



Production, characterization and bio-functional properties of multi-functional peptides from fermented plant-based foods: A review

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ABSTRACT

Bioactive peptides derived from fermented plant-based foods have gained increasing attention due to their potential health-promoting properties. Fermentation of plant-based substrates leads to the manufacturing of various peptides with bioactive properties through the enzyme's activity in microorganisms or endogenous plant enzymes. These peptides exhibit diverse biological activities such as antioxidant, antihypertensive, antimicrobial, anti-inflammatory, anti-diabetic and anti-cancer effects. This review provides an overview of the health beneficial properties of peptides with biological activity derived from fermented foods mainly plants, including their modes of operation and prospective uses in promoting human health. Understanding the health benefits and ways of behaviour about bioactive specific protein fragments from fermented plant-based meals can contribute to the creation of dietary supplements and culinary products with enhanced health-promoting properties.

1. Introduction

Consumers prefer healthy foods which have impact on their health and it has led to significant changes like alterations of the eating patterns, most notably, demand of plant-based meals. The primary objectives behind this trend are to safeguard animal welfare, enhance environmental sustainability, and improve individual well-being (Nychas et al., 2021). Growing awareness among consumers about adopting plant-based diets is being supported by ethical concerns, campaigns by animal rights and welfare organizations aimed at lowering the use of livestock and meat consumption and increased the emissions of greenhouse gases (GHG) linked to the production of foodstuff derived from livestock (Kumar et al., 2022). Additional factors that are contributing to these patterns consist of the excessive use of resources, urbanization as well as the unequal distribution of protein (excessive intake) that is primarily seen in developed countries and has a negative impact on health as well as the continuing rise in the worldwide population (Tachie et al., 2023).

Several research demonstrate that plant-based foods in one's diet might effectively retard commencement to lower the danger and possibly perhaps avoid the occurrence of a number of health problems (Bowman, 2020). Plant-based diets provide a broad range of carbohydrates in addition to important macronutrients like proteins, each of which has a unique function in promoting a healthy gut flora. In addition, they offer a wide range of micronutrients that support overall wellness and health, including as vitamins, minerals, and bioactive substances (Samtiya et al., 2021). For instance, meals based on soy deliver essential minerals like calcium and isoflavones, which support bone health and can reduce the risk of osteoporosis (Qin et al., 2022).

Conversely, plant-based diets are categorized as incomplete protein sources because they frequently lack adequate amounts of several important amino acids (Kumar et al., 2022). A diet that includes plant-based foods may help a person's cardiovascular health, lower their risk of cardiovascular disease, improve their glycaemic management, and lower their blood pressure, cholesterol and obesity, according to research findings (Kim et al., 2019). Studies done by van Nielen et al.

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(2014) showed that substituting soy products for beef could improve insulin sensitivity. Furthermore, a study performed by Kahleova et al. (2017), these plant-based diets can also help improve digestive reactions, which in turn helps regulate glycaemic index levels.

Bioactive peptide is discrete, small fragments of protein that have specific biological functions besides their role as food. These peptides may be generated through the body's digestive process, which breaks down proteins, or they may be produced by special processing methods such fermentation or *in vitro* enzymatic hydrolysis. They have been shown to have a wide range of documented benefits, including antihypertensive, antidiabetic (Wang et al., 2019), hypocholesterolemic, immunomodulatory, anti-inflammatory, opiate (Maestri et al., 2016), antioxidant, antimicrobial (Tkaczewska, 2020), hepatoprotective, appetite regulatory (Kaneko, 2021), memory and cognitive improving activities (Katayama et al., 2021). As stated, by Xu et al. (2019), peptides with bioactive properties have the capacity to penetrate the intestinal wall and enter the bloodstream while maintaining their structural integrity, which enables them to carry out their biological actions. There are countless bioactive peptides that have been found in a variety of food sources over time, with dairy, eggs, meat, fish and soybeans receiving special attention (Tang et al., 2021).

A growing number of research is being done on the extraction of bioactive peptides from different plant sources. These include fruits, vegetables (Sosalagere et al., 2022), legumes and grain (Maestri et al., 2016), nuts (Daroit & Brandelli, 2021), mushrooms (Zhou et al., 2020). Numerous health benefits have been linked to plant-derived peptides, such as improved absorption of trace minerals (Zhou et al., 2020), cytoimmunomodulation, opioid activity, blood pressure reduction (Lee & Hur, 2017), antioxidant effects (Durand et al., 2021), lowering of cholesterol levels (Nagaoka et al., 2021), and antimicrobial properties (Liu, Sameen, et al., 2021). Plant-derived bioactive peptides are becoming more and more popular in the fields of functional foods and pharmaceuticals. They are becoming known as good alternatives to supplies produced from animals because they are non-toxic, inexpensive, widely available, and have a variety of uses. An additional benefit of using bioactive peptides generated from plants is that there is no social or religious bias against their use (Sosalagere et al., 2022).

Food proteins can be broken down by fermentation, a useful method that hasn't been studied as much as enzymatic hydrolysis for producing bioactive peptides from dietary sources. Food ingredients may now be effectively extracted and transformed into useful products like organic acids, enzymes, and other biotechnological goods thanks to this technique. According to research, microbial fermentation implies the ability to enhance dietary habits makeup, reduce antinutrients levels and increase the number and types of food products that are available (Tamang et al., 2020). The two main techniques in fermentation technology are submerged fermentation (SmF) and solid-state fermentation (SSF). SSF involves the development of products and microbiological growth on a solid substrate with a small amount of free water present. Even if there isn't much free water, the solid matrix must have enough moisture and nutrients to enable microbial growth and metabolism. SSF settings are ideal for filamentous fungi since they mimic their natural habitats. Furthermore, bacteria and yeasts may survive on solid substrates because they need less moisture. SSF has been widely used to produce bioactive peptides from a variety of dietary sources, including maize gluten meal, quinoa, oats, lupins, and red beans (Jiang et al., 2020).

A number of traditional foods processing techniques, including fermentation, heating, soaking, puffing and germination, have been utilized to reduce antinutritional elements and enhance the protein's digestibility (Samtiya et al., 2020). Lactic acid bacteria (LAB), which include several species including *Pedococcus*, *Lactobacillus*, *Streptococcus*, *Lactococcus*, *Leuconostoc* and *Enterococcus*, are accountable for the manufacture of fermented foods. One of the main metabolites produced by these bacteria is lactic acid. Yeast, filamentous fungus, bacteria, or a combination of these microbes may all be involved in the fermentation process (Kanwar & Keshani, 2016). Microbial metabolism helps to

improve the bioavailability of macro and micronutrients, as well as phytochemicals, during fermentation by making macromolecules more digestible. Furthermore, it is known that fermentation is a very effective way to remove toxins, allergies, and antinutrients from food items (Shiferaw Terefe & Augustin, 2020).

1.1. Objectives

The objective of this study is to investigate and elucidate the health beneficial properties associated with bioactive peptides derived from fermented plant-based foods. This review aims to comprehensively analyze the bioactive peptides present in fermented plant-based foods, explore their potential health-promoting effects, including but not limited to antioxidant, antimicrobial, antihypertensive, anti-cancer and antidiabetic activities and provide insights into their mechanisms of action. Additionally, this study seeks to evaluate the impact of fermentation processes on the generation and bioavailability of these bioactive peptides, contributing to a deeper understanding of their potential applications in functional foods and nutraceuticals for promoting human health and wellness.

2. Materials and methods

A systematic search was performed in, google scholar, PubMed, Web of Science, Springer- Link, and Science Direct related online searching databases using keywords and related terms. It used certain keywords, i. e., plant based bioactive peptides (PDBPs), and combined such terms with the following keywords: enzymatic hydrolysis, extraction, fermentation, bioactivity, characterization of PDBPs structure-activity relationship related to health benefits in and their applications were sought in the literature and in the titles of the works in that respective article and a thorough review of the entire literature. The maximum reference has been gathered from 2020 to 2024. The language for the literature search was English, and references were chosen based on their relevancy. Nevertheless, a summary of information regarding the extraction and analysis of plant protein sources for plant-based bioactive peptides (PDBPs) is shown below, as per review-related sources.

2.1. Plant protein sources for plant based bioactive peptides (PDBPs)

Plant protein sources, such as fruits, legumes, cereals, pseudocereals, seeds, nuts, shoots, and leaves, can deliver high-quality proteins. Globally, individuals consume roughly 21 g of beans daily. Legumes, including 20–45% protein and a high concentration of the essential amino acid lysine, are an excellent protein source. Lupins and soybeans possess a higher protein content (38–45%), whilst beans and peas exhibit comparatively lower levels (17–20%) (Ambika et al., 2022).

Leguminous proteins comprise albumins and globulins, categorized as vialin and legumin. The primary protein component of most legumes, vialin, exhibits a low concentration of sulfur-containing amino acids. Cereal proteins are obtained from grain sources such as rice, millet, barley, corn, sorghum, wheat, and its bran (Nirmal et al., 2024). The protein concentration in cereal grains ranges from 10 to 15% of dry weight, with a substantial proportion derived from storage proteins. The protein composition of legumes is as follows: beans 16–33%, soybeans 35–40%, chickpeas 17–22%, peas 15.5–39.7%, and lentils 26–30%. These storage proteins comprise prolamins, germains, and globulins (Gong et al., 2022). Consequently, the most often ingested seeds encompass chia seeds, flaxseeds, pumpkin seeds, hemp seeds, and watermelon seeds which is a rich source of protein; and several nutrients and minerals. Almonds possess a greater concentration of sulfur amino acids and lysine, the primary limiting amino acid, comparable to cashew nuts (Sá et al., 2020).

However, pseudocereals such as amaranth, buckwheat, and quinoa serve as alternatives to cereals, providing high-quality protein, vitamins, minerals and unsaturated fatty acids. However, other amino acids such

as tryptophan, arginine, and sulfur-containing amino acids are also prevalent in amaranth. Quinoa and amaranth possess high concentrations of lysine, an essential amino acid frequently deficient in grains, rendering them potentially nutritional supplements (Malik & Singh, 2022).

2.2. Green biotechnological methods for plant based bioactive peptides (PDBPs) extraction

2.2.1. Microbial fermentation

Proteolysis is frequently accomplished via bacteria or yeast that generate protease during the microbial fermentation of protein-rich foodstuffs. The proteolytic enzymes produced by these bacteria facilitate the degradation of dietary proteins into biopeptides. This procedure transpires throughout the bacterial growth phase. Fermentation, often conducted by various microorganisms in submerged or solid states, is an alternative approach employed to liberate bioactive peptides. *Lactobacillus* species are predominantly employed in the synthesis of protein hydrolysates (Nasri et al., 2022).

2.2.2. Enzymatic hydrolysis and other methods

Compared to fermentation, enzymatic hydrolysis is a more reliable approach for the extraction of peptides produced from plant proteins. This procedure is also ecologically sustainable. The hydrolysis process is influenced by various factors, including non-protein molecules. The preliminary extraction of crude protein is critically significant. It is essential to select an enzyme and enzymatic conditions suitable for the specific peptide. The yield is substantially influenced by the selected protease, the properties of the proteins, and the hydrolysis conditions. (Koira et al., 2023). Subtilisin, an endo-protease, can cleave a diverse array of peptide bonds, with a preference for methionine and aromatic residues. When hydrolyzing proteins employing numerous proteases, the pH and temperature of the process should be considered, as they will change the activity of the protease (Ospina-Quiroga et al., 2022). Nevertheless, other environmentally friendly extraction methods included microwave-assisted extraction, ultrasound-assisted extraction, pressurized liquid extraction, pulsed electric field-assisted extraction, and supercritical fluid extraction. This extraction technique can also use within combination of enzymatic extraction or other methods (Zia, Khan, et al., 2019). However, Table 1 shown the brief introduction of the advantages and Limitation of the different extraction methods for getting PDBPs.

Table 1
Advantages and limitation of different extraction method for PDBPs.

Extraction Method	Advantages	Limitation	References
Enzymatic hydrolysis	No toxic substances are produced, non-destructive amino acid, high catalytic efficiency, high specificity	Costly, high requirements on substrate environment (composition, enzyme selection, particle size)	(Sun et al., 2024; Wang et al., 2024)
Microbial fermentation	High efficiency, inhibition of Pathogens, low cost	Difficult to control, susceptible to microbial contamination, difficult separation, and purification	
Microwave-assisted extraction	Shorter extraction time, higher extraction efficiency, reduced solvent usage, lower operational costs, protection of sensitive compounds	Equipment costs, limited applicability, need for optimization among microwave power, time, and solvent type	Al Ragib et al. (2024)
Natural Deep Eutectic Solvent (NaDES)	Environmentally friendly, simple preparation, high solvency capacity, low volatility and flammability.	Limited knowledge and research, potential for inconsistency.	(Al Ragib et al., 2024; Hikmawanti et al., 2021)
Ultrasound-assisted extraction	Shorter extraction time, lower solvent consumption, energy efficiency, enhanced extraction efficiency	Optimization requirements, potential for degradation of sensitive compounds	Mehta et al. (2022)
Pressured liquid extraction	Faster extraction times, higher yields, reduced solvent consumption, no need for filtration, environmentally friendly	Optimization requirements, potential degradation of sensitive compounds	(Barp et al., 2023; Rahmanian et al., 2015)
Pulsed electric field-assisted extraction	Enhanced extraction efficiency, reduced solvent usage, shorter extraction times, non-thermal process	Optimization requirements, inconsistent results	Naliyadhara et al. (2022)
Supercritical fluid extraction	High efficiency and yield, selectivity, environmentally friendly, mild extraction condition, solvent recovery	Complexity of process optimization, limited, applicability to polar compounds	(Mora et al., 2025)

2.3. Bioactivities of plant based bioactive peptides (PDBPs) through microbial fermentation

PDBPs are hydrolysed derivatives derived from plants. PDBPs have significant effects, although their structures might be as simple as two amino acids or as complicated as twenty amino acids. The benefits of PDBPs mostly pertain to their enhanced bioavailability and their physiological actions that far surpass those of amino acids (Yuan et al., 2022) as the biological properties of PDBPs mainly include antioxidant, anti-cancer, antibacterial, antihypertensive, hypoglycemic, immunomodulation, and the regulation of intestinal flora (Shi et al., 2019). Bioactive peptides demonstrate diverse biological activities, including ACE inhibition, antidiabetic effects, cholesterol reduction, antibacterial properties, and immunomodulation, which can be utilized in the treatment and management of several health disorders (Sun et al., 2024). However, Table 2 shown the microbial fermentation of plant source to obtain PPDP with bio-functional properties. shown in Table 2.

2.4. Characterization and analysis of bio-functional properties of PDBPs

The characterization and analysis of bioactive peptides provide significant challenges and necessitate ongoing analytical advancements (Okoye et al., 2022). Numerous chromatography techniques, including membrane ultracentrifugation, high-performance liquid chromatography, electrophoresis, ultrafiltration, and precipitation, are commonly employed to separate and identify bioactive peptides and proteins. In terms of selectivity and recovery, affinity chromatography techniques represent the most successful downstream processing approaches (Vollet Marson et al., 2020). Further identification of the peptide can be achieved using mass spectrometry (Moyer et al., 2021) and combination methods (tandem mass spectrometry in combination with LC-MS) (Zhang et al., 2022).

2.4.1. Liquid chromatography-mass spectrometry

Liquid chromatography-mass spectrometry (LC-MS) is a proficient technique for the identification and quantification of peptides produced from plant proteins. Owing to its exceptional sensitivity and ability to detect even trace amounts of peptides in various matrices, LC-MS is the most promising method for ascertaining the presence of bioactive peptides (Wen et al., 2020). Due to the diverse sample types to be analyzed, it may never be feasible to provide a singular standard LC-MS technique for metabolite profiling (Alves et al., 2019).

2.4.2. Ultra-high-performance liquid chromatography–tandem mass spectrometry

UHPLC has been shown to be useful for evaluating complex mixtures

Table 2

Condition of microbial fermentation for PDBPs.

Plant source	Microorganisms	Fermentation conditions	Bioactive peptides	Bio-functional properties	References
Oat	Lactobacillus spp and Bifidobacterium spp	37 °C for 48 h in anaerobic condition	FNDRLRQGQLL, GLVYIL, GQTV, GQTVFNDRLRQGQLL, YHNAP, YHNAPGLVYIL, DVNNNANQLEPR	antidiabetic, antioxidant, antihypoxic, antihypertensive, antithrombotic, antifatigue, immunomodulator and hypocholesterolamic	Mathews and Chu (2024)
Wheat, Lupin, quinoa	Lactobacillus. reuteri and L. plantarum	35°C for 72 h	AREGETVVPQ, PVLGPVRGPFPPL	Antioxidant, anti-cancer and ACE inhibition	(Ayyash et al., 2019; Weng et al., 2023)
Barley	Rhizopus oryzae, Lactobacillus plantarum	32 °C for 36 h	Hordein, Lunasin, VLPVPQ	Antioxidant activity	Wang et al. (2020)
Grapes, Grapes seed	Lactobacillus spp. Acetobacter and Gluconobacter	70 °C for 24 h	AWPF, SWSF, YYAPF, WVPSVY, LIPPGVPY, YYAPFDGL	Antioxidant Activity, Cardiovascular Protection, Anti-inflammatory Effects, Anti-cancerous, antimicrobial	Rizwan et al. (2023a, 2023b)
Soybeans	B. subtilis	3 days at 36°C	IKAFKEATKVDKVVVLWTA, PGTAVF	Antioxidant activity, antibacterial;	Ruan et al. (2020)
Cucumber	Lactobacillus spp.	18 °C–24 °C for 3–5 days	SCAK33	Antibacterial, antiooxidant	Zhao et al. (2024)
Coffee	Bacillus clausii	37°C for 39 h	YGF, GMCC, YWRYDCQ, RMYRY	Antioxidant potential and ACE and DPP-IV inhibitory activities	Ramírez et al. (2021)
Chicks' pea,	Pediococcus pentosaceus	37°C for 72 h	Leg1 RIKTVTSFDLPALRFLKL Leg2 RIKTVTSFDLPALRWLKL	Antioxidant activity, antimicrobial	Xing et al. (2020)
cow pea	Lactobacillus brevis, Lactococcus lactis	30 °C for 72 h	GPETAFLR	Antioxidant activity, anti-inflammatory, antimicrobial, antithrombotic, antidiabetic, Antioxidant and ACE-inhibitory	Juárez-Chairez et al. (2022)
Tomato seeds	B. subtilis	37°C for 10 days	DGVVYV, GQVPP		Moayed et al. (2018a, 2018b)
Banana peels	Leuconostoc mensenteroides, Lactobacillus paracasei, Lactobacillus brevis, L. Plantarum, Pichia kluyveri LPBII-A	28°C for 24 h	BLP-7,	Antimicrobial activity	Joseph et al. (2024)
Pineapples	Aspergillus niger, Saccharomyces cerevisiae	24 °C–30 °C for 2–5 days	Bromelain (MD2 v2)	Antioxidants activity, antimicrobial	Meena et al. (2022)
Watermelon	Trichoderma virens	20 °C to 25° for 3–14 days, 2%–3% salt	CIAAP3, CIAAP6	Antioxidant activity, anti-inflammatory activity	(Okoye et al., 2022a; Zia et al., 2023)
Carrot	Lactobacillus acidophilus	15 °C–24 °C for 5 days to 2 weeks with 2–3% salt, anaerobic	STYQQYM, MFRRXVK	Antioxidant activity, antimicrobial abtivity	Mall and Patel (2024)
Water Kefir grain	Lactobacillus spp. Lactococcus spp	20 °C–25 °C for 12–48 h,	RDMPIQAFL, SLSQSKVLPVPQ, EMPFPKYPVEPF	Antioxidant, antidiarrheal activity	Okoye et al. (2022a)

in both isocratic and gradient modes, and is extensively utilized in peptide identification and isolation. The integration of UHPLC with mass spectrometry (MS) enhances sensitivity, selectivity, and high throughput in peptide analysis. UHPLC coupled with tandem MS is an effective technique for both targeted and nontargeted analysis (Nazim et al., 2023a). The UHPLC-ESI-MS/MS has recently proven to be an effective method for the concurrent analysis of plant-derived bioactive peptides, owing to its rapid separation capabilities, high throughput, minimal solvent consumption, exceptional resolution, specificity, selectivity, and the ability to detect multiple ions through selective ion fragmentation. (Okoye et al., 2022b).

2.4.3. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry

The Matrix-Assisted Laser Desorption/Ionization (MALDI) technology analyzes protein fingerprints by comparing them to a library of reference spectra through several integrated algorithms (Welker and Moore, 2011). A TOF analyzer, which quantifies the mass of intact peptides, is commonly employed alongside MALDI. MALDI-TOF MS is classified as a “soft” ionization technique due to its minimal fragmentation, enabling the detection of molecular ions in complex biopolymer mixtures (Umemura et al., 2024). MALDI-TOF-MS has shown to be a versatile technology for the analysis of biological materials, especially

proteins and peptides (Alves et al., 2019).

2.4.4. Other chromatographic methods

Gas Chromatography (GC) is particularly effective for analyzing low molecular weight analytes, as it separates them based on their volatility and interactions with the stationary phase. Capillary Electrophoresis (CE) is an analytical method that segregates and examines charged molecules according to their charge, mass, and atomic radius. The particle's migration velocity is directly related to the intensity of the applied electric field; increased field strength results in enhanced mobility (Nazim et al., 2023b).

3. Fermented plant-based food multifunctional peptides: production, characterization, and bio-functional properties

Numerous beneficial benefits on human health have been demonstrated for bioactive peptides derived from fermented plant-derived meals. These peptides are protein fragments with a low molecular weight that have positive physiologically consequences for human beings. They are created by the highly active metabolic processes of microbes during fermentation, which change food matrix macromolecules into bioactive elements, such as bioactive peptides (Toutirais et al., 2023). In a nutshell, the health benefits of bioactive peptide compounds

are as follows.

- Effects against Hypertension:** It has been discovered that bioactive peptides from fermented foods, especially plant-based sources, have an effect on hypertension. It has been observed that peptide fractions derived from fermented milk and soybean products can lower systolic blood pressure in both mildly hypertensive human volunteers and spontaneously hypertensive rats (Zaky et al., 2022).
- Antioxidant Effects:** These peptides have antioxidative properties that can aid in the body's defence against oxidative stress (Zaky et al., 2022).
- Antimicrobial Effects:** It has been discovered that fermented plant-based diets contain bioactive peptides with antimicrobial qualities (Toutrais et al., 2023).
- Immunomodulatory Effects:** They also display immunomodulatory effects, which have the potential to improve and regulate immunity (Rizwan, Masoodi, Wani, & Mir, 2023a, 2023b).
- Additional Possible Effects:** These peptides may also have anti-osteoporotic, mucin-stimulating, opioid, and insulin-mimetic properties (Rizwan et al., 2023a, 2023b).

This review focuses on the potential use, nutritional value and beneficial health effects of plant based fermented bioactive peptide as a source of food protein that exposes its beneficial effect on human health, schematically shown in Fig. 1. Both microbial fermentation and enzymatic hydrolysis with pepsin, trypsin, and papain are used in the biologically active peptide's synthesis via fermented plant-derived diets. Combining these approaches has been used in several researches to create peptides with biological activity (Amigo & Hernández-Ledesma, 2020). More thorough research is needed on the optimization of fermentation strains, sequence modification of bioactive peptides, and enhancement of bioaccessibility and bioavailability in order to enable the production and implementation of bioactive peptides. The most advantageous plant-based bioactive peptides for human health have been shown to come from a variety of plant sources and display a variety

of advantageous health traits (Chakrabarti et al., 2018; Chaudhary et al., 2021). These peptides are thought to be an assisting compound for the creation of nutritional supplements or dietary supplements. In the live organism, they have antioxidative, anti-inflammatory, anticancer, antibacterial, immunomodulatory, and antihypertensive properties, among other important roles. As an illustration, oilseeds are one type of plant protein that can be utilized as a substitute in a human diet (Toutrais et al., 2023). According to the data available, oilseeds have a protein concentration of up to 40% and a very balanced amino acid profile, with sulfur-containing amino acids making up a portion of the profile. However, their anabolic potential is lower than that of animal proteins because they typically lack lysine and are high in anti-nutritional factors (ANFs). Furthermore, plant-based bioactive peptides with antihypertensive, hypocholesterolemic, immunomodulatory, antioxidant, antibacterial, anti-diabetic, opiate, and others properties are being recognized for maintain the health benefits and disease prevention capacity (Rizwan et al., 2023a, 2023b).

The peptides produced from multiple kinds of plants and their derived compounds serve significant physiological advantages (Chaudhary et al., 2021). Soy milk and seeds are known to contain bioactive peptides with antibacterial qualities (Kangari et al., 2018). Furthermore, biologically active peptides containing ACE inhibitory abilities have been revealed in cultured soy products like natto (Dwivedi et al., 2024). Multiple bioactive peptides have been discovered within cereal grains such as barley, millet, sorghum, corn, rice, and wheat; barley and wheat are especially known to have potent ACE inhibitory qualities. Further, whole grains have a good track record of helping against chronic illnesses like cancer, diabetes, and heart disease (Chauhan et al., 2024).

The wide range of proteins obtained from plants, which are essential parts of the diets of both humans and animals, are referred to as plant protein sources. In contrast to animal proteins, which are mostly found in meat, dairy products, and eggs, plant proteins can be found in a variety of plant-based foods, such as grains, legumes, nuts, seeds, and some vegetables. Plant protein sources are becoming more and more popular

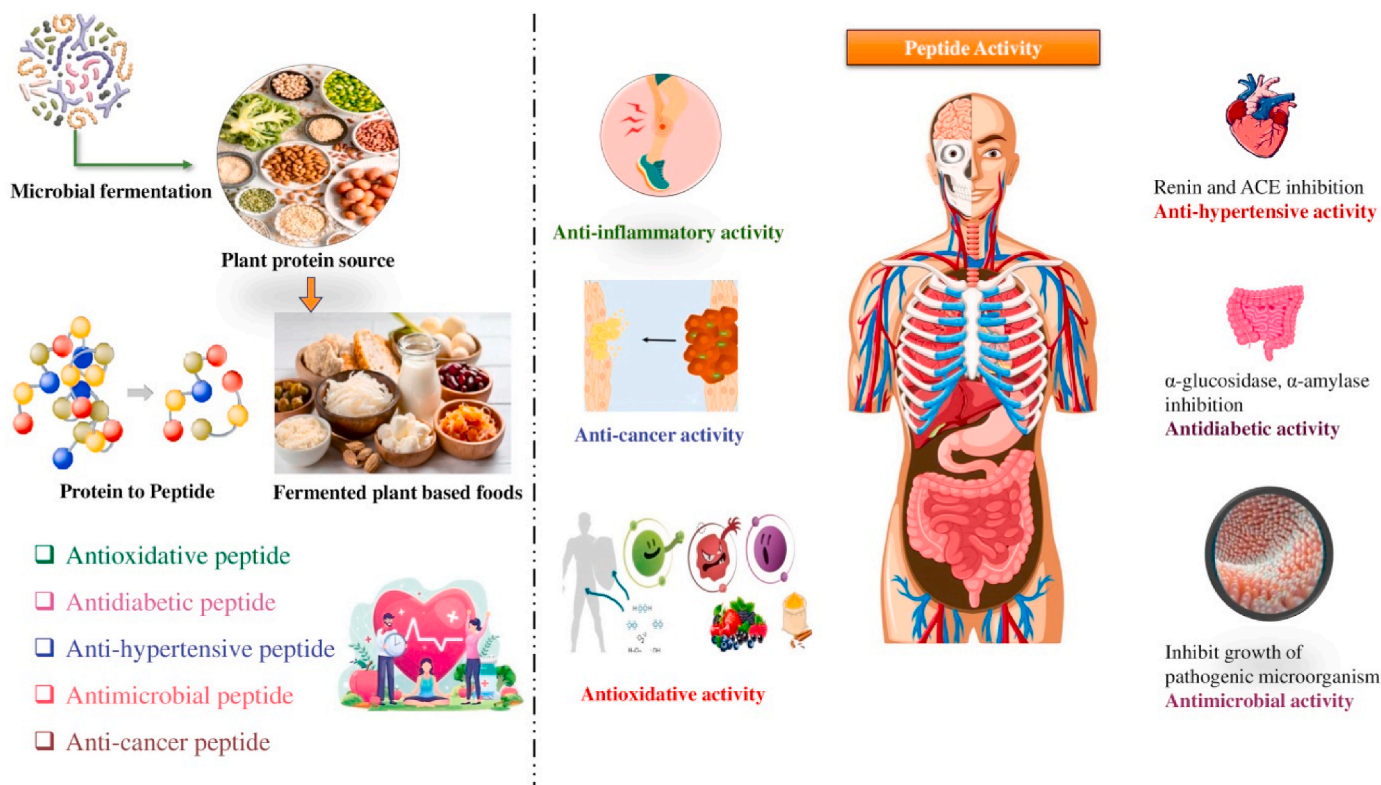


Fig. 1. Beneficial activity of peptide on human health.

and significant for a variety of reasons, such as ethical concerns, environmental sustainability, and health benefits. For reasons including reduced environmental impact, resolving animal welfare concerns, and lowering the risk of chronic diseases, plant-based diets have become more popular among people looking for alternatives to traditional animal-based diets. Typical plant protein sources are shown in Fig. 2. As the building blocks of proteins, amino acids are essential for numerous bodily functions such as immune system function, hormone synthesis, and muscular growth. The human body requires 20 distinct amino acids to function correctly; these can be found in a variety of dietary protein sources. Amino acid concentrations and kinds vary throughout dietary protein sources. Complete proteins are derived from sources involving animals, including meat, fish, poultry, eggs, and dairy products. These sources provide all nine essential amino acids, which the body is unable to manufacture on its own. Histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine are among these essential amino acids. On the other hand, incomplete proteins—those that lack one or more of the necessary amino acids—may be found in plant-based protein sources (Table 3). However, people can receive all needed amino acids and produce a complete protein by combining several plant-based protein sources (such as grains and legumes). Plant-based sources of protein include quinoa, lentils, beans, tofu, tempeh, almonds, and seeds.

4. Bioactive peptides

Small fragments of protein referred to as “bioactive peptides” have beneficial impacts on many different kinds of body systems as well as general human health. These peptides usually consist of two (di-peptides) to twenty amino acid residues, with a molecular mass between 0.4 and 2 kDa. Longer peptides are not frequent, although they do occur. Lunasin, a soy-derived peptide with 43 amino acids, is one such instance. It has been demonstrated that lunasin possesses hypocholesterolemic and anti-cancer effects (Ulug et al., 2021). Food-derived peptides, which are recognized for their bioactivity, are very promising for the creation of functional foods and nutraceuticals that are intended to treat a range of chronic medical conditions. Peptides show ability in treating diseases like obesity, oxidative stress, inflammation and

hypertension, providing avenues for both therapeutic and preventive approaches (Chakrabarti et al., 2014).

4.1. The technique for generating bioactive peptides

There are multiple techniques for obtaining accessible peptides. Although these plant-derived peptides are able to be derived from plant's organs and tissues, using the method of extraction which is not effective in creating particular peptide medications with special medicinal advantages. Extraction is a separate method for bioactive peptide included innovative thermal and non-thermal extraction techniques, such as microwave-assisted extraction, ultrasound-assisted extraction, pressured liquid extraction, pulsed electric field-assisted extraction, and supercritical fluid extraction (Zia et al., 2020). Another possibility is chemical synthesis, although it is expensive as well as filled with undesirable consequences. As an alternative, DNA recombination technology can be used to create bioactive peptides. In recent years, fermentation by microorganisms and hydrolysis using digestive or proteolytic enzymes are among the most employed techniques (Jao et al., 2015). Chemical or enzymatic hydrolysis and fermentation extract bioactive peptides with specific length of the amino acid residues. Microwave, ultrasound-assisted extraction, ohmic heating, pulsed electric fields, and subcritical water hydrolysis have been studied to increase bioactive peptide hydrolysis (Bamdad et al., 2017) FermentDB (<https://webs.iitd.edu.in/raghava/fermentdb/>) is a meticulously maintained database of bioactive peptides sourced from a diverse array of foods, providing extensive information on peptides and the fermentation process (Chaudhary et al., 2021). Future research on bioactive peptides can focus on optimizing fermentation techniques, such as adjusting temperature, pH, and time, and exploring novel microorganisms or co-cultures. Combining fermentation with methods like enzymatic hydrolysis or ultrasound-assisted extraction may improve yields and bioactivity. Enzyme engineering and recombinant technologies can provide better control over peptide size and sequence, enhancing their bioactivity. In silico peptide design using computational tools can accelerate the discovery of bioactive peptides, while high-throughput screening technologies allow for efficient testing of large peptide libraries. Sustainable peptide production can benefit from green extraction methods, and advanced delivery systems can enhance bioavailability. Research into post-translational modifications and the role of the gut microbiome in peptide bioactivity may also open new therapeutic possibilities.

4.1.1. Physical methods

Physical approaches for food production are preferred over chemical or enzymatic ones due to fewer changes. The aforementioned methods are generally simpler to implement and cheaper to use for industry. Peptide extraction methods that are often used in homogenization, freeze-thaw cycles, high-speed blending, colloidal milling, and the use of high pressure. (Amagliani et al., 2017).

Physical methods for bioactive peptide production, such as high-pressure processing, ultrasonication, and heat treatment, offer advantages like being non-toxic, chemical-free, and scalable system. These techniques enhance peptide release and minimize the need for processing additives, making them cost-effective and suitable for large-scale production while retaining peptide functionality. However, drawbacks include high energy consumption, lack of specificity compared to enzymatic methods, potential protein denaturation, and the high cost of equipment. Additionally, the variability in peptides production may challenge to control the bioactivity consistently (Han et al., 2018).

4.1.1.1. Microwave-assisted extraction. An innovative method that uses electromagnetic waves with wavelengths between 1 mm and 1 m and frequency between 300 MHz and 300 GHz is a microwave heating. Its features, which include uniform heating, quick heating rates, safety,

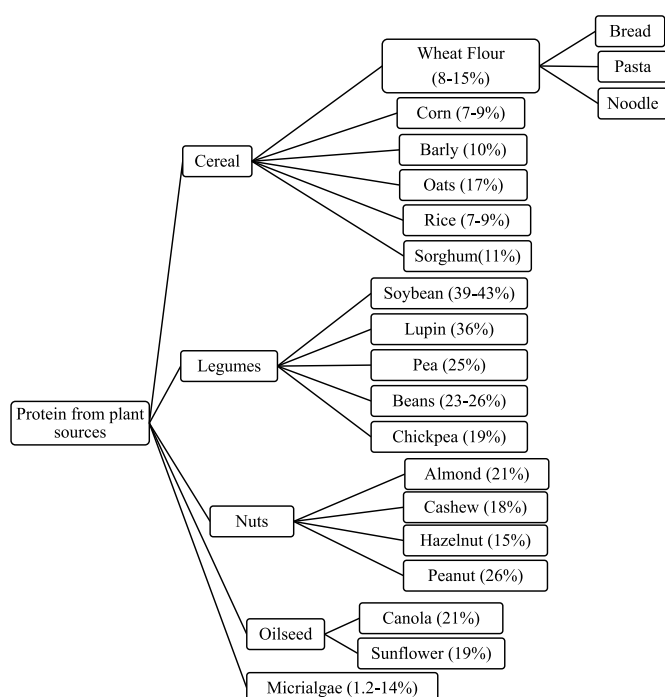


Fig. 2. Protein contents from plant sources (Melton et al., 2018).

Table 3

Amino acid content of various dietary protein sources.

EAA	Basic		Neutral	Neutral (Branched chain)			Aromatic		Sulfur AA
Protein source	Histidine	Lysine	Threonine	Isoleucine	Leucine	Valine	Phenylalanine	Tryptophan	Methionine
Wheat flour	26	27	31	37	75	47	85	13	41
Corn	27	29	36	39	128	51	100	8	44
Oat	25	43	35	39	76	54	91	9	43
Rice	26	28	37	45	88	63	82	14	44
Sorghum	18	16	29	34	127	43	76	n.d.	7
Soybean	28	73	47	54	90	55	99	16	31
Soy protein isolate	25	56	39	49	56	51	94	13	26
peanut	25	39	22	35	70	40	88	7	16
Soy protein concentrate	25	62	36	45	76	47	86	12	28
Fava beans	31	63	39	35	85	48	77	8	27
Chickpea	23	55	32	38	65	37	82	10	22
Lupin	39	45	40	40	69	41	80	10	13
Hemp protein isolate	32	27	34	36	65	47	83	10	32
Microalgae	26	66	52	45	93	61	98	n.d.	27
Milk	28	84	47	53	99	65	95	14	30
Egg	25	78	51	57	91	64	98	12	47

(Essential Amino Acid = EAA-mg/g protein, n.d. = not determine) (Day et al., 2022).

simplicity of use, effectiveness, cleanliness, and affordable maintenance, have led to its widespread use in the food processing industry. These microwave heating radiations are conveyed as waves that can infiltrate the matrix and directly interact with polar molecules, such as water, through dipole rotation and ionic conduction to produce heat. MAE is employed for the extraction of several nutritional components and antioxidants from food matrices and trash. It is a more economical and expedient extraction approach compared to traditional methods (Chuyen et al., 2018). This heating method also affects food flavour and nutrition less than regular heating. This method has the capability to disrupt proteins by disrupting both disulfide and hydrogen bonds, which are non-covalent interactions. Consequently, it can lead to the unfolding of proteins, thereby impacting their secondary and tertiary structures (Han et al., 2018). The microwave approach improved chia seed protein enzymatic hydrolysis by increasing bioactivity (antioxidant activity) and functionality (emulsification and foaming capabilities) in less time (Urbizo-Reyes et al., 2019).

In recent times, peptides that are bioactive compounds comprising of cricket (Hall & Liceaga, 2020), chia (Urbizo-Reyes et al., 2019), trout frames (Ketnawa et al., 2018), and rainbow trout frames (Nguyen et al., 2017) which have been prepared via microwave-assisted enzymatic hydrolysis. Higher protein breakdown as well as the formation of peptides with smaller molecular weights were among the reasons of microwave-assisted hydrolysis using enzymes proved more biologically active than heat treatment during hydrolysis. When compared with the traditionally heated hydrolysed cricket protein ($IC_{50} = 0.20$ mg/mL and 0.65 mg/mL, respectively), the microwave-assisted enzymatic hydrolysed cricket protein significantly demonstrated higher DPP-IV and ACE inhibition ($IC_{50} = 0.096$ mg/mL and 0.27 mg/mL, respectively) (Hall & Liceaga, 2020). In comparison with water bath supported traditional (87.54%) or ordered (80.06%) hydrolysis products, microwave-assisted singled enzyme (alcalase; 93.13%) or sequential (alcalase in addition to flavourzyme; 94.76%) hydrolysed chia proteins considerably demonstrated greater cellular antioxidant capacities (Urbizo-Reyes et al., 2019).

4.1.1.2. Ultrasound-assisted extraction method. Ultrasound waves (US), a green technology above 20 kHz, are employed in extensive industrial applications such as degassing, homogenization, particle size reduction, defoaming, crystallization, bioactive component extraction, low-

temperature pasteurization, and eco-friendly extraction. The UAE permits comprehensive compound extraction within minutes, achieving elevated extraction rates, diminished solvent consumption, increased product capacity, efficient work-up and handling, elimination of post-extraction wastewater treatment, and reduced fossil energy usage compared to maceration, distillation, and Soxhlet methods. Organic acids, antioxidants, pigments, and inorganic compounds have been extracted from various food matrices. The United States amplifies mechanical agitation, perturbing cellular membranes. Disruption of cell walls enhances solvent infiltration and mass transfer (Barati et al., 2020; Zia, 2019).

Sonication represents an innovative, environmentally friendly, and sustainable approach, heralding a new era in processing methods that uses undetected high sound waves (>16 kHz). Compared to typical thermal methods, this method is more efficient, faster, cheaper, easier to apply and operate, less contaminated, and produces better food (Urbizo-Reyes et al., 2019). Zhao et al. (2022) demonstrated that subjecting walnut protein isolate to sonication at power levels of 200, 400, or 600W for durations of 15 or 30 min led to alterations in its secondary and tertiary structures, while the primary structure remained unaffected due to the inability to disrupt covalent bonds. Similarly, Vanga et al. (2020) observed a 52% decrease in trypsin inhibitor activity of soymilk protein along with enhanced digestibility following ultrasonic treatment at 25 kHz and 400W for durations ranging from 1 to 16 min.

In recent years, numerous types of food proteins, such as the protein from whey (Wu et al., 2018), protein from milk (Uluko et al., 2015), rapeseed protein (Wali et al., 2017), along with corn protein, have been hydrolysed using an ultrasound to generate food-derived biologically active peptides or hydrolysis products. UAE by itself is likewise insufficient to dissolve peptide linkages; thus, hydrolysis using enzymes and ultrasonography have been used in tandem to increase the synthesis of peptides with biological activity. By making enzymes more accessible into the peptide bonds, sonication improves the extraction of bioactive peptides from meals. Recently, Wu et al. (2018) conducted a thorough investigation of the thermodynamic characteristics of whey protein hydrolysed by alcalase with ultrasonic pretreatment (20 kHz, 300 W for 15 min). Whey protein began to unfold as a result of the ultrasonic pretreatment procedures, which also raised the amount of free sulfhydryl. Additionally, modifications to conformity were brought about by ultrasound, exhibiting a boost in the β -sheet and β -turn formation.

Whenever the enzymatic breakdown of whey protein was aided by the ultrasound, the ACE regulatory and immunomodulatory properties of the hydrolysates were dramatically strengthened (Wu et al., 2018).

4.1.1.3. Ohmic heating. Ohmic heating uses alternating electric currents to heat semi-conductive material. The first use was milk pasteurization in 1920. Joule's law states that a moderate and alternating electric current, which acts as resistance in an electrical circuit, generates direct or volumetric heat in products. Thus, Li et al. (2018) compared the ohmic and traditional heating of soybean milk protein's structure and techno-functionality. Ohmic heating shortened heating time and improved protein emulsification. As surface hydrophobicity decreased, protein foaming is also decreased. Ohmic heating has been utilized to prompt protein aggregation and gelation by causing protein unfolding and denaturation, as demonstrated by Pereira et al. (2017). In the processing of soy milk, the application of ohmic heating has been noted to elevate the levels of free amino acids, indicating potential protein decomposition during high-voltage processing, as observed by Li et al. (2018). Sweet whey processed with ohmic heating at a low electric field (between 2 V and 4 V cm⁻¹) showed much higher ACE inhibitory activity compared to traditional pasteurization at 72–75 °C. However, this effect decreased when the field strength reached 5 V cm⁻¹ (Costa et al., 2018). A similar pattern was also observed for antioxidant activity. The authors suggested that bioactive compounds were released during ohmic heating, but they did not study the formation of peptides, which would be important to explore further to understand how ohmic heating influences the production of bioactive peptides.

Ohmic heating is an efficient technique that uses electric current to uniformly heat-treated food materials, offering several benefits for processing plant proteins to produce bioactive peptides. Its key advantages include uniform heating, which reduces hotspots and ensures consistent peptide release while preserving heat-sensitive components. The method also provides shorter processing times, enhancing the retention of bioactive properties and minimizing nutrient loss. Additionally, ohmic heating is energy-efficient and scalable for industrial use, improving peptide yield and bioavailability by facilitating the breakdown of plant cell walls.

However, challenges include the high initial equipment cost, potential electrode corrosion, and the complexity of controlling process parameters due to variations in electrical conductivity. There are concerns about the long-term effects on peptide integrity in large-scale applications, as well as risks of unwanted electrochemical reactions affecting product quality. Despite reducing processing time, precise temperature control is still required to prevent degradation of heat-sensitive compounds.

4.1.1.4. Pulsed-electric field (PEF). This extraction technique employs a liquid solvent at pressures ranging from 2 to 20 MPa and a temperature of 200 °C to isolate certain compounds. This extraction technique employs a solvent at a temperature exceeding its boiling point to enhance solubility and mass transfer. Pressurized or accelerated solvent extraction and pressurized hot water extraction (PHWE) utilize H₂O as a solvent. This eco-friendly technique employs pressurized water at high pressures (3.5–20 MPa) and elevated temperatures (313–473 K) to enhance extraction kinetics without the use of organic solvents. Water is the selected solvent for this procedure. PLE is conducted in a sealed extractor to protect the chemical from light and oxygen exposure (Zhou et al., 2022).

PEF inactivation of microbes and enzymes is non-thermal. In this method, food samples undergo brief electrical pulses (μs or ms) between

electrodes. PEF systems have a chamber, electrodes, high-voltage pulse generator, and computer for device monitoring and control. A strong electric field forms between two electrodes due to their electrical potential difference. Electrical energy from PEF may unfold proteins and increase solute interactions (Zhang et al., 2017). This increases peptide/protein solubility, which affects function. However, PEF treatment of canola seeds improved protein solubility, emulsifying, and foaming. The strength and duration of the PEF process might cause denaturation and aggregation, decreasing solubility. The PEF technique alters plant-derived peptides and proteins' secondary and tertiary structures. The antioxidant activity and secondary structure of pine nut protein peptides were also revealed (Liang et al., 2018).

4.1.1.5. Supercritical fluid extraction (SFE). Supercritical fluid extraction (SFE) isolates designated components from solid or liquid food matrices utilizing supercritical fluid as a solvent. It can extract the requisite chemical or eliminate undesirable components from products on a larger scale. This extraction process offers numerous advantages over conventional approaches due to its utilization of supercritical fluid solvent. Diverse solvents possess distinct dielectric constants, diffusivities, densities, and viscosities. Supercritical fluids exhibit elevated diffusivity, reduced viscosity, and enhanced transport properties, enabling them to permeate solid matrices and augment extraction rates. The solvent utilized in this extraction technique is mostly CO₂, occasionally supplemented with ethanol or methanol. Standard supercritical fluid extraction (SFE) operating conditions require carbon dioxide at temperatures exceeding 31 °C and a pressure of 7.4 MPa. Nonetheless, modifications may alter these conditions. This technique uses high-pressure carbon dioxide to extract important bioactive compounds from food (Alvarez et al., 2019).

4.1.2. Chemical methods

The most common approach for plant protein extraction uses alkalis like sodium hydroxide. It solubilizes rice bran proteins by breaking hydrogen and amide bonds. Although this technique is effective at making most proteins soluble, it causes structural modifications that render a protein inactive. Enzymatic hydrolysis presents an alternative approach to segregating dietary proteins and their resulting hydrolysates or peptides. (Yan et al., 2015). Cell wall disintegration, starch-bond protein release, and protein solubility enhancement are all ways enzymes help extract protein from meals isolated protein from rice bran using phytase and xylanase and found that carbohydrates might increase soluble protein output (Wang et al., 1999). In the past, the majority of hydrolysed proteins were made in the USA by acid hydrolyzing vegetable proteins for use as flavourings in processed meat, crackers, and soup mixes. Fish proteins have been hydrolysed using the acid hydrolysis method. When compared to protein hydrolysates generated by enzymes, those obtained via acid hydrolysis exhibited fewer nutritious qualities (Wisuthiphaet et al., 2016).

4.1.2.1. The enzyme-assisted extraction (EAE) method. Enzyme extraction and fermentation are efficient biological techniques for the extraction of bioactive components from agro-industrial waste. Enzyme-assisted extraction (EAE) enhances extraction time, reduces solvent usage, improves product quality and purity, and decreases manufacturing costs in comparison to traditional extraction methods (Lemes et al., 2022). Various enzymes such as cellulases, hemicellulases, pectinases, amylases, proteases, and lipases may be utilized. Operating parameters such as pH, temperature, enzyme and substrate concentrations, solid/liquid ratio, substrate particle size, and reaction duration

influence enzyme activity (Hernández Becerra et al., 2021). Consequently, pectinase, alpha-amylase, hemicellulase, cellulase, and glucoamylase enzymes, where Solvent-biomass ratio: 5 ml/g; solvent: water; temperature: 50–70 °C; enzyme loading: 0.1–1% v/v biomass; duration: 5.5 h; agitation: 200 rpm for caffeine and tannin metabolites (Chen, Ning, et al., 2021). Consequently, the solvent-biomass ratio is 20 ml/g, utilizing a solvent composed of a 12.5% ethanol solution with 4 g/L tartaric acid, at a pH of 3.6, within a temperature range of 20–30 °C, with an enzyme loading of 15 mg/L, for a duration of 72 h to extract anthocyanins and flavanols metabolites (Nogales-Bueno et al., 2020).

4.1.2.2. Natural deep eutectic solvent (NADES) method. NADES is a quaternary ammonium salt or hydrogen-bond acceptor (HBA) combined with a hydrogen-bond donor (HBD) yields NADES, an innovative eco-friendly solvent akin to ionic liquids. NADES are among the most efficient green solvents regarding extractability, stability, recovery yield, bioactivity, solubility, and bioavailability. Moreover, NADES is cost-effective, straightforward, and rapid. NADES exhibits few adverse effects and is utilized to enhance the extraction of bioactive components from plants. Choline chloride has been utilized as a principal constituent of NADES in historical literature because to its cost-effectiveness and biodegradability (Ivanović et al., 2020).

4.1.3. Production of bioactive peptides using fermentation

Selecting the appropriate fermenting strains is essential for producing bioactive peptides. Fermented product's characteristics are greatly affected by the composition and interaction of microorganisms. Common strains used in microbial fermentation nowadays include strains of single-cell, *Lactobacillus* and *Lactococcus* bacteria, acetic acid bacteria, several strains of yeast and even kefir (Fan et al., 2019). Lactic acid bacteria have become the most popular option for dietary protein fermentation, which accelerates the process of breakdown. One novel bioconversion method is the fermentation process that uses the LAB protein. Through the application of specific laboratory strains, it produces greater quantities of proteolytic enzymes, which in turn generate bioactive peptides. Important components of the lactic acid bacteria's proteolytic framework comprise enzymes that hydrolyse protein into oligopeptides, certain peptides that aid in the transportation of the intracellular peptidases and oligopeptides that hydrolyse further peptides (Chaves-Lopez et al., 2014).

5. Identification and purification of bioactive ingredients

5.1. Identification and analysis of plant protein-derived peptides

The peptide structure must be established via several separation and purification processes. Common methodologies for peptide identification encompass DNA translation, mass spectrometry, and peptide sequencing systems. The benefits of great efficiency, sensitivity, and excellent reproducibility have resulted in the extensive application of mass spectrometry (MS) for peptide sequence identification. It is vital to highlight that these usages have been prevalent. Fast atom bombardment, electrospray ionization (ESI), and matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) are the three principal categories of mass spectrometers (Minkiewicz et al., 2019). Mass spectrometers are categorized based on the several principles that control their operation. The most often employed techniques for identifying antioxidant peptides are ESI and MALDI-TOF MS. This is due to the elevated sensitivity and minimal detection threshold inherent in these approaches. Utilizing the mass spectrometer, we successfully obtained the mass-to-charge ratio (m/z) of the peptide subfractions (Wen, Zhang, Feng, et al., 2020). The data was subsequently input into the online protein database to determine the amino acid sequence. The peptide sequencer can be utilized for de novo sequencing of peptide sequences when the online protein database lacks information about the primary structure of the

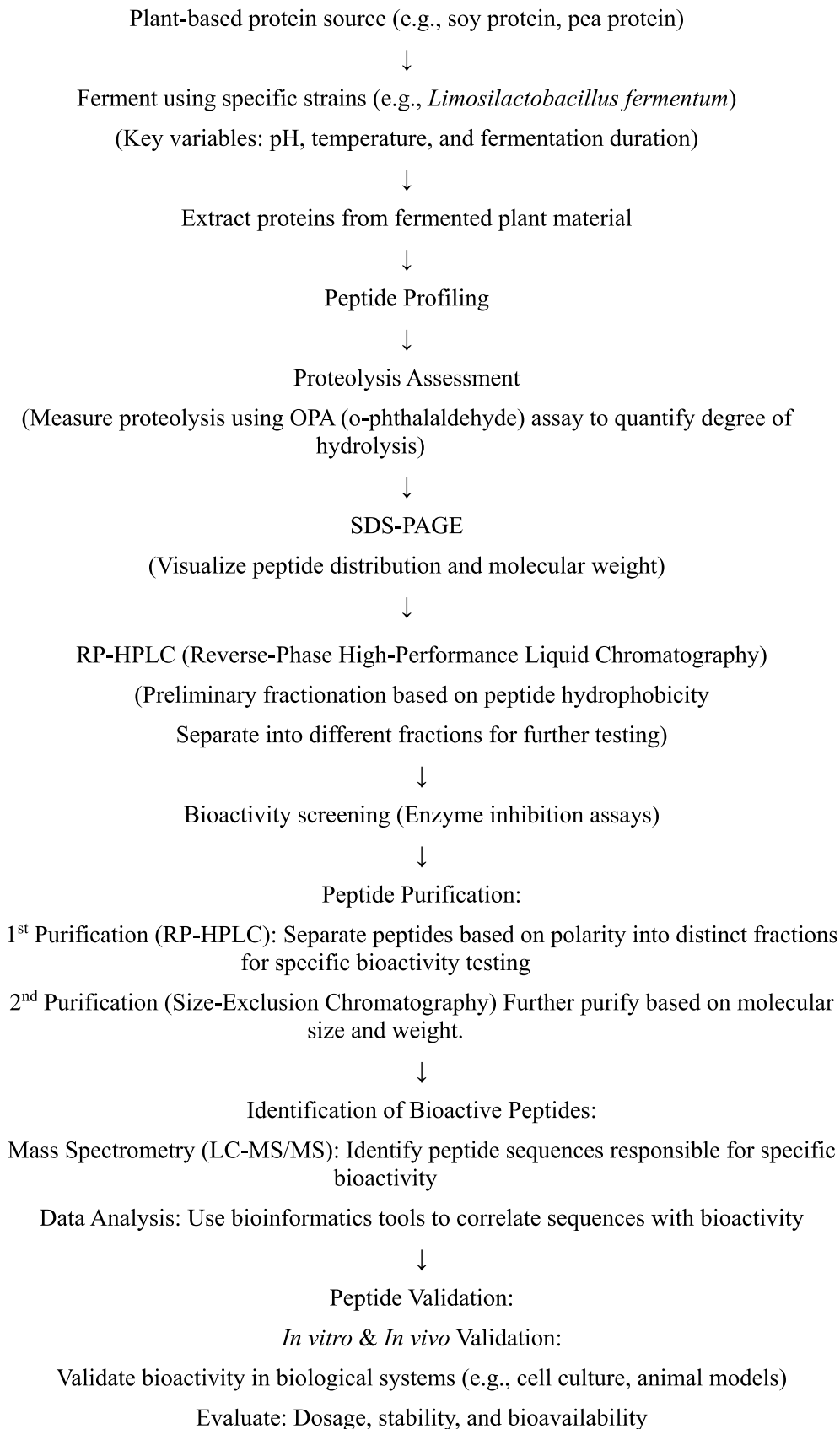
original protein. The peptide sequencer is ineffective for analyzing peptide samples with low purity levels. Moreover, liquid chromatography (LC) in conjunction with mass spectrometry (MS/MS) is commonly employed in peptide sequence analysis (F. Zhu et al., 2023). This encompasses LC-ESI-MS/MS, LC-MALDI-TOF-MS/MS, LC-quadrupole TOF-MS/MS, and LC-FTICR-MS/MS. The investigation of antioxidant peptides has gained prominence, along with the utilization of certain internet resources. The effective prevention of oxidative stress and food oxidation by antioxidant peptides has been achieved by three principal methods for assessing the antioxidant potential of peptides. These methodologies encompass chemical techniques, *in vitro* cell biology methods, and *in vivo* animal testing (Nirmal et al., 2024). The primary mechanisms of antioxidant peptides include: (1) direct scavenging of reactive oxygen free radicals; (2) chelation of metal ions; (3) suppression of lipid peroxidation reactions; and (4) activation of the body's antioxidant defense system (Sun et al., 2024).

5.2. Purification and analysis method for plant protein-derived peptide

The current technologies employed for peptide separation and purification mostly include chromatography (GPC, IEC, and RP-HPLC), membrane separation, and their combinations. Techniques such as mass spectrometry, tandem mass spectrometry, and bioinformatics are commonly employed to identify protein hydrolysates. The separation of protein hydrolysates is often achieved using ultrafiltration membranes in the purification process. The membrane separation technique is categorized into several types, including nanofiltration, ultrafiltration, and microfiltration, based on differing pore diameters (Wen, Zhang, Zhang, et al., 2020). Ultrafiltration membranes are among the most favored methods for the separation and purification of protein hydrolysates or peptides. This is due to its ease of operation, low energy usage, and environmental protection benefits. The purification of protein hydrolysates or peptides is predominantly achieved via chromatographic separation techniques (Aderinola et al., 2018). Chromatography technology, which relies on the variances in the physical and chemical properties of the peptide components, enables separation by differentially distributing these components between the stationary and mobile phases. This category encompasses several procedures, including GPC, IEC, and RP-HPLC. GPC is often referred to as size exclusion chromatography and molecular sieve chromatography. The separation efficacy of this approach is predominantly affected by many factors, including the packing type and the chromatography column volume, among others (Z. Zhu et al., 2024). This technology is frequently employed for the separation of proteins, peptides, and polysaccharides because it is easy to operate, environmentally benign, and cost-effective. The principle of separation in RP-HPLC relies on the disparity in hydrophobicity between the peptide mixture and the stationary phase, predicated on the larger polarity of the mobile phase compared to the stationary phase. Initially, the weak hydrophobic peptide component is eluted, followed by the elution of the strong hydrophobic fraction. In RP-HPLC, silica gel is commonly used as the stationary phase packing for columns, whereas ultrapure water, methanol, and acetonitrile are the predominant solvents available (Y. Zhu et al., 2022).

Purification and identification of bioactive peptide procedures are the requirement for commercial use. Ultrafiltration, RP-HPLC, size exclusion, and ion-exchange chromatography can purify bioactive the peptides. Mass spectrometry (MS), electrospray ionization MS, matrix-assisted laser desorption ionization time-of-flight MS, liquid chromatography-MS/MS, and HILIC are also used for protein identification (Zaky et al., 2021).

Below is a flowchart that illustrates the process of identifying and purifying bioactive peptides from fermented plant-based foods.



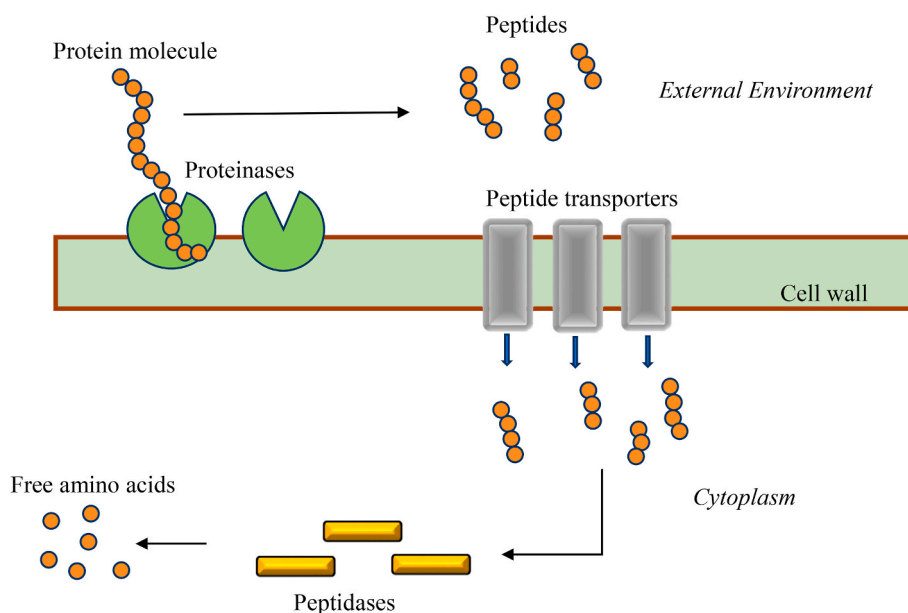


Fig. 3. Proteolytic system of LAB (Ji et al., 2021).

6. LAB proteolytic system

Several essential amino acids that are required for growth, which include valine, histidine, methionine, glutamic acid, glycine, leucine, and isoleucine are not synthesized by LAB. As a result, they must utilize outside protein sources to meet their needs for amino acids. A well-defined proteolytic mechanism (Fig. 3) is employed by LAB to convert amino acids are produced by the breakdown of the proteins in the fermentation medium. These amino acids are essential sources of nitrogen that help LAB grow and multiply while producing different peptide sequences at various phases. The three fundamental elements render up the extensively examined proteolytic system that exists in lactic acid bacteria (LAB), specifically *Lactococcus lactis*, *Lactobacillus helveticus*, and *Lactobacillus delbrueckii* subsp. *bulgaricus*: (1) intracellular peptidases, (2) peptide transporters and (3) cell envelope proteinase (CEP).

They begin the process of breaking down proteins by cleaving the molecules into peptides that have between four and thirty amino acid residues. Thus far, scientists have recognized about six different kinds of CEPs in LAB: The initial one to be identified in *Lactobacillus paracasei* subsp. *paracasei*, *Lactococcus lactis* subsp. *cremoris* and *Lactobacillus lactis* subsp. *lactis* was PrtP; the others were PrtB in *Lactobacillus delbrueckii* subsp. *bulgaricus*, PrtH in *Lactobacillus helveticus*, PrtS in *Streptococcus thermophilus*, PrtR in *Lactobacillus rhamnosus*, and PrtL in *Lactobacillus delbrueckii* subsp. *lactis*. A few CEPs have also been found in *Lactobacillus plantarum*, *Lactobacillus acidophilus*, *Lactobacillus casei* and *Lactobacillus sanfrancisco* (Ji et al., 2021). LAB are generally recognized for having a single distinguishable CEP. Even so, among all *Lactobacillus* species, *Lactobacillus helveticus* is the most proteolytic; it possesses four CEPs with different specificities. *L. helveticus* may be able to produce a broad range of bioactive thanks to this characteristic (Raveschot et al., 2018). Peptides produced via CEP cleavage are discharged into the extracellular space and then taken up by three different active transport systems: ABC transporter (Dpp), ion-linked transporter (DtpT), which is intrigued from a proton motive force and oligopeptide permease (Opp), an ATP-driven active transport system. Numerous peptidases, which involve tripeptidases, dipeptidases, endopeptidases, and aminopeptidases are some of these enzymes, that help to further break down these peptides into amino acids inside the cell. Whereas aminopeptidases extract amino acids that come from peptide's ends of a protein chain ends, endopeptidases break intracellular peptide bonds. Tripeptidases

and dipeptidases utilize the breakdown products that are produced as substrates. In the end, the hydrolysis process generates abundant free amino acids in LAB utilizes nitrogen for their metabolic functions (Kieliszek et al., 2021). The peptides that are formed during fermentation can be easily ingested by adding them directly into fermented foods or by extracting them using a simple purification practice (Rizzello et al., 2016).

7. The positive aspects of producing bioactive peptides by way of microbial fermentation

With the notable exception of relatively low cultivation costs, this technique is less expensive compared to enzymatic hydrolysis because it doesn't require costly commercial enzymes (Ayala-Niño et al., 2019). Although peptides are derived from accessible dietary proteins, microbial fermentation produces bioactive peptides thought to be enhanced safety along more health-promoting. To further ensure their safety and acceptability for ingestion, these peptides are derived from microorganisms that are classified as Generally Recognized as Safe (GRAS) (Fan et al., 2019). By enhancing the value of the substrate utilized, microbial fermentation offers the potential to improve organoleptic, nutritional, and health properties while also promoting microbial safety. One environmentally conscious technique that contributes to environmental sustainability is microbial fermentation, as it produces no hazardous byproducts (Görgüç et al., 2020).

7.1. Methods of fermentation for plant source

A diverse array of microorganisms facilitates bioprocesses, which convert complex substrates into simple, value-added products (Sadh et al., 2018). Fermentation is one of the oldest techniques still employed for converting materials into value-added products through microbial action. Solid state fermentation, submerged fermentation, and liquid fermentation are the three predominant types of fermentation processes employed. Product-specific decisions can be taken during the fermentation process. Bioactive compounds of industrial significance can be extracted from diverse substrates, including waste materials, via either solid-state or submerged fermentation processes.

7.1.1. Solid-state fermentation (SSF)

In solid-state fermentation (SSF), bacteria proliferate on solid

substrates without the presence of free liquid. The basic objective of solid-state fermentation (SSF) is to maximize nutrient extraction from the substrate using microorganisms such as fungi or bacteria for fermentation purposes. This is achieved through the use of biological stuff. In terms of the seed culture utilized for fermentation, solid-state fermentation (SSF) can be categorized as either pure or mixed (Chilakamarry et al., 2022). In pure culture solid-state fermentation (SSF), specific strains are employed, but in mixed culture, a diverse array of microorganisms is utilized during the fermentation process. The SSF can be categorized into two separate types, each defined by the properties of the solid phase. In the initial form of SSF, the solid not only sustains the organism but also serves as a food supply (Verduzco-Oliva & Gutierrez-Urbe, 2020). The second type of solid-state fluid (SSF) is distinguished by the solid support being saturated with a liquid medium, including carbohydrates, lipids, and organic acids. SSF can attain elevated volumetric productivity by increasing the concentration of products while concurrently minimizing effluent generation (Saeed et al., 2022).

7.1.2. Sub Merged/liquid fermentation

Submerged fermentation (SmF) is a fermentation technique in which the substrate is either liquefied or suspended in a liquid medium. SmF is predominantly utilized in industrial operations due to its high yield, cost-effectiveness, and minimal contamination. Nevertheless, SmF presents some disadvantages, including the requirement for physical space, energy, and water, among other factors (Ramesh et al., 2022). In contrast to SSF, the enzyme manufacturing by SmF has been employed during the past century owing to its numerous advantages. Pectinase, an enzyme generated by fungi, can be described via submerged fermentation (SmF) (Premalatha et al., 2023). Pectinases are a collection of related proteins that facilitate the degradation of pectin from diverse plant sources. Pectinases hold considerable importance in numerous commercial and industrial applications. Process factors encompass variables such as incubation duration, incubation temperature, starting pH, starch concentration, supplementary nitrogen source, and concentrations of nitrogen and phosphorus (Pyter et al., 2022).

8. Functional properties of plant-derived peptides

Plant-derived peptides are more promising with their wide range of functional benefits. Numerous investigations have shown that these peptides might improve particular technological features of food items. It has been demonstrated that these bioactive peptides affect critical factors like emulsification activity and stability, oil and water retention capacity and the capacity to form and maintain foams in the final product which are intended to improve overall product quality.

Bioactive peptides may serve as an emulsifier naturally by binding themselves to the water-oil interface. They efficiently stabilize the oil-water interface by interacting to create a barrier close to oil or air droplet like particles. Their amphiphilic nature is responsible for this functionality (Yesiltas et al., 2021). Peptide's ability to emulsify depends on many different kinds of structural characteristics, including their length, isoelectric point, hydrophilic/lipophilic balance, and conformational flexibility (Pham et al., 2019). Yesiltas et al. (2021) declared that peptides that adopted an α -helical shape demonstrated improved emulsifying properties although their length allowed a stable helical structure to form. On the other hand, peptides with an assumed β -strand shape showed better emulsifying properties within a smaller range of lengths, usually between 14 and 16 amino acids. A further investigation was carried out by Cui et al. (2020) determined that applying a treatment with high hydrostatic pressure enhanced the ability of sweet potato peptide to emulsify through modifying peptide's structural versatility. Improved protein flexibility in conformation enables easier structural rearrangements to occur smoothly and for adsorption to happen in the stratum between, which improves the procedure of emulsification.

The foam structure is commonly utilized within numerous types of aerated systems, among which are ice cream, sponge cakes and desserts. They play a vital role to improving the intended texture and sensory attributes of the finished product. Because they are amphiphilic, bioactive peptides have significant surface activity, particularly around the interface between water and air. These peptides are great foamers, which makes them ideal foam stabilizer candidates. As a result, adding them to food formulations has the potential to drastically alter structures as well as textural traits of food products (Hou et al., 2017). Soybean hydrolysates produced through pre-heating and then regulated enzymatic hydrolysis possesses remarkable foaming and emulsifying properties that are comparable to modern proteins. Likewise, peptides originating from gliadin's hydrophilic central domain (CD) demonstrate significant foaming stability at pH values close to neutral (Liang et al., 2020). The CD-derived peptide contained a greater amount of hydrophobic amino acids, which improved its foaming capabilities. On the other hand, peptide fractions derived from gliadin's terminal domain (TD) illustrated superior foaming stability at alkaline pH levels. According to a study by Tang et al. (2021), adding soy peptides (SP) increased the flexibility of the egg white protein's secondary structure, which in turn improved the powder's ability to foam and its stability. Zein peptide is one of the amphiphilic plant-derived peptides whose ability to self-assemble into spherical micellar nanoparticles has been demonstrated by recent studies. These new peptide-based nanoparticles exhibit promise as novel particulate stabilizers for the creation of food emulsions and foams of the Pickering type, since they demonstrate substantial interfacial activity (Liu, Wang, et al., 2021).

9. Utilizing peptides derived from plants in the development of functional foods

The demand for innovative products that emphasize safety and health first is rising as a result of growing awareness among consumers. This requirement also applies to goods with certain features or attributes. Nutraceuticals and functional goods containing bioactive peptides derived from plants have been receiving a lot of attention lately. They are receiving attention because of their numerous bioactive potential and useful qualities, which have few adverse consequences. Puchalska et al. (2014) found 120 antioxidant peptides in an infant formula that can be purchased commercially that are produced out of soybeans. This finding the capability of peptides that possess antioxidant properties generated from soybeans to be a strong substitute for conventional newborn formulae made with cow's milk. Furthermore, the benefits of soybean peptides were acknowledged, including strong acid and heat stability, low viscosity and good emulsification. Consequently, there has been a notable improvement in endeavours to leverage soybean peptides for enhancing the stability of functional beverages. Zhang, He, et al. (2021) achieved an important advancement in this field when they successfully combined soybean peptides and yeast that had been enriched with selenium to develop a healthy drink that targeted selenium supplementation. According to Tkaczewska (2020), various hydrolysed proteins and bioactive peptides with antibacterial antioxidant and antibacterial qualities could be serving as natural food preservatives. Furthermore, such peptides show potential with functioning components for the formulation of coatings and edible films that are used as active packaging materials. de Oliveira Filho et al. (2019) discovered that the alginate films with cotton protein hydro lysate enhanced the film's the capability to inhibit *Staphylococcus aureus* and *Rhizopus oligosporus* whereas preserving the film's oil barrier, moisture content, biodegradability and solubility. According to a study, several amphiphilic peptides, which include pine nut peptide (KDHCH) and zein peptide, are capable of creating three-dimensional hydrophilic matrix gels in aqueous solutions. Both within and between molecule's self-assembly processes are responsible for this phenomenon (Ma et al., 2020). Hydrogels that are made of self-assembling peptides are frequently used in biological contexts, especially for drug delivery. Fu

et al. (2021) have highlighted the exceptional injectability, significant loading capacity and remarkable biocompatibility of these materials. Liu, Wang, et al. (2021) developed three lipid-based carriers: a solid lipid nanoparticle, a nano-emulsion and a nano-structured lipid carrier to encapsulate lutein while stabilizing it with zein peptides. It was discovered through their analysis that carriers stabilized with zein peptides had better storage stability. Additional research carried out by Lan et al. (2021) demonstrates that rapeseed peptides, which are produced by the hydrolysis of rapeseed meal by enzymes, have an intrinsic capacity to self-assemble, making them appropriate vehicles for encapsulating β -carotene.

10. The health benefits linked to bioactive peptides derived from plants

The proteins are necessary during many biological activities, including growth and maintenance. Biologically active peptides are becoming increasingly recognized due to their potential as regulators of various body activities. Certain bioactive peptides have different kinds of biological effects that are reliant upon sequence, chemical weight and amino acid composition makeup, net charge and secondary structures.

10.1. Antioxidant peptides

The presence of bioactive substances such as vitamins, peptides and phytochemicals in functional foods makes them beneficial for health. These beneficial components are introduced intentionally from outside sources, develop organically in food product during processing, or both (Butnariu & Sarac, 2019). Amongst other bioactive substances, antioxidant peptides have emerged as the primary focus of interest in the food revenue in recent years. These peptides have received a lot of concern because of their ability to mitigate oxidative damage. Because their

unique protein fragments have antioxidant qualities, they present viable ways to improve human health and ensure the calibre of security of food commodities. These peptides are essential for reducing oxidative stress and preventing lipid peroxidation due to their ability to neutralize the effects of free radicals produced during oxidation processes in the human body and dietary products (Hema et al., 2017).

10.1.1. Mechanisms of antioxidant peptides

Antioxidative peptides play a crucial role in neutralizing free radicals and reducing oxidative stress within cells. Their mechanisms of action include scavenging free radicals by donating hydrogen atoms or electrons, thereby preventing damage to cellular components such as lipids, proteins, and DNA. Additionally, some peptides can bind to metal ions like iron or copper, reducing their ability to catalyse reactions that produce harmful reactive oxygen species (ROS). These peptides also inhibit lipid peroxidation, which protects cell membranes and preserves cellular integrity. Furthermore, antioxidative peptides may enhance the body's natural defense by stimulating the production of endogenous antioxidants like glutathione and further supporting the reduction of oxidative stress. The primary ways in which peptides are antioxidants prevent oxidation via neutralizing ROS, scavenging free radicals, binding prooxidative transition metals, and reducing hydroperoxides. ROS are extremely reactive substances that may happen endogenously as natural outcome of metabolic processes or exogenously as a result of external causes. Examples of these molecules are hydrogen peroxide (H_2O_2), superoxide anion ($\bullet O_2^-$), and hydroxyl radicals ($\bullet OH$). In living things, oxidases and lipoxygenases, among other intracellular enzymes, mediate metabolic oxidation circumstances that lead to the generation of ROS (Ahmad et al., 2023). ROS can also be produced artificially as a result of exposure to certain stimuli from the environment, for example UV light, ionizing radiation and pollution.

In cellular physiology, endogenous ROS are essential for improving reproductive activities, modifying intercellular communication pathways and protecting cells from pathogens (Wagner et al., 2018). Oxidative stress is triggered by an excess of ROS in living things, particularly if those organisms are not adequately protected against antioxidants. Diabetes, atherosclerosis and cancer are just a few of the non-communicable diseases that are more common globally as a result of this imbalance. Moreover, oxidation-sensitive ingredients and chemicals in food products are severely deteriorated by ROS-induced oxidative damage, such as lipid peroxidation (Zou et al., 2016).

Although plant proteins are relatively rare in phytophagous crops or their agricultural sector by-products, plants are an amazing source of natural bio-peptides. However, phytochemical proteins are coming under more and more scrutiny as the best precursors for bifunctional peptides because of their remarkable biological efficacy, high-volume production potential, affordability, quick yield cycles and lack of local religious or sociocultural restrictions. To identify and characterizing antioxidant peptides, many different kinds of byproducts of phytophagous crops, including husks, leaves, seeds, veggies, fruits, legumes, and grains have been researched (Table 4). The importance of rapeseed, grains and legumes as key sources of phytochemical proteins is highlighted by the substantial global presence of cultivation and protein content (Görgüç et al., 2020). Using this framework, there exists an increased possibility for improving the diversity as well as accessibility of bioactive peptides, which includes peptides with antioxidant properties. The significant both qualitative and quantitative relationships that have been found coupling biopeptides produced from animals along with their corresponding precursors of proteins serve as evidence of this (Bechaux et al., 2019). Research has repeatedly demonstrated that, in comparison to proteins from other crop sources, cereal and legume proteins include an abundance of peptide fragments with strong antioxidant properties. Among the major cereal crops, oats and rye have been found to contain abundant amounts of antioxidant sequences of peptides (Leung et al., 2018). Other crops that have been found to contain high amounts of these sequences include wheat, buckwheat, rice, corn, millet, and canary

Table 4
Antioxidative peptides sequence from plant protein sources.

Sources	Antioxidative peptides sequence	Activity (IC ₅₀)	References
Black soybean	LIVPK, IVPK, YSK, RDPEER	0.216 μ M (RDPEER)	Wen et al. (2020)
Kinema	SEDDVFVIAAYPF, CHMYWF, VLLY	3 mM/mL (VLLY)	Ma et al. (2020)
Defatted walnut meal	VEGNLQVLRPR, QQRQQQL, WSREEQEREE, YVPHWDL	0.173 μ M/mL (YVPHWDL)	Sheng et al. (2019); Wang et al. (2016)
Soybean cheese	KFVPKQPNMIL, AVPYPQR, EMPFPK, VLPVPQK, KELEEK	0.216 μ M/mL (KELEEK)	Wen et al. (2020)
Rice bran protein	TVAQGEVVA,	0.62 μ M (TLAGMN)	Zaky et al. (2019)
Soy protein isolate	WNLNAN, FESFL, SLLDFPALW, LDDDGRL	0.54 μ M/mL (LDDDGRL)	Wen et al. (2020)
Wheat germ protein	TVGGAPAGRIVMQ, AREGETVVPV	0.5 mg/mL (SGGY)	Feng et al. (2019)
Quinoa protein	LWREGM, DKDTPK, IFQETI	9.80 μ g/mL (DKDTPK)	Mudgil et al. (2019)
Mulberry leaf proteins	SVL, EAVQ, RDY, KWFCT	1.09 mg/mL (KWFCT)	Sun, Tang, et al. (2019)
Fermented grains (Jiupai)	VNP, YGD, AGI/LPM	0.79 μ g/mL (AGI/LPM)	Jiang et al. (2018)
Oat bran protein	WGYDGDAPVVA, EARQIEPKRA, LHGQFPI	0.13 μ M/mL (LHGQFPI)	Esfandi et al. (2019)
Wheat gluten	LY, OY, YQ, APY, RGGY, YQAPSY	0.31 mM/L (YQAPSY)	Liu, Zhang, et al. (2021)
Amaranth Seed Proteins	LVRW, PKLTL	0.26 μ M/L (PKLTL)	Ayala-Niño et al. (2019)
Palm kernel cake protein	YLLK, WAFS, YGIKVGAI, KVFLGLK, GVQQGAGHYALL	4 mg/mL (KVFLGLK)	Arulrajah et al. (2020)

seeds (Chen, Ning, et al., 2021). Meanwhile, soybean peptides have been the focus of much interest among legumes, with chickpea, faba, and mung bean peptides coming in close second (Zhang, Wang, et al., 2021).

It is commonly known that vegetables and fruits are abundant sources containing naturally occurring antioxidant substances, among which are terpenoids, alkaloids, phenols, flavonoids, and saponins, which have robust physiological effects. In recent times, there has been a growing emphasis among academics to look into the biological characteristics of peptides containing antioxidant capacity that are obtained from fruits and vegetables. Studies on a variety of sources, including watermelon (Wen et al., 2020), sacha inchi (Zhan et al., 2021), perilla, mulberry leaves (Sun, Tang, et al., 2019), tricholoma matsutake singer (Li et al., 2021) and amaranth, have notably revealed certain amino acid sequences or peptide fragments with noteworthy internal antioxidant capabilities (Sandoval-Sicaireos et al., 2021).

10.1.2. Free radical scavenging activity

Reactive oxygen and nitrogen species can be neutralized by peptide molecules possessing antioxidant characteristics through two different mechanisms: single electron transfer (SET) reactions and hydrogen atom transfer (HAT) actions (Lorenzo et al., 2018). The evaluation methods for antioxidant ability, among which are the total radical capture antioxidant parameter (TRAP) assay and oxygen radical absorbance capacity (ORAC), are particularly emphasized by Ashaolu (2020). The above methods are based on the hydrogen HAT concept, which quantifies antioxidant's capacity for eliminating radicals that are free by giving protons. Evaluating the antioxidant's hydrogen atom donor group's bond dissociation energy and ionization potential (IP) is crucial for determining the HAT reaction's degree of intensity. The potential of an antioxidant to decrease radicals, metals or carbonyls by donating an electron is measured using techniques based on SET, such as DPPH (α , α -diphenyl- β -picrylhydrazyl) radical scavenging capacity, ferric reducing antioxidant capacity (FRAP), likewise ABTS (2,2-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid) radical scavenging capacity. Reactive functional group's deprotonation and IP influence the relative reactivity of these techniques. However, research has demonstrated that the mechanisms of HAT and SET coexist nearly every specimen, with prevailing method ascertained through the antioxidant's structural properties, including its partition coefficient and systemic solubility. Additionally, to the previously referred to all-inclusive assessment methods, peptide's antioxidative the ability can also be evaluated by their capacity to scavenge additional free radicals, such as the superoxide anion ($\text{O}_2^{\cdot-}$) and the hydroxyl radical ($\text{OH}^{\cdot}$). Molecular oxygen in a reduced state with poor oxidizing capabilities is called a superoxide anion. But it is capable of breaking down into more powerful and ROS, such as hydroxyl radicals, which could be harmful to living things. Superoxide radical scavenging assays and other widely used techniques like ABTS, DPPH and FRAP have been shown to positively correlate in studies, demonstrating their complementary nature. Moreover, antioxidant's capacity to transfer hydrogen atoms is intimately linked to the hydroxyl radical quenching mechanism (Munoz-Rugeles et al., 2017). Numerous antioxidative peptides derived from plant proteins have been identified and characterized.

In a study by Coscueta et al. (2016), the antioxidative activities of chickpeas and African yam beans were examined using various assays. Chickpeas exhibited a 5% scavenging activity against the DPPH radical, while African yam beans demonstrated a significantly higher activity of 40%. In terms of scavenging the hydroxyl radical, chickpeas showed a substantial activity of 81%, whereas African yam beans exhibited a lower but still notable activity of 40%. Similarly, for scavenging the superoxide radical, chickpeas displayed a considerable activity of 69%, whereas African yam beans demonstrated a somewhat lower activity of 30%. These results indicate that both chickpeas and African yam beans possess antioxidative properties, with African yam beans generally showing higher scavenging activity across the tested radicals compared to chickpea.

10.2. Antidiabetic peptides

Diabetes seems a chronic condition associated with the fastest worldwide rate of growth, therefore becoming a critical global health concern. Unfortunately, that is one of the top 10 worldwide reasons for deaths (Lin et al., 2020). Additionally, there was an officially documented 3% increase in diabetes-related fatalities between 2000 and 2019 (WHO, 2022). It was estimated that 537 million people worldwide, aged 20 to 79, would have diabetes mellitus in 2021. According to projections, this figure will increase to 643 million by 2030 and 783 million by 2045. (IDF, 2020).

The following three primary types of diabetes are accounted for in the diabetic condition: gestational diabetes, diabetes mellitus type 1 (T1DM) and type 2 diabetes (T2DM). Amongst people with diabetic complications worldwide, T2DM is the common kind, affecting between 85 and 95% of the population (IDF, 2020). Chronic T2DM is characterized by peripheral tissue insulin resistance and inadequate insulin synthesis, which raise blood sugar levels in the body. Dietary patterns rich in quickly broken-down carbs, such as starch, and consuming a lot of processed food have a major impact on the prevalence of type 2 diabetes. Sedentary lifestyles can increase the chance of getting type 2 diabetes (Ardisson et al., 2014).

Managing T2DM hinges upon fostering a wholesome lifestyle. Key components encompass adopting a nutritious diet, engaging in consistent physical activity, abstaining from smoking, and sustaining an optimal body weight (IDF, 2020). For those who are suffering from T2DM, leading a healthy lifestyle might be difficult. Throughout the extended duration of the disease, balancing blood glucose levels with lifestyle changes alone becomes more difficult. As a result, people with type 2 diabetes turn to using hypoglycemic medications. Metformin, miglitol, rosiglitazone, acarbose, voglibose, gliptins likewise sulfonylureas are the oral hypoglycemic medicines that are available. These oral medications assist in regulating blood sugar via lowering insulin resistance (metformin) levels, increasing the hormone insulin synthesis, improving rosiglitazone, an insulin sensitivity agent, functions as a Dipeptidyl peptidase-IV (DPP-IV) inhibitor (gliptins), functioning as acarbose, an α -amylase inhibitor, and serving as inhibitors of α -glucosidase (miglitol, voglibose and acarbose) (Awosika & Aluko, 2019). Moreover, should the administration of oral hypoglycemic medications fail to achieve the targeted blood glucose levels, individuals with T2DM might necessitate the use of insulin injections.

10.2.1. Mechanisms of antidiabetic peptides

Antidiabetic peptides derived from fermented proteins, such as whey protein isolate (WPI) and soy protein isolate (SPI), regulate blood glucose levels and improve insulin sensitivity through multiple mechanisms. One key mechanism is the inhibition of carbohydrate-hydrolyzing enzymes, such as α -amylase and α -glucosidase. By blocking these enzymes, the peptides reduce the breakdown of carbohydrates into glucose, which slows glucose absorption in the intestines. This helps prevent postprandial blood glucose spikes and enhances glycemic control. Another important mechanism is the enhancement of insulin sensitivity. Certain peptides activate signaling pathways involved in glucose uptake, including the insulin receptor substrate (IRS) and the PI3K-Akt pathway, facilitating glucose transport into cells, particularly in muscle and adipose tissues, thereby reducing circulating blood glucose levels (Lu et al., 2012).

Additionally, these peptides modulate gut hormones like glucagon-like peptide-1 (GLP-1), which increases insulin secretion in a glucose-dependent manner, reduces glucagon release, and delays gastric emptying. These actions collectively improve glycemic control by slowing the release of glucose into the bloodstream. Antidiabetic peptides also exhibit anti-inflammatory and antioxidant properties, helping to reduce oxidative stress and inflammation, both of which contribute to insulin resistance and the progression of type 2 diabetes. By decreasing levels of pro-inflammatory cytokines like IL-6, IL-1 β , and TNF- α , these

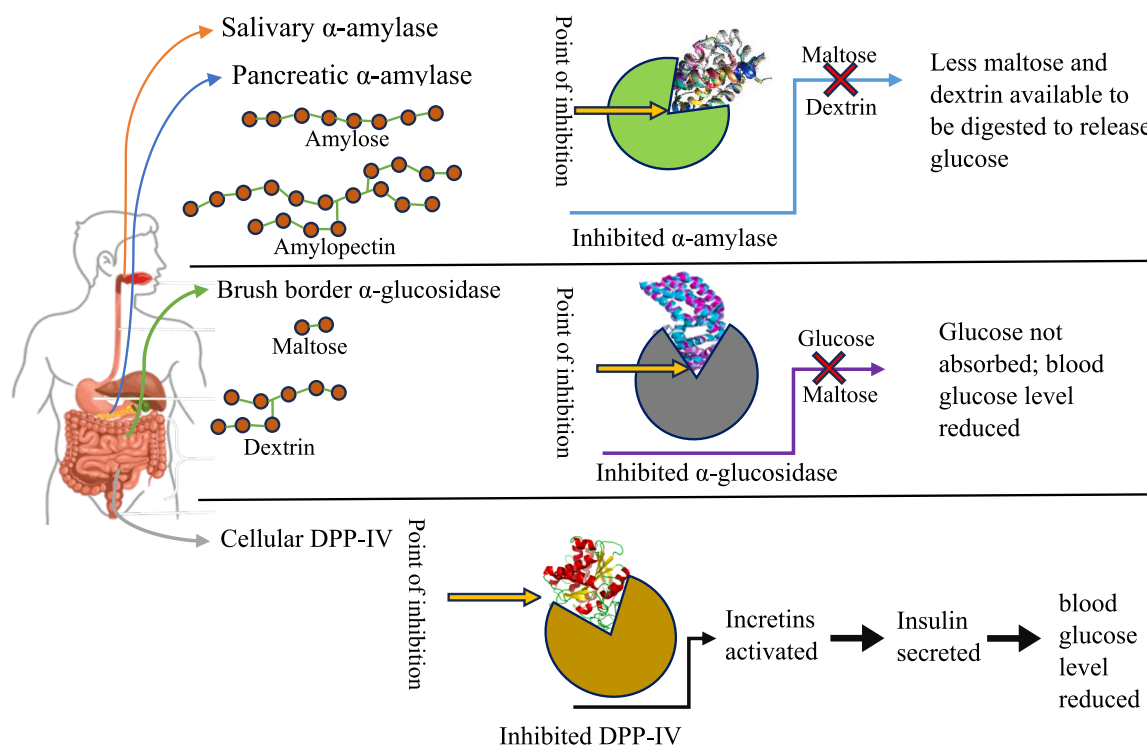


Fig. 4. Peptides' mechanisms of inhibition of α -amylase, α -glucosidase, and DPP-IV in the management of diabetes (Wang et al., 2019).

Table 5
Antidiabetic activities of plant derived peptides.

Sources	Peptides	α -Amylase	α -Glucosidase	DPP-IV
Soybean	NNDDRDS and IKSQSES	85%	25%	IC ₅₀ : 0.8 mg/mL
Lupin	LTFPGSAED, SPRRF, FE	20%	21%	IC ₅₀ : 0.75 mg/mL
Common bean	LAPPG and KLLRLRLQ	5%	30%	IC ₅₀ : 0.60 mg/mL
Black bean	AKSPLF and ATNPLF	30%	49%	IC ₅₀ : 0.87 mg/mL
Pinto bean	PPHMLP and PLPWGAGF	62%	NR	NR
Oat protein	GDVVALPA and DVVALPAG	57%	78%	IC ₅₀ : 0.413 mg/mL
Hard-to-cook bean	LLSL, QQEG and NEGEAH	49%	76%	55%
<i>Chenopodium quinoa</i> Wild.	IQAEGGLT, DKDYPK, GEHGSDGNV	IC ₅₀ : 0.48 mg/mL	IC ₅₀ : 0.32 mg/mL	IC ₅₀ : 1.64 mg/mL
<i>Cannabis sativa</i> L.	LR and PLMLP	–	27%	–

(NR= Not Reported) (Fuentes et al., 2021; Oseguera-Toledo et al., 2015; Vilcacundo et al., 2017; Wen et al., 2020).

peptides help restore insulin sensitivity and protect pancreatic β -cells (Wang et al., 2019).

Moreover, some peptides regulate the expression of glucose transporters, such as GLUT-4, on cell surfaces, which enhances glucose uptake, especially in skeletal muscle and adipose tissue. Lastly, certain peptides directly support the function of pancreatic β -cells, improving their viability and insulin production, which helps maintain healthy insulin secretion and prevents the progression of diabetes. Through these combined actions—enzyme inhibition, insulin sensitivity enhancement, modulation of incretin hormones, reduction of inflammation, regulation of glucose transporters, and preservation of β -cell function—antidiabetic peptides contribute to improved glycemic control and

overall diabetes management (Wang et al., 2019).

Prohibiting the activity of pancreatic and salivary α -amylases causes certain diet bolus's starch and polysaccharides to pass through the small intestine undigested. As a result, this reduces the number of substrates that α -glucosidase can use. Additionally, when α -glucosidase is inhibited, digested amylose and amylopectin are converted into forms that are not easily absorbed by intestinal cells for bloodstream transfer, such as maltose, maltotriose, branching oligosaccharides, and isomaltose. Blood glucose levels fall when α -amylase and α -glucosidase are inhibited, which can help with postprandial blood glucose regulation (Wang et al., 2019). Preventing the action of DPP-IV enzymes extends the duration of action of incretins, which are comprised of the hormone's glucose-dependent insulinotropic peptide and glucagon-like peptide 1 (GLP-1) (Wang et al., 2019). Insulin secretion is stimulated by these hormones. When DPP-IV is inhibited or incretins are active, they increase the production of insulin and successfully control blood glucose levels. How peptides inhibit α -amylase, α -glucosidase, and DPP-IV are illustrated in Fig. 4, which is relevant to the control of diabetes.

Continuous intake of hypoglycemic synthetic drugs can have negative side effects that involve gas, diarrhoea, inflammation in the abdomen, water retention, osteoporosis, hypoglycemia, impaired liver function, increased risk of kidney damage and possible heart failure (González-Montoya et al., 2018). Studies on natural food components have been prompted by the growing need for safer alternatives with effects comparable to traditional treatments. The possibility that these substances could reduce the absorption of glucose and the digestibility of starch has sparked research efforts all around the world. This is accomplished by processing or incorporating more resistant starch into different goods in order to increase it (Kumar et al., 2019). Incorporating soluble fibre (Jia et al., 2020), lipids (Ye et al., 2018), proteins (Chi et al., 2018), protein hydrolysates and bioactive peptides (Toledo et al., 2016) are some of the strategies that have been used. The above compounds work through a number of various mechanisms. These include decreasing the amount of digestible starch (also known as resistant starch), raising the lumen's fluid viscosity to prevent starch-digesting

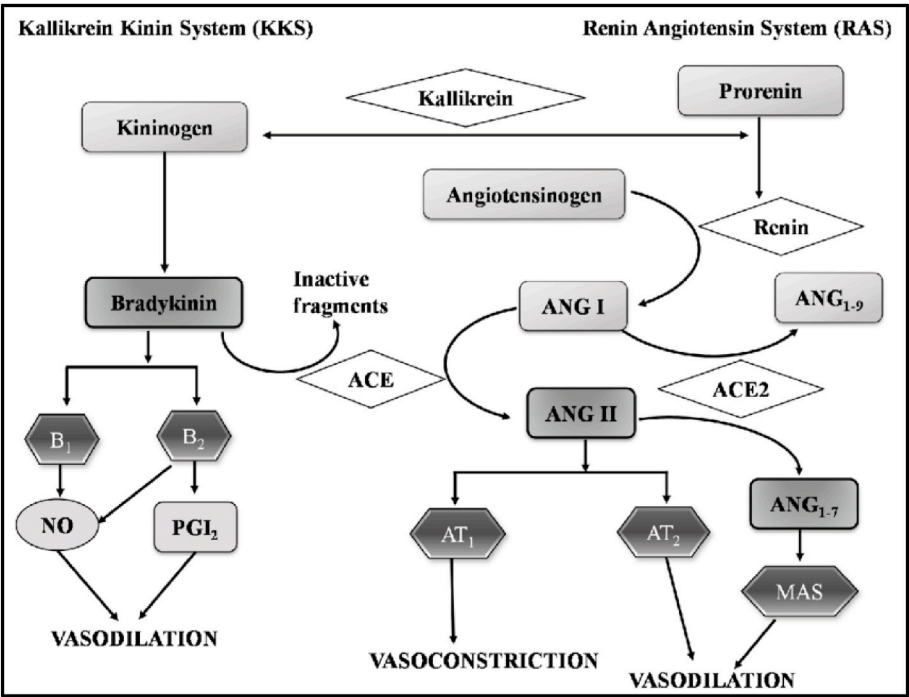


Fig. 5. Regulation of blood pressure through the renin-angiotensin and kallikrein-kinin systems (Pihlanto & Mäkinen, 2017).

enzymes from binding to their substrates (via protein and fibre that is soluble), accumulating a layer on the surface of starch granules to prevent lipids and proteins from being bound by the enzymes, preventing the actions of α -amylase, α -glucosidase, and DPP-IV, so postponing the small intestine's glucose absorption and reducing the rate at which cells absorb glucose through amino acids and peptides (Table 5) (Kumar et al., 2019).

10.3. Antihypertension peptides

One of the most prevalent medical disorders affecting adults across the globe is hypertension, which is defined as 140/90 mmHg and higher in both systolic and diastolic levels. An enormous number of humans who are impacted by chronically high blood pressure is a global concern (WHO, 2022). The amounts reported from 2010 to the present have remained mostly similar despite multiple attempts to lower this number because of rising rates of obesity and increased intake of alcohol, tobacco and other substances, all of which are major causes of hypertension (Louca et al., 2020). One of the primary factors contributing to death from noncommunicable diseases are hypertension and health problems that it entails. Even with the effectiveness of current antihypertensive drugs, there is still a subgroup of hypertensive patients for whom these therapies do not lower blood pressure. Moreover, the prohibitive cost as part of these drugs and their adverse effects, which include hypotension and raised levels of potassium in the body make it harder for patients to follow their prescribed treatment plans, which increases their risk of developing other chronic conditions linked to uncontrolled high blood pressure, causing cardiac events and failure of the organs (Leoncini et al., 2020). For that reason, persistently looking for safer substances that may successfully regulate blood pressure is still crucial and could benefit those who are not responding to current antihypertensive drugs. To effectively treat cases of hypertension, traditional medicine relies on natural treatments produced from dietary proteins (Wang et al., 2021) and plants (Verma et al., 2021).

The extracts of plants, notably those that are rich in chemicals known as polyphenols have drawn a lot of interest because of their capacity to reduce blood pressure in mice treated with spontaneous hypertension, as well as in normotensive and experimentally generated animals (Kim

Table 6
Renin inhibitor peptides derived from plant source.

Source	Renin inhibitor Peptides	Activity (IC ₅₀)	References
Papain	IRLIIVLMPILMA, SLR, YY, ER, FR	100 μ M (IRLIIVLMPILMA)	Kontani et al. (2014)
Porphyra yezoensis	IQP, PYGP, PPY	0.41 mM (FNLPIRL)	Nardo et al. (2020)
Rapeseed	RALP, GHS and RIY	1.932 mM (TNLDWY)	Ma et al. (2019)
Tofu	LIVTQ, IFL and HHL	44.8 μ M (IFL)	Martinez-Villaluenga et al. (2017)
Moringa oleifera leaf	LGFF and GLFF	1.88 mM (LGFF) 2.80 mM (GLFF)	Ma et al. (2021)
Amaranth proteins	SFNLPIRL, FNLPIRL and AFEDGFVWSKF	0.41 mM (FNLPIRL)	Nardo et al. (2020)
Rice	WEDIGTIK and FNVPSRYGIY	2.7 mg/mL (WEDIGTIK) 0.87 mg/mL (FNVPSRYGIY)	Pincirolu et al. (2019)
Hemp seed protein	SVYT, WYT and IPAGV	0.063 mM (SVYT) 0.574 mM (WYT) 0.093 mM (IPAGV)	Girgih, He, and Aluko (2014)
Oat protein	PFL, FLLA and WWK	1.0 mg/mL (WWK)	Bleakley et al. (2017)
<i>P. palmata</i>	IRLIIVLMPILMA, AGGEY, VYRT, IKGHY	3.34 mM (IRLIIVLMPILMA)	Aluko (2019)
Amaranth	QAFEDGFVWSFK, SFNLPIRL	0.6 mg/mL (SFNLPIRL)	Quiroga et al. (2017)
Rape seed protein	LY, RALP, GHS	0.97 mM (RALP)	He et al. (2019)
Ochrophyta	IY, IW, VY, AW, FY, VW, YGCC	7.63 μ g/mL (YGCC)	Sun et al. (2016)

et al., 2020). There is increasing evidence from clinical trials that dietary proteins have a beneficial effect on hypertension. Consuming dietary proteins can change a person's genetic predisposition to hypertension, as demonstrated by the POUNT Lost Trials, which demonstrated that

Table 7
ACE inhibitor peptides derived from plant source.

Sources	ACE inhibitor Peptides	Activity (IC ₅₀)	References
Wheat gluten	LY, LVS, YQ, APSY and RGGY	0.31, 0.60, 2.00, 1.47 and 1.48 mmol/L, respectively	Liu, Li, et al. (2021)
Amaranth grains	SFNLPLR and AFEDGFEWVSKE	2.50 and 1.47 mM, respectively	Nardo et al. (2020)
Ginkgo biloba	TNLDWY, RADFY and RVFDGAV	1.93, 1.35 and 1.01 mM, respectively	Ma et al. (2019)
Cottonseed	FPAIGMK, YRKISQL	46.7 µg/mL	Gao et al. (2019)
Ginkgo biloba seed	RVFDGAV, TNLDWY, RADFY	1.006 mM	Ma et al. (2019)
Longan seed	ETSGMKPTEL, ISSMGLVCL	2.15 µM	Nuchprapha et al. (2020)
Sesame seed	GHITVAR, ISGAQPSLR, VVISAPSK	3.60 µM	Wang et al. (2019)
Bitter melon seed	VSGAGRY, APLVSW	8.64 µM	Priyanto et al. (2015)
Moringa oleifera leaf	LGF, IPPAYSK, ILVDR	0.29 mM	Ma et al. (2021)
Moringa oleifera leaf	GLFF, FFFPK, LLDPR	0.31 mM	Ma et al. (2021)
Pearl Oyster	HLHT, GWA	458.06 µM	Liu et al. (2019)

individuals on high-protein diets had a significantly lower chance of developing hypertension than those on a placebo regimen (Sun, Zhou, et al., 2019).

According to Daiber et al. (2020), bioactive peptides exhibit effectiveness in lowering blood pressure by fostering an equilibrium harmony between both constriction and dilatation processes of major arteries that carry blood, particularly in situations when there is vascular damage and the production of clots. The potential of angiotensin-converting enzyme (ACE) inhibitory peptides, among other bioactive peptides, has been extensively studied; in particular, their potential for controlling and preventing hypertension has been researched (Metchi Donfack et al., 2021).

10.3.1. Mechanisms of antihypertension peptides

The primary endocrine mechanism influencing blood pressure regulation is the RAS (shown in Fig. 5). Reduced salt intake and sympathetic nervous system activation cause renin synthesis to occur. The conversion of angiotensinogen to angiotensin-I, which has a minimal biological function, is catalysed by this enzyme. Angiotensin-Converting Enzyme-I mediates the conversion of angiotensin-I into angiotensin-II in the circulatory system of the lungs. Vasoconstrictive effects of angiotensin-II are well known, and they raise blood pressure. Additionally, angiotensin-II stimulates the adrenal gland to secrete aldosterone, which promotes water and salt retention and exacerbates hypertension. The latest study has demonstrated that non-circulating local renin-angiotensin paracrine systems, as opposed to the circulating RAS, make a major contribution to controlling the pressure of blood in hypertensive individuals. A significant proportion of hypertensive individuals has been discovered to have low levels of renin and angiotensin-II and therapeutic inhibitors of the circulating RAS are less beneficial. It has been documented that the kidney and the heart's local paracrine systems have functional roles for regulating the circulation of blood. Table 6 and Table 7 indicates that both renin inhibitor peptides and ACE inhibitor peptides derived from plant sources could serve as natural and potentially safer alternatives to synthetic drugs for the management of hypertension and cardiovascular diseases.

The kallikrein-kinin system (KKS) is essential for blood pressure regulation through the production of vasoactive peptides called kinins, which work closely with the renin-angiotensin-aldosterone system

(RAAS) and the endothelial system to balance vasodilation and vasoconstriction (Fig. 5). Kallikreins, serine proteases found in plasma and tissues, are activated by physiological or pathological triggers like inflammation or endothelial injury. Plasma kallikrein cleaves high-molecular-weight kininogen (HMWK) to release bradykinin, while tissue kallikrein acts on low-molecular-weight kininogen (LMWK) to produce kallidin. These kinins exert their effects by binding to G protein-coupled receptors, including B2 receptors, which are constitutively active, and B1 receptors, which are upregulated during inflammation. Kinins promote vasodilation by stimulating endothelial nitric oxide (NO) release, prostacyclin (PGI₂) production, and endothelium-derived hyperpolarizing factor (EDHF) activity, which collectively reduce vascular resistance. The KKS also counteracts the RAAS by enhancing vasodilation and promoting natriuresis, helping maintain normal blood pressure. Kinins are rapidly degraded by enzymes such as angiotensin-converting enzyme (ACE), neutral endopeptidases, and aminopeptidases. This tightly regulated system plays a vital role in vascular homeostasis and offers therapeutic potential in managing hypertension and cardiovascular diseases (Mohammadi et al., 2024).

10.4. Antimicrobial peptides (AMP)

Antimicrobial resistance (AMR) due to infectious microorganisms is rising on a worldwide scale, and this trend is cause for concern (Ferri et al., 2017). The commencement of extensive and long-term measures has been suggested by global health authorities as a critical way to begin mitigating the threat posed by AMR bacteria. A major public health concern of the twenty-first century is bacterial AMR, which is defined as decreased treatment efficacy against bacteria as a result of genetic changes in the microorganisms. According to statistics, AMR bacteria were responsible for 4.95 million deaths in the year 2019. Some of the primary bacteria responsible for these deaths are *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* (Murray et al., 2022).

The possible use of bioactive peptides as intrinsic antibacterial agents has been addressed by lately conducted research, which provides notable benefits over other naturally occurring substances (Tang et al., 2015). A significant number of antibacterial peptides that have been examined in earlier studies were produced by the enzymatic degradation of proteins obtained from plants. Alcalase-derived peptides from palm kernel cake showed significant antibacterial activity against a range of *Bacillus* species (Tan et al., 2013). Several different peptides showed moderate antibacterial effectiveness against *Salmonella enterica* and *Escherichia coli* in another research (Kobbi et al., 2015). Furthermore, peptides that are produced via LAB fermentation demonstrated remarkable antibacterial properties alongside a wide range of activity (Chai et al., 2019). Arulrajah et al. (2020) reported the exceptional antimicrobial properties of bioactive peptides obtained during fermentation of the Kenaf seeds. Particularly, even at extremely low concentrations, against harmful microbes, five peptides-AKVGLKPGGFFVLK, GSTIK, HGDRPR, TAHDDYK, and LLLSK-displayed exceptional antibacterial action. Moreover, bioactive peptide's versatility has generated an abundance of attention in recent years because of their various biological roles, most notably their antioxidant potential (Gluvić & Ulrih, 2019). Peptides that are produced through SSF have potent antioxidant qualities, as demonstrated by their capacity to scavenge DPPH and show ferric reducing power. Furthermore, Jakubczyk et al. (2019) discovered through several studies that fermenting diverse bean varieties produces peptides with a small molecular mass, shorter chains, and great antioxidant and antibacterial effects.

Research has demonstrated that plants provide an abundant source of bioactive peptides with antibacterial properties that successfully extend the lifespan of the shelf life of a range of items. Furthermore, it has been discovered that a number of proteins, including lipid transfer proteins, glycine-rich proteins, napkins, and snaking, have antibacterial

Table 8
Antimicrobial peptides derived from plant source.

Source	Peptide sequence	Target bacteria	MIC	Reference
Sichuan pepper seeds	AGDKKKIKINGFGFRIGRL	<i>E. coli</i> and <i>S. aureus</i>	0.064 mg/mL	Hou et al. (2019)
Chia seeds	GDVIAIR, KLKKNL	<i>E. coli</i> , <i>S. enterica</i> and <i>L. monocytogenes</i>	0.512 mg/mL	Aguilar-Toala et al. (2020)
Moringa oleifera seed	MCNDCGA, FFFLLTN, SGGGPS	<i>S. aureus</i>	2 mg/mL	Wang et al. (2023)
Moringa oleifera seed protein	HVLDTPLL	<i>S. aureus</i>	1.99 mg/mL	Zhao et al. (2022)
Fermented Bitter Beans	EAKPSFYLK, AIGIFVKPDATV and PVNNNAWAYATNFVPGK	<i>S. typhi</i> and <i>S. aureus</i>	250 and 125 µg/mL	Muhialdin et al. (2020)
Barnyard grass Seeds	GSAPSAGCC	<i>Pseudomonas syringae</i>	5 µM	Rogozhin et al. (2012)
Cycad seeds	Cy-AMP1 (44 amino acids), Cy-AMP2 (44 amino acids), Cy-AMP3 (90 amino acids)	<i>Clavibacterium michiganensis</i> and <i>Curtobacterium flaccumfaciens</i>	6–260 µg/mL	Shwaiki et al. (2021)
Vicia faba seeds	GGQHQQEESEEQK, LSPGDVLVIPAGYPVAIK	<i>Pseudomonas aeruginosa</i>	12–35 µM	Karkouch et al. (2017)

qualities (Baindara & Mandal, 2022). Plants are a natural supply of antimicrobial peptides, which are now generated through genetic recombination in different hosts. Synthetic analogs of these chemicals are emerging as attractive substitutes, presenting improved stability, decreased toxicity, and higher bioactivity. Their prospective uses include the production of food, farming, environmental management, animal husbandry and pharmaceuticals (Erdem Büyükkiraz & Kesmen, 2022; Zakharchenko et al., 2021). The application of peptides obtained from plants to meals business presents a promising solution to the problem of food deterioration, especially for perishable goods like bread and meat products that are prone to lipid peroxidation. These peptides' bioactivity and stability are largely dependent on how they interact with different food matrix constituents, which is essential for preserving the product's effectiveness over its storage life (Rivero-Pino, 2023).

A Comprehensive antibacterial property of bioactive peptides produced by lactofermentation from Kenaf seed protein has been discovered by Arulrajah et al. (2020). Significant antibacterial activities against *Escherichia coli* and *Staphylococcus aureus* were demonstrated by a bitter bean product fermented through fermentation from Thailand (Saelim et al., 2017). Gallego et al. (2018) suggested within different research that given that bioactive peptides can tolerate heat during food processing, they might be used as natural antibacterial additions across an array of food products. Toldrá et al. (2018), however, pointed out that a variety of bioactive peptides are probably produced in food after various processing steps, guaranteeing their safety for ingestion.

Significant effectiveness against bacteria has been demonstrated by fermented bitter beans, especially against *S. typhi* ($73.41 \pm 0.08\%$) and *S. aureus* ($64.70 \pm 1.10\%$). On the other hand, raw bitter beans failed to demonstrate any detectable antibacterial action against *L. monocytogenes*, *S. typhi* or *E. coli*. The greatest antibacterial effectiveness against *S. aureus* was demonstrated by boiled bitter beans ($44.51 \pm 0.26\%$) (Muhialdin et al., 2020).

Antimicrobial peptides sourced from plants (as outlined in Table 8) present a promising avenue for the advancement of sustainable and environmentally friendly strategies to counter microbial infections, safeguard crops, and enhance food safety. Current research endeavors are directed towards deeper elucidation of their mechanisms of action, enhancement of their efficacy, and comprehensive exploration of their potential utility across diverse fields.

10.4.1. Mechanisms of AMP

Antimicrobial peptides, can interact with their targets and cause cell death via receptor-mediated or nonreceptor-mediated mechanisms (Saikia & Chaudhary, 2018). The fact that these peptides target the cell membranes rather than a particular receptor and lack specific receptors is directly related to this phenomenon (Omardien et al., 2018). On their cell surfaces, bacteria typically display a net negative charge. Because negatively charged phospholipids are present in Gram-positive and Gram-negative bacteria exhibit this trait. Additionally, Gram-negative

bacteria have lipopolysaccharides, which are likewise negatively charged substances, whereas Gram-positive bacteria have teichoic acid, which contributes to the negative charge (Bhattacharya et al., 2018). The fact that the molecules have a net positive charge is a crucial factor in the activity of AMPs. Furthermore, amphipathic qualities are frequently exhibited by AMPs, which have both hydrophobic and hydrophilic areas in their structure. Their dual nature affects how well they work as antibiotics and how they interact with cellular membranes (Patil-Sen et al., 2016).

AMPs, mainly peptides made by bacteria like bacteriocins, play a significant role in this field. Nisin, which comes from the Gram-positive bacterium *Lactococcus lactis* spp. *lactis*, is one prominent member. Because it is the only bacteriocin that the Food and Drug Administration has approved for use in food, it is widely used as a preservative in dairy products (Flynn et al., 2019). Furthermore, nisin Z, which targets Lipid II, a membrane-bound precursor of the cell wall, was the first receptor-mediated AMP to be discovered. Because Nisin Z has a great affinity for the receptor and may create pores even at very low concentrations, it is remarkably effective, especially against Gram-negative bacteria (Mitchell et al., 2018). Polymyxin B is another example of receptor-mediated AMP; it functions as an antibacterial agent by interacting with particular receptors on microbial membranes, including lipopolysaccharides, phosphatidylglycerol and cardiolipin.

10.4.2. Membrane destabilization

The main mechanism of action is transmembrane pore creation (Fig. 6). Many explanations for this phenomenon have been put out; the most well-established and investigated are the barrel-stave, carpet, and wormhole mechanisms. These mechanisms, which will be covered in depth below, serve as the foundation for our contemporary understanding of science.

10.4.2.1. The barrel-stave mechanism. One important mechanism involved in the barrel-stave mechanism is the creation of transmembrane pores in the channel configuration, which is shown in Fig. A. This process recognizes and inserts AMP molecules one after the other in a fashion similar to how staves are arranged in a barrel. To guarantee energetic favourability, AMPs must first be placed on the membrane surface before implantation. Because the hydrophilic sections of AMPs align face each other within the pore when they are abundant, this placement is crucial because it promotes aggregation and energetically facilitates the AMPs' entrance into the membrane.

10.4.2.2. The carpet mechanism. The carpet process is well-known for several of AMPs and is depicted in Fig. B as a model of membrane permeabilization similar to the effect of detergents. The peptides do not pass through the membrane as they do in the barrel-stave process; instead, they build up on its surface to create a "carpet." Peptide buildup generates a lot of electrostatic interactions in the membrane, which

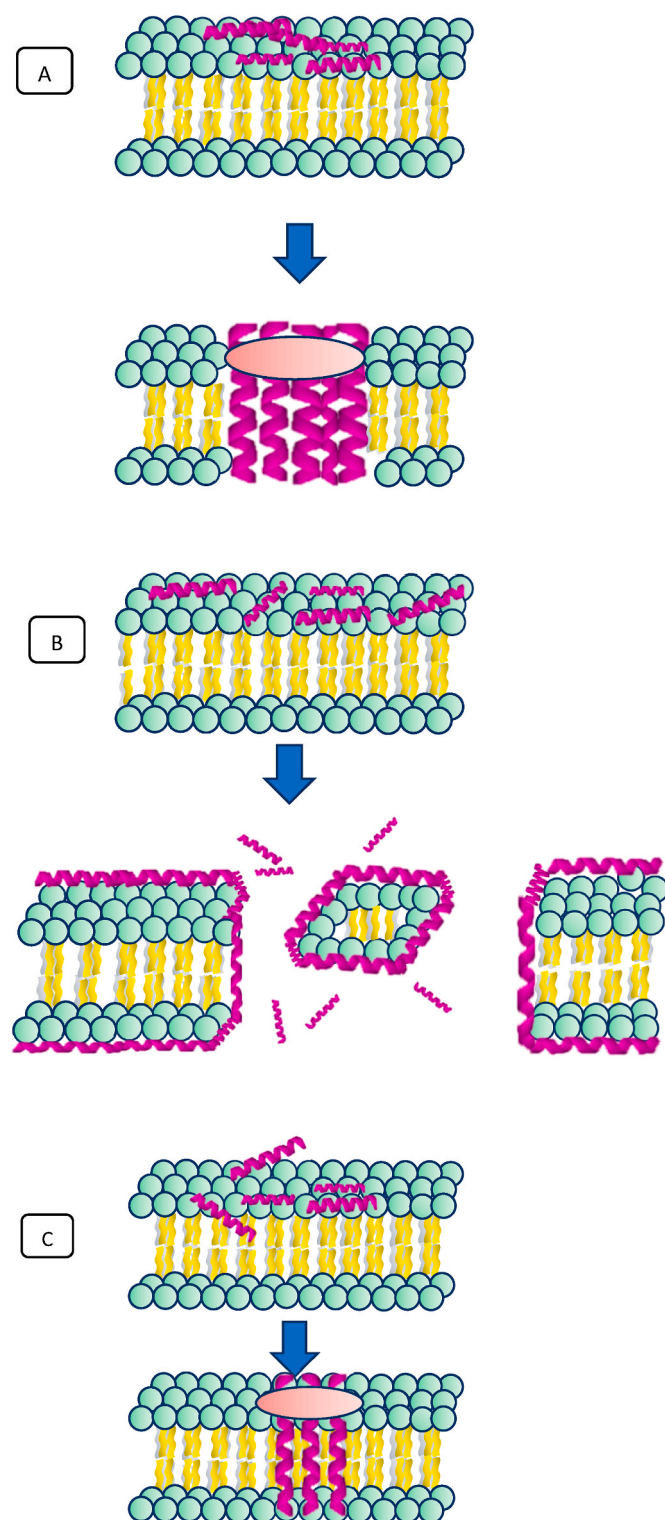


Fig. 6. Action mechanisms of antimicrobial bioactive peptides (A. Barrel-stave mechanism (Takahashi et al., 2018), B. The carpet mechanism (Perrin & Pastor, 2016) and C. The wormhole mechanism (Omardien et al., 2018)).

cause instability because the fluidity changes. The bilayer consequently breaks up into many micelles (Rajasekaran et al., 2019).

10.4.2.3. The wormhole mechanism. The wormhole, or toroidal pore, as these terms refer to the kind of pore created by this process, is a well-known mechanism in the literature (see Fig. C). The inclusion of

membrane lipids in the channel created by the wormhole mechanism, in addition to the AMPs, sets it apart from the barrel-stave mechanism's pore. As a result, the structure of the pore reveals the comparison between the heads of the polar phospholipids and the polar surfaces of the AMPs, revealing their interaction (Perrin et al., 2016).

The polar heads in the lipid structure are displaced by the hydrophobic characteristics of amino acid residues, which results in the membrane's stress due to bending. This stress causes strain to build up across the membrane's surface, which eventually causes the membrane to become unstable. Consequently, as the research by Neelay et al. (2017) shows, this instability causes the cytoplasmic contents to escape and ultimately results in lysis. Remarkable antibacterial properties were demonstrated by bioactive peptides that were obtained from the soy milk fermentation initiated by *Lactobacillus plantarum* C2 strain (LP C2). In soy milk, LP C2 showed strong growth that resulted in a significant increase in number of cells and acidity as well which in turn caused the pH to drop. The 10 kDa fraction has the highest peptide concentration of all the peptide fractions ($655.128 \pm 2.95 \mu\text{g/mL}$). The 5 kDa fraction was the most active against different pathogens; *E. coli* ($12 \pm 0.57 \text{ mm}$), *L. monocytogenes* ($10 \pm 0.57 \text{ mm}$) and *B. cereus* ($10 \pm 0.57 \text{ mm}$). The unfractionated sample did, however, show greater antibacterial activity, perhaps as a consequence of the coupled synergistic outcomes of all fractions (Singh et al., 2015).

10.5. Anti-cancer peptides

Cancer became considered among the major causes of death around the world, accountable for nearly ten million deaths, according to data released by the WHO (2022). The most prevalent cancer types are lung, colon, breast, prostate, and rectal. Approximately one-third of cancer-related deaths are attributable to tobacco use, a high body mass index, excessive alcohol use, a diet low in fruits and vegetables, and a lack of physical activity. Pulmonary, gastric, hepatic, colorectal, breast, and cervical cancers are the main cancer categories with diagnoses (Kuppusamy et al., 2014). Commonly known as malignant tumors or neoplasms, cancer is a broad category of disorders that can affect any body part. A type of cancer's primary characteristic is the fast growth of aberrant cells that go beyond normal bounds and may invade nearby tissues, which can result in metastasis, or the spread of the disease to other organs. When it comes to cancer situations, metastases are frequently the main cause of death. With a plethora of research revealing that particular meals and their constituents can lower the risk of disease and improve general health; we now have a firm understanding of the intricate relationship between diet and health.

10.5.1. Action mechanisms of anti-cancer peptides

Bioactive peptides (BPs) are being used in the rapidly expanding field of anticancer peptide therapy to target and kill cancer cells. Considering the digestive tract finds it difficult to absorb the majority of BPs, these peptides can operate directly within the intestinal tract or indirectly through interactions with receptors and cell signalling pathways in the gut. Although some research has shown that di- and tri-peptides are absorbed in the intestines, it is still unknown how BPs with bigger molecular weights pass through the intestinal epithelium. Furthermore, research is still being done to determine the precise processes by which these peptides work to inhibit cancer cells (Kuppusamy et al., 2014). Two distinctive human peptide drivers, hPepT1 and hPepT2, have been extensively observed and put through testing for functioning. The major locations of hPepT1 include the liver, proximal tubules of the kidney, small intestine, and the lysosomes of pancreatic renal cells. It is a specialized transporter of dipeptides and tripeptides. On the other hand, hPepT2, which carries out the same primary function, is expressed in the brain, lung, mammary glands, and proximal tubules.

The effectiveness of anticancer therapy varies depending on the type and stage of the cancer; nonetheless, standard treatments can be quite

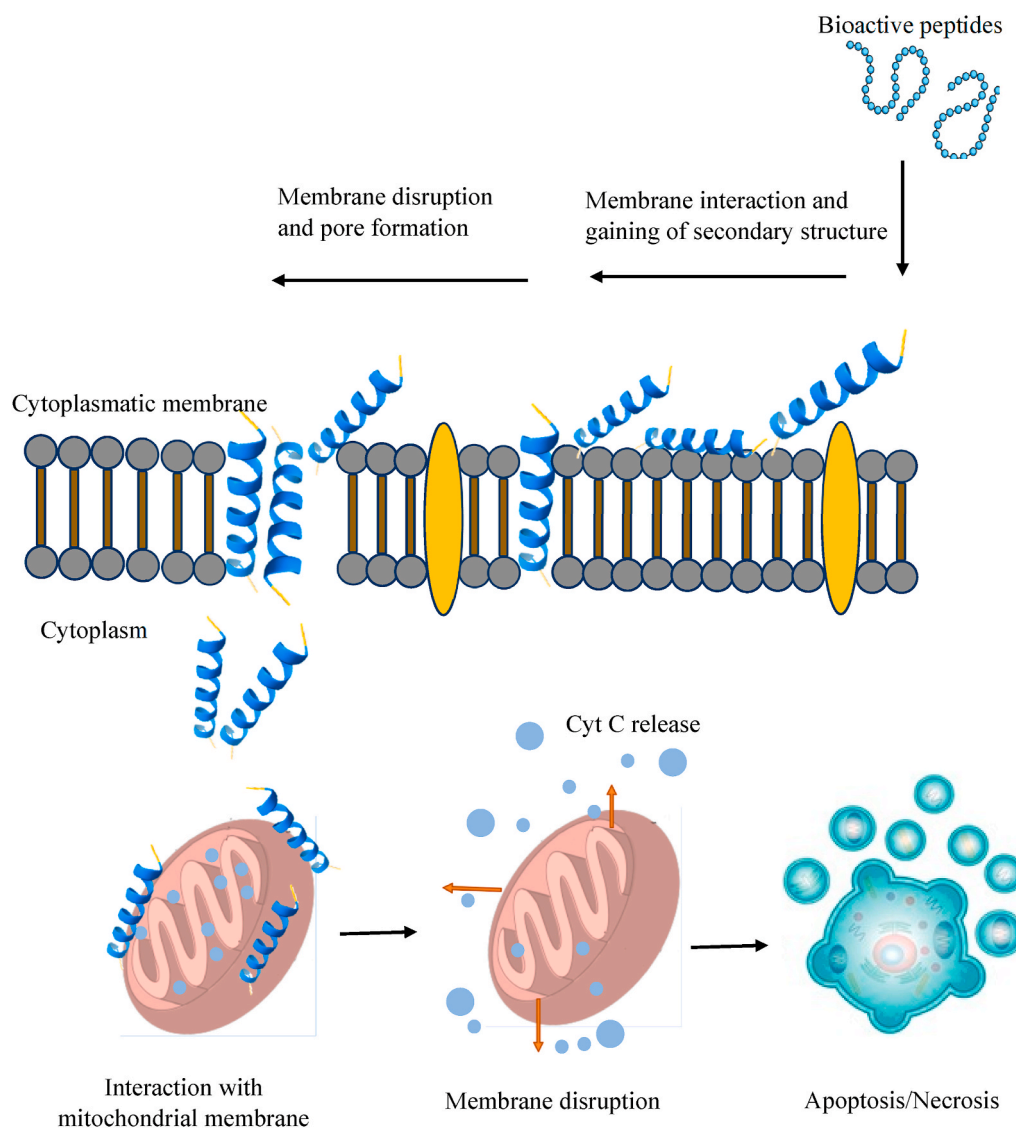


Fig. 7. Action mechanisms of anti-cancer bioactive peptide (Tyagi et al., 2015).

hazardous to patients and expensive. As a result, there is an urgent need for creative research on anticancer therapies that are less expensive and hazardous. In this context, anticancer peptides offer a viable substitute for traditional cancer medications, which are usually composed of tiny molecules. According to Kim et al. (2019), these protein fragments induce apoptosis, upset the tubulin-microtubule equilibrium, and suppress angiogenesis, among other processes that cause cell death. There has been a noticeable increase in the variety of peptides employed in anticancer therapy in recent years. This expansion is partially due to the identification of short peptides with the Glutamine-Asparagine-Serine pattern, which are well known for their capacity to block interactions with Ras-Raf proteins, which are important participants in cancer pathways. In addition, a great deal of attention is paid to peptides that are specific to tumour cells, particularly those that include the RGD motif, which has a high affinity for integrins.

RGD sequence is utilized by lunasin to help cancer cells internalize and cause apoptosis in a variety of cancer cell lines. Similarly, oligoarginine, which consists of 8–12 arginine residues, and Tat, a cell-penetrating protein derived from the human immunodeficiency virus, both show effective penetration into a variety of cell types. Proteins, nucleic acids, and nanoparticles are examples of bioactive substances that can be delivered into cells by utilizing conjugation with Cell-penetrating peptides. As a result, Cell-penetrating peptides show

promise as carriers for applications involving intracellular delivery. Additionally, a recent study showed that lunasin significantly reduced oncosphere formation *in vitro* and efficiently suppressed colony formation by isolated melanoma Cancer-Initiating Cells within soft agar. Furthermore, it demonstrated a noteworthy suppression of tumour growth in mouse xenograft models. By using in-depth mechanistic analyses, the study demonstrated that the use of lunasin treatment resulted in a decrease in the expression of NANOG, a crucial stemness factor involved in the migration, invasion, and dedifferentiation of melanoma cells, while increasing the expression of markers linked to melanocyte differentiation, such as Tyrosinase and Microphthalmia-associated Transcription Factor. The researchers produced a ground-breaking discovery: lunasin selectively targets melanoma cancer-initiating cells and induces a transition towards a differentiated non-cancer-initiating cells state, demonstrating noteworthy therapeutic promise against melanoma. According to this study, lunasin may provide a new treatment option for patients with advanced metastatic melanoma that are resistant to chemotherapy (Shidal et al., 2016). The vast majority anticancer peptides exhibit activity against tumour cells by establishing a β -sheet structure before to contact with the membrane, or by assuming a bioactive helical conformation once they reach the cell surface (shown in Fig. 7). However, peptides exist that precisely fold on the outermost layer of cancerous cells, transforming into a β -sheet configuration

Table 9Examination of bioactive peptides derived from plant sources through *in vivo* studies.

Activity	Plant source	Peptide sequence	<i>in vivo</i> models	Doses tested	Action mechanism	Reference
Antioxidative	Soybean	FDPAL	Nematode under juglone-induced oxidative stress	10 mM for 48 h	Reduced intracellular ROS production	Ma et al. (2016)
	Sesame cake	AVPWY	Male mice	20 mg/kg/d for 2 weeks	Reduced the oxidative stress status	Shu et al. (2019)
	Pine nut	QDHCH	HepG2 cell	8.45 U/mg	Possessed cellular antioxidant activity	Liang et al. (2017)
	Hemp seed meal	SVYT and WYT	Spontaneously hypertensive rats	1% (w/w) for 8 weeks	Reduced the oxidative stress	Girgih, Alashi, et al. (2014)
	Fermented grains	VNP and YGD	AAPH-treated HepG2 cells	2 g/L	Suppressed the generation of reactive oxygen species	Jiang et al. (2019)
Antidiabetic	Soybean	PCGS and CRHPSG	STZ/HFD-induced diabetic BALB/c mice	50 mg/kg/d, for 4 weeks	IR/IRS1 pathway increases insulin receptor, improving glucose absorption	Lu et al. (2012)
	Pea seed	PCGSSACR-CIPY and GLVVGNC-RHPSG	L02 cell	50 mg/kg/d for 4 weeks	Impairing glucose tolerance, restoring pancreatic function	Jiang et al. (2014)
	Black bean	AKSPLF, ATNPL and FFEELN	STZ-induced Wistar rats	100, 150 and 200 mg HPI/kg/BW for 15 d	Lowering the amount of glucose absorbed by obstructing glucose transporters	Mojica et al. (2017)
	Spiny gourd	PRILQK, PRILKK	Male Wistar rats	20 mg/kg body weight for 30 d	Reduced blood glucose levels, greater oral carbohydrate tolerance	Poovitha et al. (2017)
Antihypertensive	Broccoli	IPPAYTK, LVLPGELAK and TFQGPPHGIQVER	Spontaneous hypertensive rats (SHRs)	10 mg/kg for 2 weeks	Enhancing endothelial function	Dang et al. (2019)
	Protein Soy milk	FFYY	SHR	80 µg/kg BW/day for 3 weeks	Diminished rising in systolic blood pressure	Xiang et al. (2024)
	Rice bran	LRA, YY	Male SHR	0.25 mg/kg for 3 weeks	ACE inhibition Vasodilation	Shobako et al. (2019)
Anticancer	Wheatgerm	IVY	Male SHR	171.4 mg/kg body weight.	ACE inhibition	Lin et al. (2018)
	Common bean	GLTSK, LSGNK and GEGSGA	HCT-116	0.53 mg/ml and 0.51 mg/ml	The expression of p53 in HCT116 was dramatically elevated by both cultivars, increasing by 76% and 68%	Vital et al. (2014)
	Chickpea	ARQSHFANAQP	MCF-7 and MDA-MB-231	2.38 µM and 1.50 µM	The peptide successfully stopped breast cancer cells from proliferating	Xue et al. (2015)

Table 10

Effect of fermentation on various bioactivities of plant-based foods.

Food material	Used microorganism	Action mechanism	Reference
Wheat	<i>Lactobacillus fermentum</i> (MR13)	Antioxidative, Antihypertensive	Antognoni et al. (2019)
Wheat sourdough	<i>Lactobacillus plantarum</i> TMW 1460	Blood sugar control	Ripari et al. (2019)
Jussara pulp	<i>Lactobacillus fermentum</i> ATCC 15442	Improve digestion Strengthen immune system	Braga et al. (2018)
Faba beans	<i>Rhizopus oligosporus</i> ATCC 48012	Antioxidant activity increase bio accessibility	Polanowska et al. (2020)
Liquorice root	<i>L. plantarum</i> DSMZ 20174	Antiradical activity	Mousavi and Mousavi (2019)
Barley bran	<i>Pediococcus acidilactici</i> BaltBio01	DPPH inhibition activity	Bartkiene et al. (2018)
Oats	<i>Monascus anka</i> GIM 3.592	Antidiabetic	Bei et al. (2020)
Bergamot juice	Pure and mixed cultures of <i>L. plantarum</i> subsp. <i>plantarum</i> PTCC 1896, <i>L. plantarum</i> AF1 and <i>L. plantarum</i> LP3	Improved blood sugar regulation	Hashemi and Jafarpour (2020)
Soybean	<i>Monascus purpureus</i>	Anti-cancer	Handa et al. (2019)
Bean seed flour	<i>Lactobacillus plantarum</i> 299v	Radical scavenging activity	Jakubczyk (2018)
Lupin	<i>Lactobacillus reuteri</i> K777, <i>Lactobacillus plantarum</i> K779	Antihypertensive	Ayyash et al. (2019)
Tomato seed	<i>Bacillus subtilis</i> A14h	Antioxidant activity	Moayedi et al. (2018a, 2018b)
Oats	<i>Lactobacillus plantarum</i> B1-6	Antidiabetic activities	Wu et al. (2018)
		Antihypertensive, antidiabetic, and antioxidant activity	
		Antiproliferative activity against Caco-2 and MCF-7 cancer cell lines, α-amylase and α-glucosidase inhibition, antihypertensive, antioxidant, ACE-inhibitory and antioxidant activities	
		ACE inhibitory activity	

ultimately leads to pore creation and damage the cell membrane (Tyagi et al., 2015).

In a study, Xue et al. (2015) examined the possible anticancer effects of chickpea-derived peptides. RQSHFANAQP and ARQSHFANAQP, two peptides, were examined at different dosages. The peptide RQSHFANAQP successfully prevented breast cancer cells from multiplying, with EC₅₀ values of 2.38 µM/mL and 1.50 µM/mL. Conversely, the peptide ARQSHFANAQP exhibited an EC₅₀ of 1.50 µM/mL, indicating

encouraging outcomes in impeding the growth of colon cancer cells. These results point to the possibility of using peptides produced from chickpeas as therapeutic agents to treat various cancers.

Examination of bioactive peptides derived from plant sources through *in vivo* studies involves investigating the physiological effects and potential health benefits of these peptides when consumed by living organisms. Bioactive peptides are short sequences of amino acids found in various food sources, including plants, and have been shown to

exhibit biological activities such as antioxidant, antidiabetic, antimicrobial, antihypertensive, anti-cancer properties (Table 9). *In vivo* studies involve conducting experiments on living organisms, such as animals or humans, to evaluate the bioavailability, bioactivity, and safety of bioactive peptides derived from plants. These studies typically involve administering the peptides orally or through other routes of administration and monitoring their effects on various physiological parameters (Table 10).

11. Conclusion

Consuming food derived peptides from fermented plants that are rich in bioactive peptides has the potential to improve both human health and environmental sustainability. Throughout the fermentation process, several microbes release proteases that hydrolyse proteins in the food matrix and transform them into other bioactive components, including bioactive peptides. The current review of the literature reveals that consumers are very interested in bioactive peptides derived from plants that can improve human health. Numerous studies have demonstrated the potential advantages of these components which include biologically active peptides, carotenoids, polyphenols, phytosterols, biogenic amines, and organosulfur compounds in reducing the risk of chronic diseases. The scientific evidence of the regulation, prevention, or treatment of cancer, diabetes, cardiovascular disease, blood pressure, oxidative stress, brain health, and gut health by plant-derived bioactive peptides is reviewed in this study. This field of research is becoming progressively more renowned with the discovery of novel molecular targets for human disorders. However, more studies are necessary to determine the exact mechanisms, recommended dosages and long-term impacts of the bioactive peptides present in these products. Further, functional fermented plant-based products rich in bioactive peptides may be developed for the management of various lifestyle disorders.

12. Challenges and recommendations

The generation and usage of Peptides with bioactive properties sourced from fermented plant-based diets present some obstacles, notwithstanding their potential health benefits. It is noteworthy to acknowledge that although bioactive peptides can be chemically manufactured (Szymanowska & Baraniak, 2019); there exists uncertainty regarding the classification of completely synthetic peptides as food or nutrients (Zaky et al., 2022). There is a scarcity of research papers on bioactive peptides generated through microbial fermentation in comparison to those produced through alternative methods. The investigation into bioactive peptides derived from fermented foods has predominantly been limited to laboratory settings, necessitating the expansion of research efforts to facilitate the development of health-enhancing products based on bioactive peptides (Chakrabarti et al., 2018). However, based on the analysis of multiple papers, it has been demonstrated that bioactive peptides derived from fermented plant-based meals exhibit diverse health-enhancing characteristics, such as antihypertensive, antioxidant, and antibacterial capabilities. Peptides have the potential to play a role in promoting health through various mechanisms, including immunomodulation, stimulation of mucin, insulin-mimetic actions, and antistheoporotic activity. Fermented foods serve as a direct and superior reservoir of bioactive peptides, with a diverse array of bioactive peptides being directly extracted from fermented plant-derived food products. Bioactive peptide-rich alternatives derived from plant proteins, such as rice and soy, have the potential to meet the increasing demand for vegetarian diets in the worldwide market. Subsequently, the commercialization of bioactive peptides derived from fermented foods has garnered significant attention, prompting the need for additional research to validate their potential advantages for human health.

CRedit authorship contribution statement

Brijesh Bhuvu: Writing – review & editing, Writing – original draft, Visualization, Validation, Conceptualization. **Kunal M. Gawai:** Writing – review & editing. **Brij Pal Singh:** Writing – review & editing. **Preetam Sarkar:** Writing – review & editing. **Md Zakir Hassan:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis. **Elena G. Kovaleva:** Writing – review & editing, Writing – original draft, Visualization, Investigation, Formal analysis. **Subrota Hati:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Investigation, Formal analysis, Conceptualization.

No conflict exists

Author **Brijesh Bhuvu** declares that he/she has no conflict of interest.

Authors **Kunal M Gawai, Brij Pal Singh, Preetam Sarkar, Md. Zakir Hassan, Elena G. Kovaleva, Subrota Hati** declare that he/she has no conflict of interest.

Ethical approval

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Ethical approval

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Data availability

Data will be made available on request.

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