-2 fraction demonstrated scavenging rates of 79.3 %, 63.6 %, and 96.1 % for DPPH, hydroxyl, and ABTS radicals, respectively. Another study focused on *Boletus edulis* Bull.: Fr. peptides hydrolysed by bromelain, with ultrafiltration fractions assessed for ACE inhibitory and antioxidant activities. KBMPHF4, the smallest fraction with a MW below 1 kDa, displayed the highest DPPH and ABTS radical scavenging capabilities, along with ACE inhibition with an IC_{50} of 0.12 mg/mL (Kaprasob et al., 2022).

However, acknowledging limitations such as minimal membrane specificity leading to the co-partitioning of peptides with similar masses is crucial for fungal BP research. This lack of selectivity can complicate the isolation of specific BPs, while interactions between peptides and the membrane surface may impact separation efficiency based on physicochemical properties. Another challenge is membrane fouling, hindering the transfer of low MW components and diminishing filtration effectiveness over time. High material volume requirements sometimes pose challenges, especially in fungal hydrolysates, where material availability may be limited. Researchers often use laboratory-scale membrane filtration as an initial enrichment step before more sophisticated analytical techniques (i.e. chromatography, mass spectrometry (MS)), addressing the lack of specificity and fouling issues. However, membrane filtration has demonstrated its efficacy as the initial separation stage in many instances, indicating its potential when carefully considering system-specific factors. Furthermore, based on the properties of the peptide mixture, membrane filtration methods can be used in combination with chromatography and the high-performance liquid chromatography (HPLC) method to separate small molecules and short peptides with similar molecular weights.

4.3. Electrophoresis and capillary electrophoresis

Electrophoresis is a separation technique that relies on the movement of charged particles in an electric field. This method is particularly effective because of its ability to separate molecules based on their charge and size. The pH of the solution plays a crucial role in this process by influencing the net charge of the substances and the degree of dissociation. For proteins and peptides, which are amphoteric electrolytes, the pH relative to their isoelectric point (pI) determines their charge. When the solution pH exceeds the pI, proteins are negatively charged. While the pH is less than the pI, they are positively charged. The greater the difference between the pI and pH, the more charges the particle acquires, leading to faster movement.

Two-dimensional electrophoresis (2D-GE) is commonly used in proteomics for separating complex protein mixtures. This method combines two types of electrophoresis: transverse isoelectric focusing (IEF) and longitudinal sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE). IEF separates proteins according to isoelectric points, where each protein stops moving at its pI. This first-dimension resolves proteins based on their charge differences. The SDS-PAGE separates these proteins based on their molecular weight. Combining these two techniques, 2D-PAGE provides high-resolution separation, making it possible to purify and analyse BPs with great precision (Liu et al., 2020). Zanutto-Elgui et al. (2019) employed 2D-PAGE combined with electrospray quadrupole time-of-flight MS (ESI-MS/MS) to identify antimicrobial and antioxidant peptides in goat milk and bovine samples produced using proteases from *Aspergillus flavipes* and *Aspergillus oryzae*.

Capillary electrophoresis (CE) is a technique for liquid-phase separation, utilising a capillary tube as the separation pathway and propelled by a high-voltage direct current electric field. In CE, the inner surface of the quartz capillary column becomes negatively charged at pH levels above 3, creating a double layer upon contact with the buffer solution. The electric field causes the positively charged particles in the buffer to migrate towards the negative electrode, resulting in the formation of an electroosmotic flow (EOF). Simultaneously, within the buffer solution, the electric field causes charged particles to travel at varying speeds in the direction opposite to their charge polarity, thus generating electrophoresis. The migration speed of charged particles in the capillary buffer is determined by the combined effects of electroosmotic flow and electrophoresis, causing negative, positive, and neutral ions to move at different rates. Additionally, the capillary surface can be tailored to match the properties of various proteins, or specific substances can be introduced to the buffer to alter its distribution characteristics (Naseem et al., 2024). Du Ming et al. (2008) used CE coupled with size exclusion chromatography (HPLC), Edman degradation, and matrix-assisted laser desorption (MALDI)-TOF-MS to purify an antioxidant selenium-containing protein from Se-enriched *Ganoderma Lucidum*.

4.4. Chromatographic separation

Chromatography techniques capitalise on variations in the physicochemical properties (such as affinity, adsorption capacity, shape, size, molecular polarity, and solubility) among the mixture's components for fraction and purification. These differences cause the components to move at varying rates influenced by the resistance of the stationary phase and the thrust of the mobile phase, resulting in their concentration in distinct regions and effective separation. For fungal peptides, common chromatographic methods encompass sieving techniques such as gel filtration chromatography (GFC), charge-based techniques like ion-exchange chromatography (IEC), and methods based on variations in hydrophobic interactions such as reversed-phase chromatography (RP-HPLC) (Table 3).

4.4.1. Gel filtration chromatography

GFC separates peptides according to molecular mass (Fig. 4B), aiding peptide identification with minimal risk of denaturation or degradation. A study compared antimicrobial peptide purification from *Pichia pastoris* X33 using free-flow electrophoresis and GFC, with GFC achieving 93 % purity (Xia et al., 2017). In another study, following a purification approach that combined ammonium sulfate precipitation, GFC, and IEC, a *Penicillium digitatum*-derived serine protease was purified to homogeneity and exhibited a 13-fold increase in activity (Aissaoui et al., 2014). GFC enables rapid isolation without extensive optimisation, making it a time-efficient method. A challenge lies in the potential co-elution of peptides with similar MW, reducing resolution and purity. This limitation is pronounced in complex mixtures, requiring complementary techniques for purification. GFC combined with MS enhances sensitivity and specificity, contributing to accurate characterisation (Nikolov et al., 2012). Novel gel matrix materials can optimise GFC for a broader range of peptides in complex mixtures (Ó'Fágáin et al., 2017).

4.4.2. Ion-exchange chromatography (IEC)

The IEC method relies on the differential binding of charged molecules to a resin with oppositely charged groups, facilitating the isolation of peptides based on their net charge (Fig. 4C). Its selectivity makes it suitable for purifying peptides with diverse isoelectric points, leading to the effective separation of complex mixtures from fungal cultures. In a study by Rizk et al. (2018), IEC was evaluated for the purification and isolation of the peptide fraction ranging from 5 to 10 kDa, obtained from 60–80 % ammonium sulfate precipitation of *Saccharomyces cerevisiae* protein hydrolysates, and isolate specific BPs with inhibitory effects on malolactic enzyme activity *in vitro*. In another investigation, IEC isolated a distinct peptide fraction with increased bioactivity from *Boletus edulis* Bull.: Fr. mushroom protein hydrolysate (Kaprasob et al., 2022). The chromatographic separation was based on ionic interactions, allowing positively charged peptides to elute first. Negatively charged peptides, however, needed a higher salt concentration to be eluted. Among the eluted fractions, fraction 51 exhibited the highest surface

Table 3
Separation, identification, and quantification techniques applied to some fungi-derived bioactive peptides[1].

Fungi Strains	Separation/ Purification/ Fractionation Method	Detection/ Quantification	Peptide sequences	Function Validation	Functional activities	References
Aspergillus oryzae Aspergillus flavipes	2D-PAGE	ESI-MS/MS	HPINHQGLSPEVLNENLLR, FALPQYLK	*In vitro*	Antimicrobial, antioxidant	(Zanutto-Elgui et al., 2019)
Boletus edulis Bull.: Fr.	Ultrafiltration, Ion-exchange-chromatography, RP-HPLC	LC-MS/MS	SETGGGHTTSCETDLGEFL, AAPLPGP, GTDPTGEMLT, DLDLLEKGIRKT, NGGNAPI, VSWNVLQEP, DTGRGLGASH, IDDNLDNLIIKL, LIYAQGFSK	*In vitro*	Antioxidant, ACE inhibitory	(Kaprasob et al., 2022)
Yamadazyma triangularis	Ultrafiltration, gel filtration chromatography, RP-HPLC	LC-MS/MS	YPLP, AGPL, GPFP, APGGF	*In vitro*	Antioxidant	(Cai et al., 2022)
Yarrowia lipolytica	Ultrafiltration, gel filtration chromatography, prep-HPLC	LC-MS/MS	QSLVSVPGMS	*In vitro* *In silico*	Antioxidant, ACE inhibitory	(Eckert et al., 2019)
Kluyveromyces marxianus	Ultrafiltration, RP-HPLC	–	LPGSVHLAL, VLSTSPPPL	*In vitro* *In silico*	Antioxidant, ACE inhibitory	(Mirzaei et al., 2018)
Saccharomyces cerevisiae	Ion-exchange-chromatography	LC-MS/MS	DDDDDNDDDDEEGNxKTKSAAT and CNATQACPEDKPCCSQYGEcGTG	*In vitro*	Malolactic fermentation inhibition	(Rizk et al., 2018)
Schizophyllum commune	Ultrafiltration	RP-HPLC	MYSEKHGSGGT, PGTRGAIAASSPQV, MVSTLAVLGIREP, EKEAAELGKGSF, MSVTLLLLFISLVWVTISGLN	*In vitro*	Antioxidant	(Wongaem et al., 2021)
Agaricus bisporus	Ultrafiltration, gel filtration chromatography, RP-HPLC	UPLC-Q-TOF-MS	GLPA, GHGA	*In vivo*	Kokumi peptides	(Feng et al., 2019)
Agaricus bisporus	Ultrafiltration, gel filtration chromatography, RP-HPLC	Nano-LC-ESI-MS/MS	ENDIVIGPEKPEG, RIPDDEAEKPEG, GVTEDSVGTIHPDGG, VLAEATDIEK, AVDSNGEDDEDEEKPK, AIDDEVEGESVPR, IPDDEAEKPEG, EDPKPEEPAPA	*In vitro*	Cytoprotective effect	(Kimatu et al.. 2020)
Stropharia rugoso-annulata	Ultrafiltration, gel filtration chromatography	UPLC-Q-Orbitrap-MS/MS	EPLCNQ, SGCVNEL, PHEMQ, SEPSHF, ESCAPQL	*In silico*	Umami-flavorings	(Chen et al., 2022)
Volvariella volvacea	Ultrafiltration, gel filtration chromatography, RP-HPLC	UPLC-Q-TOF/MS	ASNMSDL, YYGSNSA, LQPLNAH	*In vivo*	Umami-flavorings	(Xu et al., 2019)
Trachinotus ovatus	Ultrafiltration, gel filtration chromatography, RP-HPLC	Nano-HPLC-MS/MS	APAP, ASEFFR, AEASALR, LGDVLVR, WDDMEK	*In silico*	Umami-flavorings	(Deng et al., 2021)
Leccinum extremiorientale	Gel filtration chromatography, RP-HPLC	Nano-LC-MS/MS	DQEDLDESLIGVK, FNEIIKETST, YNEYPPLGR	*In vivo*	Umami-flavorings	(Liang et al., 2021)
Morchella esculenta	Ultrafiltration	LC-MS/MS	EYPPLGRFA, TVIDAPGHRDFI	*In silico*	Umami-flavorings	(Wang et al., 2023c)
Hericium erinaceus	Ultrafiltration, RP-HPLC	ESI-Q-TOF-MS/MS	TVNSPWHGA, WVVYHVHLV, WPVTTHK, LVAHRGPHAGH	*In vitro*	Antioxidant	(Sangtitanu et al., 2020)
Lentinus edodes	Gel filtration chromatography	LC-MS/MS	EGEPKLP, KDDLRSP, TPELKL, LDYGKL	*In silico*	α-glucosidase inhibition activity	(Zhang et al., 2023a)
Ganoderma lucidum	Ultrafiltration, RP-HPLC	MS	QLVP, QDVL, QLDL	*In vitro,* *In silico*	Anti-hypertensive activity	(Wu et al., 2019)
Hypsizygus marmoreus	Ultrafiltration, gel filtration chromatography, RP-HPLC	UPLC-Q-TOF-MS/MS	EGTAG	*In silico*	Umami-flavorings	(Chang et al., 2023)
Boletus griseus-Hypomyces chrysospermus	Ultrafiltration	UPLC-Q-Orbitrap-MS/MS	KYPHVF, LFRPE	*In silico*	ACE inhibitory	(Renjuan et al., 2022)
Agrocybe aegerita	Ultrafiltration, HPLC	RPLC-ESI-MS/MS	PAQPPPPPNR, SPSKPPPPPSS, VAPEEHPVLLTEAPLNPK, LGGLGNPHFI, VTYPSRLSASPAYN, PRPPPPPPP, PPPPPPPPPPPPPPP, PQYGAPPPP	*In silico*	Antioxidant	(Song et al., 2020)
Amanita rimosa	Ultrafiltration	UPLC-Q-Orbitrap-MS		*In silico*	Cyclopeptide toxins	(Lei et al., 2020)
Cordyceps militaris	Ultrafiltration, RP-HPLC	UPLC-Q-Orbitrap-MS	VPRKL(*Se*)M, RYNA(*Se*)MNDYT	*In vivo*	Neuroprotective	(Wu et al., 2022)

[1] 2D-PAGE: Two-dimensional polyacrylamide gel electrophoresis, RP-HPLC: Reversed-phase high-performance liquid chromatography, ESI-MS/MS: electrospray ionization tandem mass spectrometry, LC-MS/MS: Liquid chromatography tandem mass spectrometry, UPLC-Q-TOF-MS: ultra-high performance liquid chromatography with quadrupole time-of-flight mass spectrometry, RPLC-ESI-MS/MS: Reversed-phase liquid chromatography/electrospray ionization tandem mass spectrometry, ESI-Q-TOF-MS/MS: Electrospray quadrupole time-of-flight mass spectrometer, prep-HPLC: preparative-scale high-performance liquid chromatography, Nano-LC-ESI-MS/MS: Nanoscale liquid chromatography coupled to tandem mass spectrometry, LC-Q-TOF-MS: liquid chromatography–quadrupole time-of-flight

mass spectrometry. UPLC-Q-Orbitrap-MS/MS: Ultra-high-performance liquid chromatography coupled with quadrupole-orbitrap mass spectrometry, Nano-HPLC-MS/MS: Nanoscale high-performance liquid chromatography coupled to tandem mass spectrometry.

hydrophobicity (1,061 ± 26), antioxidant (DPPH: 12 ± 2 mmol Trolox/mg protein; ABTS: 94 ± 4 mmol Trolox/mg protein) and ACE inhibitory (IC_{50}: 0.12 mg/mL) activities compared to other fractions. A notable advantage of IEC is its ability to separate samples based on ionic charge, which differs from the principles of GFC that rely on other interactions for separation. This capability can potentially reduce analysis time and improve the accuracy of results. Notwithstanding, IEC encounters two main challenges: firstly, peptides with similar or identical total charges may exhibit comparable affinity to the stationary phase, hindering effective separation. Consequently, achieving the desired resolution necessitates employing additional complementary separation techniques. Secondly, gradient elution relies on salt solutions that may introduce unwanted impurity ions. Techniques like dialysis are essential to alleviate this challenge and safeguard downstream applications, which is a form of membrane filtration (Zhang et al., 2023).

4.4.3. Reversed-phase chromatography

Numerous studies have investigated how eluting molecules interact with the stationary phase in RP, showing that hydrophobic interactions play a dominant role in retaining the molecules (Fig. 4D). Both adsorption and partition processes influence this interaction. Typically, stationary phases, including polymeric-bonded or silica-bonded varieties like octyl (C8), octadecyl (C18), or other alkyl phases, find application in RP. While monomeric bonded stationary phases provide better separation performance, polymeric stationary phases with a cross-linked network structure offer more stability and resistance to operational conditions, especially when used with aqueous mobile phases. Among the various stationary phases, RP C18 columns are the most widely utilised in fungal BP purification. For example, a study by Feng et al. (2019) identified a peptide fraction with the highest antioxidant activity from a trypsin-hydrolysed *Saccharomyces cerevisiae* protein hydrolysate with a MW below 3 kDa. This fraction was further purified using RP-HPLC, resulting in a purified peptide with an ABTS radical-scavenging activity of 8 μM TE/μg protein. Other purified fractions displayed activity ranging from 7 to 27 μM TE/μg protein. These results demonstrated a substantial increase in antioxidant activity after the purification step, indicating a significant improvement in purity. A different study aimed to reduce the complexity of gel permeation chromatography-isolated kokumi taste fractions from *Agaricus bisporus*. Fraction-5 was further separated using RP-HPLC with a C-18 semi-preparative column, yielding five fractions. These fractions were analysed to assess their kokumi tastes, leading to the identification of the fraction exhibiting the highest kokumi intensity (Feng et al., 2019). RP-HPLC has some limitations for fungal peptide analysis due to their complex nature, leading to co-elution or incomplete separation. Due to the complexity of fungal peptide mixtures, meticulous optimisation of chromatographic conditions, including stationary phases and mobile phase gradients, is required. RP-HPLC sensitivity poses another constraint, especially for low-abundance peptides, potentially overlooking bioactive compounds. Advancing stationary phase technologies with novel materials that have enhanced selectivity can significantly improve resolution and efficiency. Integrating RP-HPLC with MS enhances sensitivity and specificity in peptide identification. Incorporating artificial intelligence (AI) and machine learning (ML) algorithms in data analysis promises to expedite fungal BP identification and overall efficiency.

5. Identification and validation of fungi-derived bioactive peptides

Isolating individual fungal peptides requires clarifying their structural characteristics to understand bioactive attributes for use in functional foods and other applications. Analysing peptide sequences, including amino acid composition, quantity, and arrangement, is vital for understanding biological activity. Correlating peptide sequences and biological activities can disclose the mechanism of action, laying a theoretical foundation for developing more potent peptides. Researchers have employed various approaches (i.e. peptide mapping, *de novo* sequencing, peptide databases, MS imaging, *in-silico* resources, and bioinformatics tools) to identify and characterise fungal peptide sequences.

5.1. Mass spectrometry

MS is the predominant method, evidenced by its widespread use in fungal peptide identification (Table 3). Peptide identification commonly utilises liquid chromatography (LC) coupled with electrospray ionisation (ESI). ESI introduces charge, and subsequent mass analysis employs systems like ion trap, time of flight (TOF), quadrupole (Q), TOF-TOF, Q-TOF, and Q-ion trap to separate the peptides by their mass-to-charge (*m/z*) ratios. Tandem MS/MS allows peptide sequencing by detecting, fragmenting, and analysing ions. Challenges may arise, especially when certain amino acids undergo modification, such as oxidation during sample preparation. Furthermore, structural identification of fungal peptides involves techniques like nuclear magnetic resonance method, circular dichroism, infrared spectroscopy (IR), visible spectrum (UV), ultraviolet, and MS. However, difficulties also exist in precisely determining specific conformations of fungal peptides. Therefore, revealing peptides possessing modified amino acids and characterising their spatial conformations are key considerations in peptide research.

5.2. Databases

Most MS analysis software employs a database search tool to compare and match peptide sequences, but challenges arise due to the limited number of fungal protein sequences in public sequence repositories. Database searching helps to identify amino acid sequences of fungal-derived peptides by comparing tandem mass spectra with theoretical spectra. Screening for short peptides involves creating a database with all possible di-, tri-, and tetra-peptide combinations. Regular peptides (≥6-7 amino acids) are typically identified using protein sequence repositories like UniProt and NCBI but may lack information on unannotated proteins and short open reading frame (sORF)-encoded peptides (SEPs). To address these issues for fungal-derived peptides, custom databases based on genome and transcriptome sequences can be generated. Constructing a reference sequence database is crucial for identifying SEPs, especially in peptidomics. To facilitate the identification of unannotated SEPs through MS, the improvement of high-resolution reference genome sequences using proteogenomic tools becomes crucial. These approaches integrate genomic and proteomic techniques to identify the expression of unannotated peptides with the goal of augmenting or refining genome annotations. Moreover, transcriptome sequence translation serves as a comprehensive database for the identification of both annotated and unannotated peptides, including SEPs. In a study by Zhou et al. (2021), transcriptome analysis using PacBio and Illumina sequencing revealed 46 unveiled MSDIN genes in the fungal species *Amanita rimosa*, *Amanita exitialis*, *Amanita subjunquillea*, and *Amanita pallidorosea*, and a combination of genomics and MS demonstrated that 12 of these MSDIN genes produced novel cyclic peptides. To reduce the likelihood of false positives, exploring diverse approaches like spectral library searches or *de novo* sequencing while implementing multiple hypothesis testing and adjusting for the false discovery rate (FDR) is essential. Employing both *de novo*- and homology-based prediction approaches, Tong and Guo (2022) identified 8,541 potential antioxidant peptides in *Ophiocordyceps sinensis*. They concluded that the high degree of similarity between the expression

profiles of artificially cultivated and wild mature fruiting bodies suggests the trustworthiness of the predictions.

5.3. De novo peptide sequencing

In contrast to database searching, *de novo* sequencing can provide comprehensive information on peptide sequences, particularly in sophisticated samples exhibiting multiple modifications or absent in the database. Furthermore, *de novo* sequencing shows promise in explaining the amino acid sequences of peptides with sequence variations, isoforms, unknown modifications, or atypical shapes, such as cyclotides. *De novo* sequencing from MS/MS data can be performed manually, but numerous software tools exist to automate this process and improve throughput. For instance, Chen et al. (2022) employed UHPLC-Q-Orbitrap-MS/MS to obtain tandem mass spectra of umami-active peptides in *Stropharia rugoso-annulata* mushroom. Subsequently, they utilised Peaks Studio 7.5 to perform automatic peptide sequencing based on the highest peptide ion intensity, leading to the identification of the following amino acid sequences: EPLCNQ, SGCVNEL, PHEMQ, SEPSHF, and ESCAPQL. In another study, analysis of the F3 sub-fraction of *Ganoderma lucidum* revealed the presence of two novel antioxidant peptides, DRVSIYGWG and ALLSISSF, identified through *de novo* peptide sequencing using ESI-Q-TOF-MS/MS (Aursuwanna et al., 2022). *De novo* peptide sequencing encounters significant challenges, including resolution and low accuracy. In addition, the accuracy of peptide identification in MS using available software is currently limited to a range of 30–50 %, primarily due to challenges such as interference from generated peaks, overlapping cleavage profiles of different peptides, and

Table 4
Analysis of fungal-derived bioactive peptides using *in-silico* resources and bioinformatics tools.

	Tools and Databases	Software Type	Web Address	Fungal Strains	References
Physiochemical characteristics	ExPASy-ProtParam tool	Web-server	https://web.expasy.org/protparam/	*Lignosus rhinocerus*	(Goh et al., 2022)
	BIOPEP-UWM	Web-server	https://www.uwm.edu.pl/biochemia/index.php/pl/biopep	*Pleurotus pulmonarius*	(Manoharan et al., 2017)
	HAPPEN	Web-server	https://research.timmons.eu/happenn	*Agaricus bisporus*	(Fatima et al., 2023)
Peptide activity / Solubility/ Toxicity/ Allergenicity	Proteomics tools	Web-server	https://www.innovagen.com/proteomics-tools	*Stropharia rugoso-annulata*	(Chen et al., 2022)
	ToxinPred	Web-server	https://webs.iiitd.edu.in/raghava/toxinpred/	*Stropharia rugoso-annulata*; *Ganoderma lucidum*	(Chen et al., 2022); (Aursuwanna et al., 2022)
	AdmetSAR	Web-server	https://lmmd.ecust.edu.cn/admetsar1	*Boletus griseus-Hypomyces chrysospermus*	(Renjuan et al., 2022)
	SADP, Allermatch, and Allergen Online	Web-server	https://fermi.utmb.edu/, https://www.allermatch.org/, https://www.allergenonline.org/	*Lentinula edodes*	(Vashisht et al., 2023)
	ExPASy-ProtParam, Allergen FP	Web-server	https://web.expasy.org/protparam/, https://ddg-pharmfac.net/AllergenFP/	*Agaricus bisporus*	(Fatima et al., 2023)
	Peptide 2.0	Web-server	https://www.peptide2.com/main_about.php	*Ganoderma lucidum*	(Aursuwanna et al., 2022);
	ADMETlab 2.0	Web-server	https://admetmesh.scbdd.com/	*Termitornyces albuminosus*	(Yu et al., 2023)
Molecular docking of peptides	AutoDock vina-1.1.2	Software	https://vina.scripps.edu/	*Stropharia rugoso-annulata*; *Boletus edulis* Bull.: Fr.; *Boletus edulis*; yeast extract	(Chen et al., 2022); (Kaprasob et al., 2022); (Song et al., 2023); (Wang, et al., 2023b)
	SYBYL-X 2.0	Software	https://www.certara.com/	*Boletus griseus-Hypomyces chrysospermus*	(Renjuan et al., 2022)
	Discovery Studio 20	Software	https://www.3ds.com/products/biovia/discovery-studio	*Lentinula edodes*	(Paisansak et al., 2021)
	MOE (2009)	Software	https://www.chemcomp.com/Products.htm	*Lentinula edodes*	(Zhang et al., 2023a); (Li et al., 2022a)
	SWISS-MODEL	Web-server	https://swissmodel.expasy.org/	*Hypsizygus marmoreus*	(Dong et al., 2023)
	CDOCKER tool	Software	https://www.3ds.com/products/biovia/discovery-studio	*Agaricus bisporus*	(Wang et al., 2022a)
	HADDOCK	Web-server	https://wenmr.science.uu.nl/	*Kluyveromyces marxianus*	(Mirzaei et al., 2018)
Databases and identification of peptide sequences	UniProt	Web-server	https://www.uniprot.org	*Stropharia rugoso-annulata*; *Lentinula edodes*; *Boletus edulis*; yeast extract	(Chen et al., 2022); (Vashisht et al., 2023); (Song et al., 2023); (Wang et al., 2023b)
	RCSB PDB	Web-server	https://www.rcsb.org/	*Lentinula edodes*; *Kluyveromyces marxianus*	(Paisansak et al., 2021); (Mirzaei et al., 2018)
	NCBI	Web-server	https://www.ncbi.nlm.nih.gov/	*Lentinula edodes*; *Lignosus rhinoceros*; *Boletus edulis*	(Vashisht et al., 2023); (Goh et al., 2022); (Song et al., 2023)
	Ensembl	Web-server	https://fungi.ensembl.org/index.html	*Pleurotus ostreatus*	(Landi et al., 2020)
	JGI MycoCosm	Web-server	https://mycocosm.jgi.doe.gov/	*Pleurotus eryngii*	(Yuan et al., 2017)
Prediction of potential tastant and bioactivity	Peptide Ranker	Software	https://distilldeep.ucd.ie/PeptideRanker	*Boletus griseus-Hypomyces chrysospermus*	(Renjuan et al., 2022)
	AHTpin	Web-server	https://webs.iiitd.edu.in/raghava/ahtpin/	*Boletus griseus-Hypomyces chrysospermus*	(Renjuan et al., 2022)
	AMPA tool	Web-server	https://tcoffee.crg.cat/apps/ampa/do	*Agaricus bisporus*	(Fatima et al., 2023)
	UMPred-FRL	Web-server	https://pmlabstack.pythonanywhere.com/UMPred-FRL	Yeast extract	(Wang et al., 2023b)

incomplete sequence information. Moreover, high-quality spectra are essential for accurate *de novo* sequencing, as poor MS/MS spectral quality can lead to the loss of product ion signals, especially those generated during individual peptide bond cleavages (Zhang et al., 2023). AI algorithms, particularly ML models, can be trained to recognise complex patterns in MS data, thereby improving the ability to distinguish true peptide signals from noise and interference. These models can learn to differentiate overlapping cleavage profiles, mitigate the impact of incomplete sequence information, and enhance the overall quality of peptide-spectrum matches. AI-aided software tools have the potential to significantly increase the accuracy of *de novo* sequencing. They can adapt to the complex nature of MS data, deciphering nuanced patterns and optimising the interpretation of incomplete or mosaic spectra.

5.4. In-silico resources

In certain cases, a significant number of peptides are identified in a fungal hydrolysate, prompting the utilisation of *in-silico* approaches for peptide selection. Table 4 includes a compilation of *in-silico* databases and bioinformatics tools used for the analysis of BPs derived from fungi. The bioinformatics exploration of BPs typically commences with obtaining amino acid sequences from databases such as UniProt-KB (Chen et al., 2022; Vashisht et al., 2023; Song et al., 2023; Wang et al., 2023b), NCBI (Paisansak et al., 2021; Mirzaei et al., 2018), and RCSB (Vashisht et al., 2023; Goh et al., 2022). After selecting proteins with known sequences, *in-silico* digestion is performed using protein digestion databases like BIOPEP (Manoharan et al., 2017), HAPPENN (Fatima et al., 2023), and ExPASy-ProtParam tool (Goh et al., 2022), enabling the generation of peptides based on cleavage specificities. Post-digestion databases like ExPASy PeptideCutter offer cleavage site maps or BIOPEP provides peptide lists for further analysis. Peptides are then characterised for biological (potential allergenicity, toxicity, bioactivities), physicochemical (average hydropathicity, aliphatic index, theoretical pI, MW, etc.), and sensory properties (umami, bitter, presence of sweet, and other taste-evoking peptide sequences), using chemometrics and sequence homology (Table 4). To overcome the limitation in the number of sequences that can be analysed with online tools for *in-silico* digestion, researchers often turn to algorithms implemented in programming languages such as R and Python. R and Python are widely used in the bioinformatics community, providing numerous packages and libraries specifically designed for sequence data analysis. Researchers can develop custom scripts or use existing algorithms to perform *in-silico* digestion on a broader set of protein sequences, enabling more extensive analyses than what may be feasible with online tools. Additionally, ML algorithms, implemented using programming languages like R and Python, can enhance the efficiency and accuracy of tasks related to peptide identification and analysis. These tools may assist in predicting cleavage patterns, identifying post-translational modifications (PTMs), and improving the overall reliability of *in-silico* digestion results. *In-silico* methodologies, including molecular docking and quantitative structure–activity relationship (QSAR), aid in studying structure–activity relationships and predicting biological activities (Zhang, Chen et al., 2023). Nevertheless, challenges arise, notably in the context of similarity searches involving short-chain fungal peptides, where the distinctive characteristics of these peptides pose difficulties in accurately identifying similar sequences. This underscores the necessity for employing multiple tools in the analysis, as a singular tool may not suffice to comprehensively address all aspects of the task (Wang et al., 2023b). The *in-silico* approach predicts bioactivity based on amino acid residues, but assumptions may not fully represent *in vitro* conditions due to protein tertiary structure, and environmental factors. Moreover, peptidomics provides strategies to overcome challenges in separating and identifying BPs in complex matrices (Paisansak et al., 2021). While peptidomics has advantages, challenges like peptide co-elution are addressed through tandem fragmentation techniques.

6. Application of fungal bioactive peptides

6.1. Food applications

Fungal BPs are useful in developing health-focused functional diets. These peptide isolates can help to achieve specific technical qualities in functional products. It is well documented that protein and lipid oxidation are crucial to forming flavour compounds in meat processing and storage. However, excessive oxidation can result in poor flavour and potential health risks. Researchers have found that controlled oxidation helps in developing flavour by breaking down proteins and lipids. Several recent studies have focused on edible macrofungi to investigate their effect on meat tenderness, oxidative stability, and flavour enhancement. For instance, a study examined the effectiveness of four edible mushrooms – *Agaricus bisporus*, *Hypsizygus marmoreus*, *Pleurotus ostreatus*, and *Volvariella volvacea* – on beef paste quality. These mushrooms significantly altered the texture and colour of the beef paste. The possible reason is due to notably increased levels of free amino acids and enhanced accumulation of volatile compounds (Qing et al., 2021). Another study investigated the taste-enhancing properties of *Lentinus edodes*, *Flammulina velutipes*, *Hypsizygus tessellatus*, and *Pleurotus ostreatus* mushrooms on chicken soup. The chicken soup with *Hypsizygus tessellatus* scored second-highest in taste and aroma, followed by the soup with *Pleurotus ostreatus* hydrolysates, indicating their taste-enhancing effects. This enhancement is attributed to the high degree of hydrolysis of the *Hypsizygus marmoreus* protein hydrolysate, which increased the content of short peptides and free amino acids from the mushroom protein (Ang & Ismail-Fitry, 2019).

Yeast cells enhance the production of BPs during fermentation through their proteolytic activity, improving the functional properties of fermented beverages like kefir and kumis. A study explored the impact of *Kluyveromyces marxianus* in milk fermented with *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, revealing that *K. marxianus* caused significant changes in 25 protein spots, primarily bovine serum proteins and caseins. This led to a 16.3 % increase in total free amino acids. Additionally, the variety of peptides in milk fermented by *K. marxianus* was significantly higher compared to milk fermented by *Lactobacillus* (D.-D. Zhang et al., 2017). Furthermore, using a co-culture of *Candida lipolytica* MIUG D67 yeast strains (10^6 CFU/100 mL) and 2.5 g artisanal kefir grains improved the functional quality of the fermented products. The combination increased the BPs, enhancing antioxidant activity and ACE inhibitory properties, compared to products fermented solely with kefir grains (Cotârleț et al., 2019). Chaves-López et al. (2012) explored the *K. marxianus* KL26A, and *Pichia kudriavzevii* KL84A strains in fermented milk to achieve products with high ACE inhibitory activity and minimal bitterness. Sensory analysis revealed that *Pichia kudriavzevii* KL84A and *K. marxianus* KL26A were promising adjunct starter cultures for Kumis, as they significantly enhanced ACE inhibitory activity without imparting a bitter taste.

Emulsification is a common process used to evaluate protein-rich products. BPs, due to their amphiphilic nature, play a crucial role in emulsifying properties. Peptides derived from fungi have demonstrated effective emulsifying capabilities. Mazumder et al. (2023) investigated that using *Pleurotus sajor-cajun* (Fr.) Singer mushroom to develop mushroom-based emulsion-type sausages improved emulsion activity due to the higher levels of BPs. Foam formation can enhance the texture and sensory qualities of foods like pastries and sauces. Active peptides can serve as foam stabilisers because they reduce surface tension. Kupfer et al. (2017) documented the foam-stabilising properties of *Saccharomyces cerevisiae* protein PAU5 and recommended it as a gushing marker and a direct stabiliser for sparkling wine against gushing-inducing factors. Research has highlighted fungi-based protein hydrolysates' significant role in improving meat products' water-holding capacity. This enhancement is crucial for maintaining juiciness, which affects colour, texture, and flavour during processing. In this regard, Banerjee et al. (2020) stated that protein concentrate of *Flammulina velutipes* mushroom

stem wastes employed in goat meat nuggets formulation significantly improved the nuggets' water-holding capacity.

6.2. Pharmaceutical applications

Peptide-based drugs are gaining preference due to the rising incidence of multidrug resistance to non-peptidic drugs. Many non-peptidic compounds also cause adverse or toxic side effects. As a result, fungi, alongside animals, plants, and bacteria, have been studied to search for more effective and safer therapeutic peptides. Caspofungin (brand name: Cancidas) is a semi-synthetic lipopeptide and the first drug in the echinocandins class (https://go.drugbank.com/drugs/DB00520), developed by Merck & Co., Inc. It was initially derived from the fermentation of the fungus *Glarea lozoyensis* (Letscher-Bru & Herbrecht, 2003). Clinical trials have demonstrated caspofungin's effectiveness in treating invasive aspergillosis, invasive candidiasis, oropharyngeal and esophageal (Johnson & Perfect, 2003). Furthermore, the cyclic undecapeptide cyclosporin A (CsA) is well-known for its immunosuppressive properties. Its selective immunosuppressant activity has made it effective in various transplantation therapies, significantly improving survival rates after solid organ grafts. Combining CsA with other drugs has also been effective in treating rheumatoid arthritis. Although CsA is derived from fungi such as *Trichoderma polysporum*, *Fusarium oxysporum*, *Beauveria nivea*, and *Aspergillus* sp., the fungus *Tolypocladium inflatum* is predominantly used for CsA production through various fermentation techniques (Survase et al., 2011). Fungal-derived BPs have also been explored as ACE, HIV-1 reverse transcriptase inhibitors, anti-cancer, anti-diabetic, antihyperlipidemic, and anti-viral agents. For example, Kang et al. (2013)) isolated an ACE inhibitory peptide (IC_{50}: 0.19 mg/mL) from *Hypsizygus marmoreus*, and the *H. marmoreus* water extract effectively reduced hypertension in spontaneously hypertensive rats. Wong et al. (2011) found that a peptide called cordymin from *Cordyceps militaris* inhibited HIV-1 reverse transcriptase (IC_{50}: 55 μM) and selectively reduced the proliferation of MCF-7 breast cancer cells without showing any effect on HT-29 colon cancer cells. Also, CULP, a ubiquitin-like peptide from *Handkea utriformis*, showed N-glycosylase activity and inhibited protein synthesis (38 %) in a rabbit reticulocyte lysate system. It exhibited ribonuclease activity (1 IU/mg) against yeast tRNA. CULP also demonstrated antiproliferative effects on human breast carcinoma cells and antimitogenic effects on murine splenocytes, both with an IC_{50} of 0.1 μM (Lam et al., 2001). Misato Kobayashi and colleagues isolated a methylated cyclic heptapeptide (ternatin) from *Coriolus versicolor* mushroom and investigated the effects of [d-Leu^7]ternatin and ternatin on type 2 diabetes and obesity in KK-A^y mice. Both compounds suppressed hyperglycemia development. In the liver, SREBP-1c mRNA levels were lower in mice treated with [d-Leu^7]ternatin or ternatin (Kobayashi et al., 2012). Furthermore, CMPS-80, an N-glycosidic polysaccharide-peptide complex derived from *Cordyceps militaris* fruiting bodies, significantly improved plasma lipid profiles and atherosclerotic lesions in apolipoprotein E-deficient mice (Li, Miao et al., 2022). RC28, a peptide cloned from *Rozites caperata* mushroom, exhibited potent antiviral activity against herpes simplex virus-1 in Vero cells and a mouse keratitis model. In Vero cells, plaque assays revealed that RC28 reduced viral yields by 1.2 logs. In the mouse model, the peptide delayed the onset of stromal keratitis and reduced disease severity (Yan et al., 2015).

6.3. Cosmeceutical applications

The development of natural and more stable synthetic peptides has revived interest in peptide-based skincare products. Recent studies have shown that fungal peptides promote collagen synthesis, improving skin elasticity and reducing wrinkles, which is crucial for anti-aging skincare products. In this regard, Hexapeptide-11, derived from *Saccharomyces* yeast fermentation, delayed senescence in both extrinsically and intrinsically dermal papillae and aged fibroblast cells *in vitro* (Gruber et al., 2013). Additionally, Gorouhi and Maibach reviewed a placebo-controlled study involving 25 healthy volunteers who applied Hexapeptide-11 to their skin twice daily for four weeks. The treatment improved deformation response and initial skin elasticity (Gorouhi & Maibach, 2009). Furthermore, cryptocandin A, an aromatic lipopeptide derived from the *Cryptosporiopsis* cf. *quercina*, demonstrated antifungal properties. It was effective against *Trichophyton rubrum* and *Trichophyton mentagrophytes*, with plate MIC values ranging from 0.030 to 0.060 μg/ml. Notably, it showed a plate MIC value of 0.035 μg/ml against *Candida albicans*, highlighting its potential as a therapeutic agent for treating skin infections and human nail diseases such as tinea barbae, tinea corporis, tinea cruris, tinea pedis, and onychomycosis (Rahnamaeian & Vilcinskas, 2015). In addition, Dong-Uk Jo and colleagues studied the skin-whitening properties of *S. cerevisiae* fermentation residue and proposed that this residue directly inhibited tyrosinase, an enzyme essential for melanin synthesis. They identified four dipeptides (Phe-Lys, Val-Tyr, Tyr-Glu, Tyr-Pro) as competitive inhibitors, suggesting these might be the active compounds in the fermentation residue responsible for its inhibitory effect (Jo et al., 2022).

7. Challenges and future perspectives

Exploring fungi as an alternative protein source and as a viable and sustainable option for peptide production shows promise, given their diversity and the existence of several non-toxic and non-pathogenic species and strains with low nutritional needs. Only a small percentage of fungi (approximately 2 %) are genuinely toxic, while a comparably minimal percentage (around 4 % to 5 %) are recognised as desirable edible species. Efficient strain screening for optimal peptide content and amino acid composition is essential to ensure fungal peptides meet nutritional demands and possess desirable bio-functionality. The call for engineered strains underscores the potential of genetic manipulation, yet ethical and safety concerns require careful consideration, especially regarding the unintended consequences of gene editing. Promising prospects involve sORF-encoded BPs in fungi and non-ribosomally produced and post-translationally modified peptides (NRP-PTMs). Fungal sORF-encoded BPs, discovered through genome exploration, promise diverse functionalities for biotechnological applications. Genetic engineering may optimise fungal strains for efficient sORF-encoded peptide production. Furthermore, expanding the screening of fungal peptide resources to non-edible or toxic species may reveal untapped BPs. However, this exploration requires thorough toxicity assessments and an understanding of risks associated with peptides from unconventional fungal sources. Balancing benefits with safety considerations is crucial for public trust and regulatory approval.

Scientists' interest in applying novel processing technologies, specifically ultrasound, microwave, high-pressure processing, is growing due to their potential to improve enzymatic hydrolysis as well as microbial fermentation and enhance peptide yields. Yet, the lack of widespread adoption suggests a gap between scientific exploration and practical application. It becomes essential to bridge this gap through intensive research that establishes the efficiency of these technologies and evaluates the safety and efficacy of the peptides they produce. Other emerging heat processing technologies, like ohmic heating and pulsed electric fields, are yet to be explored for fungal peptide production.

Ongoing challenges include inadequately characterised enzyme preparations, non-standardised protocols for assessing bioactive properties, identifying peptides, and evaluating bioactivities *in vitro* and *in vivo*. This lack of standardisation can hinder results in reproducibility, reliability, and progress in the field. Furthermore, the insufficient investigation of short peptides and a shortage of mechanistic studies underscore the necessity for comprehensive research to understand the biological activities and mechanisms of action of fungal-derived peptides. A viable approach for preparing fungal peptides with fewer strains is suggested through microbial fermentation. Microbial fermentation for peptide production is complicated by the simultaneous generation of

bioactive compounds such as viable and non-viable bacterial cells, bacteriocins, and exopolysaccharides, which possess distinct biological properties, casting uncertainty on whether observed bioactivities are attributable to the peptides or these co-produced compounds. This complexity underscores the challenge of achieving specificity, the targeted production of peptides without by-products, and purity especially given the inadequate isolation and purification of many peptides, posing a risk to meeting industrial standards of quality.

Issues related to amino acid modification during the preparation process and identifying of PTMs peptides in fungi emphasise the need for precise and reliable identification techniques. Accurate identification of specific peptide conformations is considered critical, necessitating advanced analytical methods for comprehensive characterisation of fungal-derived peptides. Moreover, the literature on the correlation between the structure and activity of fungal peptides reveals a significant knowledge gap. The limited understanding of specific structure–activity relationships of fungal peptides calls for further investigation to unlock their complete bio-functional and therapeutic potential.

While computational biology, data science and AI-aided tools are shown to be promising for peptide research, the importance of validating and comparing different methods is vital. Establishing the predictive accuracy of *in-silico* tools and their effectiveness in the wet laboratory is crucial for instilling confidence in their application. The proposal for a comprehensive platform integrating all *in-silico* tools and steps for BP discovery and analysis is forward-looking. Such a platform presents a valuable opportunity for advancing bioinformatics in this field. Currently, spectral data from proteomics and peptidomics experiments find deposition (i.e., MS spectra repositories: PRIDE, MassIVE, ProteomeXchange: https://www.proteomexchange.org) and annotation in centralised databases like NCBI (https://www.ncbi.nlm.nih.gov/) and UniProt (https://www.uniprot.org). However, the bulk of BP data remains stored in decentralised, in-house databases. Platforms such as BIOPEP-UWM (https://www.uwm.edu.pl/biochemia/index.php/pl/biopep), CancerPPD (https://crdd.osdd.net/raghava/cancerppd/), and databases for food-derived BPs operate independently to compile BP information. It is essential to centralise the generation and storage of BP data to ensure regular backups, as certain niche servers may become archived or lost over time. An example is the Food-derived Bioactive Peptide Database (DFBP) (https://www.cqudfbp.net/), which is no longer accessible for retrieving information from the server. Additionally, establishing a peer-reviewed, central platform for researchers to contribute new peptide sequences is an innovative step forward and could lead to the creation of a comprehensive open-access database of functional peptides through a crowdsourcing approach.

Evaluating the biological activity of the novel fungal peptide necessitates the use of *in vitro* and animal models, providing a deeper understanding of its mechanism of action. ML can significantly streamline and enhance the investigation of novel fungal BPs biological activity. Through predictive modelling, ML algorithms can analyse vast datasets to identify patterns and relationships that may not be immediately apparent to human researchers. In the context of biological activity enhancement, ML can predict potential peptide modifications or structural features that might lead to improve efficacy. This predictive capability can guide the design phase, narrowing down the number of iterations required in the laboratory. Additionally, ML can assist in the selection and optimisation of *in vitro* assays, suggesting the most relevant tests for specific biological activities. Regarding animal models, algorithms can predict the likely outcomes of experiments, aiding in the design of more targeted and informative studies. Furthermore, premature loss of activity in BPs is a common occurrence attributed to factors like structural denaturation, enzyme degradation, and instability during bloodstream circulation. Modern drug delivery systems, like scaffolds and nanocarriers, could be the key to unlocking the potential of fungal BPs by delivering them directly to active sites within the organism, mirroring the potentiality achieved with peptide-based therapies. Furthermore, a key priority for future development is the effective incorporation of fungal peptides in functional foods. Questions remain about integrating these within complex food matrices, maintaining long-term biological stability, and enhancing gastrointestinal stability to deliver efficacy.

8. Conclusion

This review highlighted the recent trends in fungal peptide research, particularly emphasising their production, separation, purification, identification, validation, and application. The overview can be summarised as fermentation of various protein substrates with fungal strains releases BPs, enhancing their functional properties such as antioxidant, anti-hypertensive, and flavour enhancement. Additionally, fungal strains have been employed as sources of endogenous proteolytic enzymes to improve diverse food products' physicochemical properties, nutritional value, and sensory characteristics. Besides, various exogenous enzymes showed efficacy in releasing BPs from fungi. Combining proteases with processing technologies, specifically ultrasound, high-pressure homogenisation, microwave, and high-pulsed electric fields, shows promise in improving the release of BPs by disrupting fungal cell walls. As the separation and purification of BPs are crucial for obtaining small oligopeptides and active peptides, membrane filtration, and chromatographic methods offer advantages such as low-temperature operation, scalability, and preservation of peptide bioactivity, making them alternative to traditional methods. Furthermore, various techniques, including peptide mapping, *de novo* sequencing, peptide databases, MS imaging, *in-silico* resources, and bioinformatics tools, have been applied to identify and characterise fungal peptide sequences. Fungal BPs are also gaining serious attention in the food, pharmaceutical, and cosmeceutical industries due to their multi-target health benefits. In conclusion, research on fungal-derived BPs is still in the developmental stage. These research trends reflect a collective effort to maximise the potential of fungal BPs, focusing on identifying new compounds and understanding their diverse biological activities.

Ethics statement

The authors declare no applicable ethical considerations with regards to the information contained in this review article as no experimentation was conducted in order to create it.

Credit authorship contribution statement

Tanvir Ahmed: Writing – review & editing, Writing – original draft, Visualisation, Conceptualisation. **Angéla Juhász:** Writing – review & editing, Supervision, Conceptualisation. **Utpal Bose:** Writing – review & editing, Supervision. **Netsanet Shiferaw Terefe:** Writing – review & editing, Supervision. **Michelle L. Colgrave:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualisation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

Acknowledgment

Tanvir Ahmed acknowledges the support of Edith Cowan University (ECU), WA, Australia, for providing the higher degree by research (HDR) scholarship. This project is supported by Australian Research

Council Centre of Excellence for Innovations in Peptide and Protein Science (CE200100012).

References

Aissaoui, N., Abidi, F., Mahat, S., & Marzouki, M. N. (2014). Purification and biochemical characterization of a novel protease from Penicillium digitatum–Use in bioactive peptides production. *Journal of Basic Microbiology, 54*(S1), S178–S189.

Amorim, M. M., Pereira, J. O., Monteiro, K. M., Ruiz, A. L., Carvalho, J. E., Pinheiro, H., & Pintado, M. (2016). Antiulcer and antiproliferative properties of spent brewer's yeast peptide extracts for incorporation into foods. *Food & Function, 7*(5), 2331–2337.

Amorim, M., Marques, C., Pereira, J. O., Guardão, L., Martins, M. J., Osório, H., Moura, D., Calhau, C., Pinheiro, H., & Pintado, M. (2019a). Antihypertensive effect of spent brewer yeast peptide. *Process Biochemistry, 76*, 213–218.

Amorim, M., Pinheiro, H., & Pintado, M. (2019b). Valorization of spent brewer's yeast: Optimization of hydrolysis process towards the generation of stable ACE-inhibitory peptides. *Lwt, 111*, 77–84.

Ang, S.-S., & Ismail-Fitry, M. R. (2019). Production of different mushroom protein hydrolysates as potential flavourings in chicken soup using stem bromelain hydrolysis. *Food Technology and Biotechnology, 57*(4), 472.

Aursuwanna, T., Noitang, S., Sangtanoo, P., Srimongkol, P., Saisavoey, T., Puthong, S., Reamtong, O., & Karnchanatat, A. (2022). Investigating the cellular antioxidant and anti-inflammatory effects of the novel peptides in lingzhi mushrooms. *Heliyon, 8*(10).

Banerjee, D. K., Das, A. K., Banerjee, R., Pateiro, M., Nanda, P. K., Gadekar, Y. P., Biswas, S., McClements, D. J., & Lorenzo, J. M. (2020). Application of enoki mushroom (*Flammulina Velutipes*) stem wastes as functional ingredients in goat meat nuggets. *Foods, 9*(4), 432.

Berzosa, A., Delso, C., Sanz, J., Sánchez-Gimeno, C., & Raso, J. (2023). Sequential extraction of compounds of interest from yeast biomass assisted by pulsed electric fields. *Frontiers in Bioengineering and Biotechnology, 11*, 1197710.

Cai, J., Xing, L., Zhang, W., Fu, L., & Zhang, J. (2022). Selection of Potential Probiotic Yeasts from Dry-Cured Xuanwei Ham and Identification of Yeast-Derived Antioxidant Peptides. *Antioxidants, 11*(10), 1970.

Chang, J., Li, X., Liang, Y., Feng, T., Sun, M., Song, S., Yao, L., Wang, H., & Hou, F. (2023). Novel umami peptide from *Hypsizygus marmoreus* hydrolysate and molecular docking to the taste receptor T1R1/T1R3. *Food Chemistry, 401*, Article 134163.

Chaves-López, C., Tofalo, R., Serio, A., Paparella, A., Sacchetti, G., & Suzzi, G. (2012). Yeasts from *Colombian Kumis* as source of peptides with Angiotensin I converting enzyme (ACE) inhibitory activity in milk. *International Journal of Food Microbiology, 159*(1), 39–46.

Chen, W., Li, W., Wu, D., Zhang, Z., Chen, H., Zhang, J., Wang, C., Wu, T., & Yang, Y. (2022). Characterization of novel umami-active peptides from *Stropharia rugoso-annulata* mushroom and in silico study on action mechanism. *Journal of Food Composition and Analysis, 110*, Article 104530.

Chen, Z., Gao, H., Wu, W., Chen, H., Fang, X., Han, Y., & Mu, H. (2021). Effects of fermentation with different microbial species on the umami taste of Shiitake mushroom (*Lentinus edodes*). *Lwt, 141*, Article 110889.

Costa-Junior, R. B., Brandão-Costa, R. M. P., Albuquerque, W. W. C., Batista, J. M. S., Pedrosa, R. B., & Porto, A. L. F. (2020). Ultrasound-Assisted Enzyme-Catalyzed Hydrolysis of Collagen to Produce Peptides With Biomedical Potential: Collagenase From *Aspergillus terreus* UCP1276. *Bioelectromagnetics, 41*(2), 113–120.

Cotârleț, M., Vasile, A. M., Cantaragiu, A. M., Gaspar-Pintiliescu, A., Cruaciunescu, O., Oancea, A., Moraru, A., Moraru, I., & Bahrim, G. E. (2019). Colostrum-derived bioactive peptides obtained by fermentation with kefir grains enriched with selected yeasts. *The Annals of the University Dunarea de Jos of Galati. Fascicle VI-Food Technology, 43*(1), 54–68.

Deng, X., Lin, H., Ahmed, I., & Sui, J. (2021). Isolation and identification of the umami peptides from *Trachinotus ovatus* hydrolysate by consecutive chromatography and Nano-HPLC-MS/MS. *LWT, 141*, Article 110887.

Dong, X., Wan, C., Huang, A., Xu, H., & Lei, H. (2023). Novel Umami Peptides from *Hypsizygus marmoreus* and Interaction with Umami Receptor T1R1/T1R3. *Foods, 12*(4), 703.

Du Ming, D. M., Wang Cong, W. C., Zhang LanWei, Z. L., Hu XiaoSong, H. X., & Zhao GuangHua, Z. G. (2008). Characterization of SE-GL-P, an andtioxidant selenium-containing protein from Se-enriched *Ganoderma lucidum* mushroom. *European Food Research and Technology, 224*, 659–665.

Duarte, K., Justino, C. I. L., Gomes, A. M., Rocha-Santos, T., & Duarte, A. C. (2014). Green analytical methodologies for preparation of extracts and analysis of bioactive compounds. In *Comprehensive analytical chemistry* (Vol. 65, pp. 59–78). Elsevier.

Eckert, E., Zambrowicz, A., Bobak, Ł., Zabłocka, A., Chrzanowska, J., & Trziszka, T. (2019). Production and identification of biologically active peptides derived from by-product of hen egg-yolk phospholipid extraction. *International Journal of Peptide Research and Therapeutics, 25*, 669–680.

Fan, X., Bai, L., Zhu, L., Yang, L., & Zhang, X. (2014). Marine algae-derived bioactive peptides for human nutrition and health. *Journal of Agricultural and Food Chemistry, 62*(38), 9211–9222.

Fatima, A., Haider, F., Qaiser, J. M. A., Jan, S. U., & Bakhtiar, S. M. (2023). Insilico analysis of peptides isolated from *Agaricus bisporus* manifests potential antimicrobial therapeutic activities. *World Journal of Biology and Biotechnology, 8*(1), 25–29.

Feng, T., Wu, Y., Zhang, Z., Song, S., Zhuang, H., Xu, Z., Yao, L., & Sun, M. (2019). Purification, identification, and sensory evaluation of kokumi peptides from *agaricus bisporus* mushroom. *Foods, 8*(2), 43.

Gao, J., Fang, D., Kimatu, B. M., Chen, X., Wu, X., Du, J., Yang, Q., Chen, H., Zheng, H., An, X., et al. (2021). Analysis of umami taste substances of morel mushroom (*Morchella sextelata*) hydrolysates derived from different enzymatic systems. *Food Chemistry, 362*, Article 130192.

Gaspar-Pintiliescu, A., Oancea, A., Cotarlet, M., Vasile, A. M., Bahrim, G. E., Shaposhnikov, S., Craciunescu, O., & Oprita, E. I. (2020). Angiotensin-converting enzyme inhibition, antioxidant activity and cytotoxicity of bioactive peptides from fermented bovine colostrum. *International Journal of Dairy Technology, 73*(1), 108–116.

Gautério, G. V., da Silva, R. M., Karraz, F. C., Coelho, M. A. Z., Ribeiro, B. D., & Lemes, A. C. (2023). Cell disruption and permeabilization methods for obtaining yeast bioproducts. *Cleaner Chemical Engineering, 100112*.

Goh, N.-Y., Razif, M. F. M., Yap, Y.-H.-Y., Ng, C. L., & Fung, S.-Y. (2022). In silico analysis and characterization of medicinal mushroom cystathionine beta-synthase as an angiotensin converting enzyme (ACE) inhibitory protein. *Computational Biology and Chemistry, 96*, Article 107620.

Gorouhi, F., & Maibach, H. I. (2009). Role of topical peptides in preventing or treating aged skin. *International Journal of Cosmetic Science, 31*(5), 327–345.

Gruber, J. V., Ludwig, P., & Holtz, R. (2013). Modulation of cellular senescence in fibroblasts and dermal papillae cells in vitro. *Journal of Cosmetic Science, 64*(2), 79–87.

Guan, Y., Wang, J., Wu, J., Wang, L., Rui, X., Xing, G., & Dong, M. (2017). Enhancing the functional properties of soymilk residues (okara) by solid-state fermentation with *Actinomucor elegans*. *CyTA-Journal of Food, 15*(1), 155–163.

Gurumallesh, P., Alagu, K., Ramakrishnan, B., & Muthusamy, S. (2019). A systematic reconsideration on proteases. *International Journal of Biological Macromolecules, 128*, 254–267.

Inoue, K., Gotou, T., Kitajima, H., Mizuno, S., Nakazawa, T., & Yamamoto, N. (2009). Release of antihypertensive peptides in miso paste during its fermentation, by the addition of casein. *Journal of Bioscience and Bioengineering, 108*(2), 111–115.

Isanapong, J., Suwannoi, K., Lertlattanapong, S., & Panchal, S. (2024). Purification, characterization of laccase from Pleurotus ostreatus HK35, and optimization for congo red biodecolorization using Box-Behnken design. *3 Biotech, 14*(3), 73.

Izanloo, I., & Mahoonak, A. S. (2023). The effect of ultrasound pretreatment on hydrolysis time by pepsin enzyme to produce antioxidant peptides from edible mushroom (*Agaricus bisporus*) protein. *Iranian Food Science and Technology Research Journal., 12*(2), 191–204.

Izannloo, I., Mahoonak, S., et al. (2023). Effect of type of pretreatment and enzyme on antioxidant capacity of hydrolyzed protein of edible mushroom (*Agaricus bisporus*). *Journal of Food Technology and Nutrition, 20*(2), 29–44.

Jo, D.-U., Chin, Y.-W., Kim, Y., Kim, K.-T., Kim, T.-W., & Lim, T.-G. (2022). By-product of Korean liquor fermented by *Saccharomyces cerevisiae* exhibits skin whitening activity. *Food Science and Biotechnology, 31*(5), 587–596.

Johnson, M. D., & Perfect, J. R. (2003). Caspofungin: First approved agent in a new class of antifungals. *Expert Opinion on Pharmacotherapy, 4*(5), 807–823.

Kang, M.-G., Kim, Y.-H., Bolormaa, Z., Kim, M.-K., Seo, G.-S., & Lee, J.-S. (2013). Characterization of an antihypertensive angiotensin I-converting enzyme inhibitory peptide from the edible mushroom *Hypsizygus marmoreus*. *BioMed Research International, 2013*(1), Article 283964.

Kaprasob, R., Khongdetch, J., Laohakunjit, N., Selamassakul, O., & Kaisangsri, N. (2022). Isolation and characterization, antioxidant, and antihypertensive activity of novel bioactive peptides derived from hydrolysis of King Boletus mushroom. *LWT, 160*, Article 113287.

Kehinde, B. A., & Sharma, P. (2020). Recently isolated antidiabetic hydrolysates and peptides from multiple food sources: A review. *Critical Reviews in Food Science and Nutrition, 60*(2), 322–340.

Kimatu, B. M., Fang, D., Zhao, L., & Hu, Q. (2020). *Agaricus bisporus* peptide fractions confer cytoprotective ability against hydrogen peroxide-induced oxidative stress in HepG2 and Caco-2 cells. *Journal of Food Measurement and Characterization, 14*, 2503–2519.

Kobayashi, M., Kawashima, H., Takemori, K., Ito, H., Murai, A., Masuda, S., Yamada, K., Uemura, D., & Horio, F. (2012). Ternatin, a cyclic peptide isolated from mushroom, and its derivative suppress hyperglycemia and hepatic fatty acid synthesis in spontaneously diabetic KK-Ay mice. *Biochemical and Biophysical Research Communications, 427*(2), 299–304.

Kupfer, V. M., Vogt, E. I., Siebert, A. K., Meyer, M. L., Vogel, R. F., & Niessen, L. (2017). Foam-stabilizing properties of the yeast protein PAU5 and evaluation of factors that can influence its concentration in must and wine. *Food Research International, 102*, 111–118.

Lam, Y. W., Ng, T. B., & Wang, H. X. (2001). Antiproliferative and antimitogenic activities in a peptide from puffball mushroom *Calvatia caelata*. *Biochemical and Biophysical Research Communications, 289*(3), 744–749.

Landi, N., Clemente, A., Pedone, P. V., Ragucci, S., & Di Maro, A. (2022). An updated review of bioactive peptides from mushrooms in a well-defined molecular weight range. *Toxins, 14*(2), 84.

Landi, N., Ragucci, S., Russo, R., Valletta, M., Pizzo, E., Ferreras, J. M., & Di Maro, A. (2020). The ribotoxin-like protein Ostreatin from *Pleurotus ostreatus* fruiting bodies: Confirmation of a novel ribonuclease family expressed in basidiomycetes. *International Journal of Biological Macromolecules, 161*, 1329–1336.

Lee, K.-H., Kim, H.-K., Kim, S.-H., Kim, K.-H., Choi, Y.-M., Jin, H.-H., Lee, S.-J., & Ryu, Y.-C. (2017). Effects of mushroom extract on textural properties and muscle protein degradation of bovine longissimus dorsi muscle. *Bioscience, Biotechnology, and Biochemistry, 81*(3), 558–564.

Lei, S., Shi, P., Wu, W., Xia, B., Fu, X., Wan, Y., & Zhou, Y. (2020). Extensive screening of cyclopeptide toxins in mushrooms by ultra-high-performance liquid chromatography coupled with quadrupole-Orbitrap mass spectrometry. *Food Chemistry, 329*, Article 127146.

Letscher-Bru, V., & Herbrecht, R. (2003). Caspofungin: The first representative of a new antifungal class. *Journal of Antimicrobial Chemotherapy, 51*(3), 513–521.
Li, J., Huang, J., Yin, J., Wu, N., Song, J., Zhang, L., & Jiang, T. (2012). Rapid Purification and Characterization of γ-Glutamyl-Transpeptidase from Shiitake Mushroom (*Lentinus Edodes*). *Journal of Food Science, 77*(6), 640–645.
Li, L., Wang, L., Gao, Y., Wang, J., & Zhao, X. (2017). Effective antimicrobial activity of plectasin-derived antimicrobial peptides against *Staphylococcus aureus* infection in mammary glands. *Frontiers in Microbiology, 8*, 2386.
Li, W., Chen, W., Ma, H., Wang, J., Li, Z., Wang, Q., Zhang, Z., Wu, D., Zhang, J., & Yang, Y. (2022a). Study on the relationship between structure and taste activity of the umami peptide of *Stropharia rugosoannulata* prepared by ultrasound. *Ultrasonics Sonochemistry, 90*, Article 106206.
Li, W., Chen, W., Ma, H., Wu, D., Zhang, Z., & Yang, Y. (2022b). Structural characterization and angiotensin-converting enzyme (ACE) inhibitory mechanism of *Stropharia rugosoannulata* mushroom peptides prepared by ultrasound. *Ultrasonics Sonochemistry, 88*, Article 106074.
Li, W., Chen, W., Wu, D., Zhang, Z., & Yang, Y. (2022c). Taste peptides derived from *Stropharia rugosoannulata* fermentation mycelium and molecular docking to the taste receptor T1R1/T1R3. *Frontiers in Nutrition, 9*, Article 960218.
Li, W., Ma, H., He, R., Ren, X., & Zhou, C. (2021). Prospects and application of ultrasound and magnetic fields in the fermentation of rare edible fungi. *Ultrasonics Sonochemistry, 76*, Article 105613.
Li, X., Ye, X., Ma, H., Liu, W., Sun, L., Kong, N., et al. (2017). Stimulation of *Grifola frondosa* liquid fermentation through low-intensity alternating magnetic field and its preliminary study of biological window effects. *Food and Fermentation Industries, 43*(8), 53–58.
Li, Y., Miao, M., Yin, F., Shen, N., Yu, W.-Q., & Guo, S.-D. (2022d). The polysaccharide–peptide complex from mushroom Cordyceps militaris ameliorates atherosclerosis by modulating the lncRNA–miRNA–mRNA axis. *Food & Function, 13*(6), 3185–3197.
Liang, J., Chen, L., Li, Y., & Hu, X. (2021). Isolation and identification of umami-flavored peptides from *Leccinum extremiorientale* and their taste characteristic. *Journal of Food Processing and Preservation, 45*(3), e15255.
Liang, T., Lu, H., Ma, J., Sun, L., & Wang, J. (2023). Progress on membrane technology for separating bioactive peptides. *Journal of Food Engineering, 340*, Article 111321.
Liu, M., Zhang, M., Lin, S., Liu, J., Yang, Y., & Jin, Y. (2012). Optimization of extraction parameters for protein from beer waste brewing yeast treated by pulsed electric fields (PEF). *African Journal of Microbiology Research, 6*(22), 4739–4746.
Liu, S., Li, Z., Yu, B., Wang, S., Shen, Y., & Cong, H. (2020). Recent advances on protein separation and purification methods. *Advances in Colloid and Interface Science, 284*, Article 102254.
Manoharan, S., Shuib, A. S., Abdullah, N., Mohamad, S. B., & Aminudin, N. (2017). Characterisation of novel angiotensin-I-converting enzyme inhibitory tripeptide, Gly-Val-Arg derived from mycelium of *Pleurotus pulmonarius*. *Process Biochemistry, 62*, 215–222.
Marson, G. V., Lacour, S., Hubinger, M. D., & Belleville, M.-P. (2022). Serial fractionation of spent brewer's yeast protein hydrolysate by ultrafiltration: A peptide-rich product with low RNA content. *Journal of Food Engineering, 312*, Article 110737.
Martinez-Medina, G. A., Barragán, A. P., Ruiz, H. A., Ilyina, A., Hernández, J. L. M., Rodriguez-Jasso, R. M., Hoyos-Concha, J. L., & Aguilar-González, C. N. (2019). Fungal proteases and production of bioactive peptides for the food industry. In *Enzymes in food biotechnology* (pp. 221–246). Elsevier.
Mazorra-Manzano, M. A., Ramirez-Suarez, J. C., & Yada, R. Y. (2018). Plant proteases for bioactive peptides release: A review. *Critical Reviews in Food Science and Nutrition, 58*(13), 2147–2163.
Mazumder, M. A. R., Sujintonniti, N., Chaum, P., Ketnawa, S., & Rawdkuen, S. (2023). Developments of plant-based emulsion-type sausage by using grey oyster mushrooms and chickpeas. *Foods, 12*(8), 1564.
Mirzaei, M., Aminlari, M., & Hosseini, E. (2016). Antioxidant, ACE-inhibitory and antimicrobial activities of *Kluyveromyces marxianus* protein hydrolysates and their peptide fractions. *Functional Foods in Health and Disease, 6*(7), 425–439.
Mirzaei, M., Mirdamadi, S., Ehsani, M. R., & Aminlari, M. (2018). Production of antioxidant and ACE-inhibitory peptides from *Kluyveromyces marxianus* protein hydrolysates: Purification and molecular docking. *Journal of Food and Drug Analysis, 26*(2), 696–705.
Mirzaei, M., Shavandi, A., Mirdamadi, S., Soleymanzadeh, N., Motahari, P., Mirdamadi, N., Moser, M., Subra, G., Alimoradi, H., & Goriely, S. (2021). Bioactive peptides from yeast: A comparative review on production methods, bioactivity, structure-function relationship, and stability. *Trends in Food Science & Technology, 118*, 297–315.
Moosavi, Z. S., Mirdamadi, S., Mirzaei, M., & Laripoor, M. (2020). Effects of ultrasonic and high-pressure homogenization pretreatment on the enzymatic hydrolysis and antioxidant activity of yeast protein hydrolysate. *Journal of Food Biosciences and Technology, 10*(1), 15–26.
Nakahara, T., Sano, A., Yamaguchi, H., Sugimoto, K., Chikata, H., Kinoshita, E., & Uchida, R. (2010). Antihypertensive effect of peptide-enriched soy sauce-like seasoning and identification of its angiotensin I-converting enzyme inhibitory substances. *Journal of Agricultural and Food Chemistry, 58*(2), 821–827.
Naseem, S., Rizwan, M., Durrani, A. I., & Gillani, S. R. (2024). Green approaches to blue food (Seaweed) protein processing and advances in purification techniques. *Sustainable Chemistry and Pharmacy, 38*, Article 101496.
Nikolov, M., Schmidt, C., & Urlaub, H. (2012). Quantitative mass spectrometry-based proteomics: An overview. *Quantitative Methods in Proteomics*, 85–100.
Ó'Fágáin, C., Cummins, P. M., & O'Connor, B. F. (2017). Gel-filtration chromatography. *Protein Chromatography: Methods and Protocols*, 15–25.
Okamoto, K., Ito, R., Hayashi, J., & Tagawa, M. (2021). Production of the antihypertensive peptide Tyr-Pro from milk using the white-rot fungus *Peniophora* sp. in submerged fermentation and a jar fermentor. *Dairy, 2*(3), 452–461.
Okamoto, K., Kawamura, S., Tagawa, M., Mizuta, T., Zahid, H. M., & Nabika, T. (2020). Production of an antihypertensive peptide from milk by the brown rot fungus *Neolentinus lepideus*. *European Food Research and Technology, 246*, 1773–1782.
Paisansak, S., Sangtanoo, P., Srimongkol, P., Saisavoey, T., Reamtong, O., Choowongkomon, K., & Karnchanatat, A. (2021). Angiotensin-I converting enzyme inhibitory peptide derived from the shiitake mushroom (*Lentinula edodes*). *Journal of Food Science and Technology, 58*, 85–97.
Pokora, M., Zambrowicz, A., Zabłocka, A., D\kabrowska, A., Szołtysik, M., Babij, K., Eckert, E., Trziszka, T., & Chrzanowska, J. (2017). The use of serine protease from *Yarrowia lipolytica* yeast in the production of biopeptides from denatured egg white proteins. *Acta Biochimica Polonica, 64*(2), 245–253.
Qing, Z., Cheng, J., Wang, X., Tang, D., Liu, X., & Zhu, M. (2021). The effects of four edible mushrooms (*Volvariella volvacea*, *Hypsizygus marmoreus*, *Pleurotus ostreatus* and *Agaricus bisporus*) on physicochemical properties of beef paste. *Lwt, 135*, Article 110063.
Rahnamaeian, M., & Vilcinskas, A. (2015). Short antimicrobial peptides as cosmetic ingredients to deter dermatological pathogens. *Applied Microbiology and Biotechnology, 99*, 8847–8855.
Rai, A. K., Kumari, R., Sanjukta, S., & Sahoo, D. (2016). Production of bioactive protein hydrolysate using the yeasts isolated from soft chhurpi. *Bioresource Technology, 219*, 239–245.
Ravikumar, G., Gomathi, D., Kalaiselvi, M., & Uma, C. (2012). A protease from the medicinal mushroom *Pleurotus sajor-caju*; production, purification and partial characterization. *Asian Pacific Journal of Tropical Biomedicine, 2*(1), S411–S417.
Renjuan, L., Xiuli, Z., Liping, S., & Yongliang, Z. (2022). Identification, in silico screening, and molecular docking of novel ACE inhibitory peptides isolated from the edible symbiot *Boletus griseus-Hypomyces chrysospermus*. *LWT, 169*, Article 114008.
Rizk, Z., Rayess, Y. E., Ghanem, C., Mathieu, F., Taillandier, P., & Nehme, N. (2018). Identification of multiple-derived peptides produced by *Saccharomyces cerevisiae* involved in malolactic fermentation inhibition. *FEMS Yeast Research, 18*(7), foy080.
Rodriguez Valerón, N., Mak, T., Jahn, L. J., Arboleya, J. C., & Sörensen, P. M. (2023). Derivation of Kokumi γ-Glutamyl Peptides and Volatile Aroma Compounds from Fermented Cereal Processing By-Products for Reducing Bitterness of Plant-Based Ingredients. *Foods, 12*(23), 4297.
Sangtitanu, T., Sangtanoo, P., Srimongkol, P., Saisavoey, T., Reamtong, O., & Karnchanatat, A. (2020). Peptides obtained from edible mushrooms: *Hericium erinaceus* offers the ability to scavenge free radicals and induce apoptosis in lung cancer cells in humans. *Food & Function, 11*(6), 4927–4939.
Song, J. C., & Stevens, D. A. (2016). Caspofungin: Pharmacodynamics, pharmacokinetics, clinical uses and treatment outcomes. *Critical Reviews in Microbiology, 42*(5), 813–846.
Song, R., Liang, T., Shen, Q., Liu, J., Lu, Y., Tang, C., Chen, X., Hou, T., & Chen, Y. (2020). The optimization of production and characterization of antioxidant peptides from protein hydrolysates of *Agrocybe aegerita*. *LWT, 134*, Article 109987.
Song, S., Zhuang, J., Ma, C., Feng, T., Yao, L., Ho, C.-T., & Sun, M. (2023). Identification of novel umami peptides from *Boletus edulis* and its mechanism via sensory analysis and molecular simulation approaches. *Food Chemistry, 398*, Article 133835.
Survase, S. A., Kagliwal, L. D., Annapure, U. S., & Singhal, R. S. (2011). Cyclosporin A—a review on fermentative production, downstream processing and pharmacological applications. *Biotechnology Advances, 29*(4), 418–435.
Tavano, O. L. (2013). Protein hydrolysis using proteases: An important tool for food biotechnology. *Journal of Molecular Catalysis B: Enzymatic, 90*, 1–11.
Terefe, N. S., Buckow, R., & Versteeg, C. (2014). Quality-related enzymes in fruit and vegetable products: Effects of novel food processing technologies, part 1: High-pressure processing. *Critical Reviews in Food Science and Nutrition, 54*(1), 24–63.
Tong, X., & Guo, J. (2022). High throughput identification of the potential antioxidant peptides in *Ophiocordyceps sinensis*. *Molecules, 27*(2), 438.
Tsai, J.-S., Lin, Y. S., Pan, B. S., & Chen, T. J. (2006). Antihypertensive peptides and γ-aminobutyric acid from prozyme 6 facilitated lactic acid bacteria fermentation of soymilk. *Process Biochemistry, 41*(6), 1282–1288.
Vashisht, S., Singh, N., Sharma, A., Saini, N., Gaur, S. N., & Arora, N. (2023). In silico tools to assess the potential allergenicity of shiitake mushrooms (*Lentinula edodes*). *Journal of the Science of Food and Agriculture, 103*(2), 877–890.
Vieira, E. F., Melo, A., & Ferreira, I. M. (2017). Autolysis of intracellular content of Brewer's spent yeast to maximize ACE-inhibitory and antioxidant activities. *LWT-Food Science and Technology, 82*, 255–259.
Wang, B., Shi, Y., Lu, H., & Chen, Q. (2023a). A critical review of fungal proteins: Emerging preparation technology, active efficacy and food application. *Trends in Food Science & Technology, 104178*.
Wang, H., Wang, W., Zhang, S., Hu, Z., Yao, R., Hadiatullah, H., Li, P., & Zhao, G. (2023b). Identification of novel umami peptides from yeast extract and the mechanism against T1R1/T1R3. *Food Chemistry, 429*, Article 136807.
Wang, R., Yun, J., Wu, S., Bi, Y., & Zhao, F. (2022a). Optimisation and characterisation of novel angiotensin-converting enzyme inhibitory peptides prepared by double enzymatic hydrolysis from *Agaricus bisporus* Scraps. *Foods, 11*(3), 394.
Wang, X., Xu, M., Cheng, J., Zhang, W., Liu, X., & Zhou, P. (2019). Effect of *Flammulina velutipes* on the physicochemical and sensory characteristics of Cantonese sausages. *Meat Science, 154*, 22–28.
Wang, X., Zhang, D., Guo, Q., Pu, Y., Huang, A., & Fan, J. (2023c). Identification and Characterization of Novel Umami Peptides from Protein Hydrolysates of *Morchella esculenta* and Their Interaction with T1R1/T1R3 Receptor. *Journal of Agricultural and Food Chemistry, 71*(38), 14046–14056.

Wang, X., Zhou, P., Cheng, J., Chen, Z., & Liu, X. (2018a). Use of straw mushrooms (*Volvariella volvacea*) for the enhancement of physicochemical, nutritional and sensory profiles of Cantonese sausages. *Meat Science, 146*, 18–25.

Wang, X., Zhou, P., Cheng, J., Yang, H., Zou, J., & Liu, X. (2022b). The role of endogenous enzyme from straw mushroom (*Volvariella volvacea*) in improving taste and volatile flavor characteristics of Cantonese sausage. *LWT, 154*, Article 112627.

Wang YuFeng, W. Y., Wu BenYue, W. B., Shao JiangJuan, S. J., Jia JinXia, J. J., Tian YouQiu, T. Y., Shu Xu, S. X., Ren XiaoJie, R. X., & Guan Yue, G. Y. (2018). Extraction, purification and physicochemical properties of a novel lectin from *Laetiporus sulphureus* mushroom. *Lwt, 91*, 151-159.

Wei, C.-K., Thakur, K., Liu, D.-H., Zhang, J.-G., & Wei, Z.-J. (2018). Enzymatic hydrolysis of flaxseed (*Linum usitatissimum* L.) protein and sensory characterization of Maillard reaction products. *Food Chemistry, 263*, 186–193.

Williams, R., Dias, D. A., Jayasinghe, N., Roessner, U., & Bennett, L. E. (2016). Beta-glucan-depleted, glycopeptide-rich extracts from Brewer's and Baker's yeast (*Saccharomyces cerevisiae*) lower interferon-gamma production by stimulated human blood cells in vitro. *Food Chemistry, 197*, 761–768.

Wong, J. H., Ng, T. B., Wang, H., Sze, S. C. W., Zhang, K. Y., Li, Q., & Lu, X. (2011). Cordymin, an antifungal peptide from the medicinal fungus *Cordyceps militaris*. *Phytomedicine, 18*(5), 387–392.

Wongaem, A., Reamtong, O., Srimongkol, P., Sangtanoo, P., Saisavoey, T., & Karnchanatat, A. (2021). Antioxidant properties of peptides obtained from the split gill mushroom (*Schizophyllum commune*). *Journal of Food Science and Technology, 58*, 680–691.

Wu, Q., Li, Y., Peng, K., Wang, X.-L., Ding, Z., Liu, L., Xu, P., & Liu, G.-Q. (2019). Isolation and characterization of three antihypertension peptides from the mycelia of *Ganoderma lucidum* (Agaricomycetes). *Journal of Agricultural and Food Chemistry, 67*(29), 8149–8159.

Wu, S., Wu, Q., Wang, J., Li, Y., Chen, B., Zhu, Z., Huang, R., Chen, M., Huang, A., Xie, Y., et al. (2022). Novel selenium peptides obtained from selenium-enriched *Cordyceps militaris* alleviate neuroinflammation and gut microbiota dysbacteriosis in LPS-injured mice. *Journal of Agricultural and Food Chemistry, 70*(10), 3194–3206.

Xia, Z.-J., Liu, Z., Kong, F.-Z., Fan, L.-Y., Xiao, H., & Cao, C.-X. (2017). Comparison of antimicrobial peptide purification via free-flow electrophoresis and gel filtration chromatography. *Electrophoresis, 38*(24), 3147–3154.

Xiao, Y., Sun, M., Zhang, Q., Chen, Y., Miao, J., Rui, X., & Dong, M. (2018). Effects of *Cordyceps militaris* (L.) Fr. fermentation on the nutritional, physicochemical, functional properties and angiotensin I converting enzyme inhibitory activity of red bean (*Phaseolus angularis* [Willd.] WF Wight.) flour. *Journal of Food Science and Technology, 55*, 1244–1255.

Xu, L., Guo, S., Li, Y., Guo, W., Guo, X., Hong, S., & others. (2023). Ultrasound-Assisted Enzymatic Extraction and Bioactivity Analysis of Polypeptides from *Cordyceps militaris*. *Journal of Chemistry, 2023*.

Xu, X., Xu, R., Song, Z., Jia, Q., Feng, T., Huang, M., & Song, S. (2019). Identification of umami-tasting peptides from *Volvariella volvacea* using ultra performance liquid chromatography quadrupole time-of-flight mass spectrometry and sensory-guided separation techniques. *Journal of Chromatography A, 1596*, 96–103.

Yan, N., He, F., Piraino, F. F., Xiang, H., Chen, J., Wang, Y., & Liu, X. (2015). Antiviral activity of a cloned peptide RC28 isolated from the higher basidiomycetes mushroom *Rozites caperata* in a mouse model of HSV-1 keratitis. *International Journal of Medicinal Mushrooms, 17*(9).

Yao, H., Liu, S., Chang, R., Liu, T., Ma, D., Han, X., Lin, C., Zhou, Z., & Mao, J. (2024). Purification, characterisation and visualisation of soybean protein hydrolysis by aspergillopepsin I from mangrove *Aspergillus tubingensis*. *International Biodeterioration & Biodegradation, 191*, Article 105798.

Yu, H., Zhao, S., Yi, J., Du, M., Liu, J., Liu, Y., & Cai, S. (2023). Identification of Novel Umami Peptides in *Termitornyces albuminosus* (Berk) Heim Soup by In Silico Analyses Combined with Sensory Evaluation: Discovering Potential Mechanism of Umami Taste Formation with Molecular Perspective. *Journal of Agricultural and Food Chemistry., 71*(45), 17243–17252.

Yuan, B., Zhao, L., Rakariyatham, K., Han, Y., Gao, Z., Kimatu, B. M., Hu, Q., & Xiao, H. (2017). Isolation of a novel bioactive protein from an edible mushroom *Pleurotus eryngii* and its anti-inflammatory potential. *Food & Function, 8*(6), 2175–2183.

Zanutto-Elgui, M. R., Vieira, J. C. S., do Prado, D. Z., Buzalaf, M. A. R., de Magalhães Padilha, P., de Oliveira, D. E., & Fleuri, L. F. (2019). Production of milk peptides with antimicrobial and antioxidant properties through fungal proteases. *Food Chemistry, 278*, 823–831.

Zhang, D.-D., Liu, J.-L., Jiang, T.-M., Li, L., Fang, G.-Z., Liu, Y.-P., & Chen, L.-J. (2017). Influence of *Kluyveromyces marxianus* on proteins, peptides, and amino acids in *Lactobacillus*-fermented milk. *Food Science and Biotechnology, 26*, 739–748.

Zhang, Y., Chen, Y., Liu, X., Wang, W., Wang, J., Li, X., & Sun, S. (2023a). Preparation and identification of peptides with α-glucosidase inhibitory activity from shiitake mushroom (*Lentinus edodes*) protein. *Foods, 12*(13), 2534.

Zhang, Y., Liu, L., Zhang, M., Li, S., Wu, J., Sun, Q., Ma, S., & Cai, W. (2023b). The Research Progress of Bioactive Peptides Derived from Traditional Natural Products in China. *Molecules, 28*(17), 6421.

Zhao, R.-J., Huo, C.-Y., Qian, Y., Ren, D.-F., & Lu, J. (2017). Ultra-high-pressure processing improves proteolysis and release of bioactive peptides with activation activities on alcohol metabolic enzymes in vitro from mushroom foot protein. *Food Chemistry, 231*, 25–32.

Zhou, S., Li, X., Lüli, Y., Li, X., Chen, Z. H., Yuan, P., Yang, Z. L., Li, G., & Luo, H. (2021). Novel cyclic peptides from *lethal Amanita* mushrooms through a genome-guided approach. *Journal of Fungi, 7*(3), 204.

Zou, X.-G., Chi, Y., Cao, Y.-Q., Zheng, M., Deng, Z.-Y., Cai, M., Yang, K., & Sun, P.-L. (2023). Preparation Process Optimization of Peptides from *Agaricus blazei* Murrill, and Comparison of Their Antioxidant and Immune-Enhancing Activities Separated by Ultrafiltration Membrane Technology. *Foods, 12*(2), 251.