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Antimicrobial plant-derived peptides obtained by enzymatic hydrolysis and fermentation as components to improve current food systems

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ABSTRACT

Background: New challenges in our society are appearing following the emergence of antibiotic-resistant microorganisms and the concerns about the use of antibiotics. Furthermore, food spoilage due to microorganisms leads to food waste of around 1.3 billion tons each year. Bioactive peptides obtained from food proteins, released by enzymatic hydrolysis or fermentation, can be employed as a natural alternative to antibiotics and as preservative agents, due to their ability to inhibit the growth of several microorganisms. Plant protein use in the food industry is increasing over animal protein, due to the environmental and animal welfare concerns of the population. Considering the concerns about the use antibiotics, their exploitation as a source of antimicrobial peptides is gaining interest in recent years.
Scope and approach: This review aims to summarize the published literature on protein hydrolysates as a source of antimicrobial peptides, obtained by enzymatic hydrolysis or fermentation of plants, their mechanisms, and the use in the food industry by producing fortified products or edible coatings.
Key findings and conclusions: Antibacterial and antifungal plant peptides obtained by enzymatic hydrolysis are studied to a larger extent that antiviral peptides but further research concerning identification and applicability must be done. The results published show promising uses in the food industry in many areas such as fortification of foods or active packaging with dual bioactivity, related to the antimicrobial activity – avoiding food spoilage and antimicrobial resistance.

1. Introduction

This period of economic crisis and population growth calls for new approaches to address both food waste and deterioration on a global scale. Around a third of the production of the food destined for human consumption is lost or wasted worldwide, equivalent to approximately 1300 million tons a year (Munesue et al., 2015). This necessarily means that huge amounts of resources intended for food production are used in vain, and that greenhouse gas emissions caused by the production of that food that is not used are also wasted emissions. On the other hand, bacterial antimicrobial resistance (AMR), which implies that drugs to treat bacteria are less effective because of genetic changes in the microorganism, has appeared as one of the leading public health threats of the 21st century. It is estimated that 4.95 million deaths were associated with AMR bacteria in 2019. The six leading pathogens for these deaths are *Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Streptococcus pneumoniae, Acinetobacter baumannii,* and *Pseudomonas aeruginosa* (Murray et al., 2022).

The increasing attention being paid to food loss and waste is reflected in the Sustainable Development Goals (SDG). The SDGs are 17 interconnected global goals designed to be a "blueprint for achieving a better and more sustainable future for all". These were established in 2015 by the United Nations General Assembly and are intended to be achieved by 2030. The reduction of food loss and waste can contribute to the achievement of other SDGs, in particular the Hunger goal zero (SDG 2), for which it is required to end hunger, achieve food security and improved nutrition, and promote sustainable agriculture. Also, the effects anticipated positive environmental outcomes from reducing loss and waste of food would have an impact, among others, on SDG 6 (sustainable water management), SDG 13 (change climate), SDG 14

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(marine resources), the SDG 15 (terrestrial ecosystems, forestry, biodiversity) and many other SDGs (United Nations, 2015). However, the increasing levels of antimicrobial resistance will hinder progress towards many SDGs, particularly those that focus on health and well-being, poverty reduction, food security, the environment and economic growth (Gajdács et al., 2021).

Proteins, beyond their nutritional value, are a valuable source of hydrolysates containing peptides with biological activity. Bioactive peptides are encrypted in protein sequences and are released by enzymatic proteolysis (gastrointestinal digestion or *in vitro* hydrolysis using proteolytic enzymes), using acid/base chemicals by chemical hydrolysis, or fermentation. These peptides can be obtained from any food source, that is, from animals, plants, fungi, microorganisms and their products. Enzymatic hydrolysis and fermentation are the most widely used methods for obtaining protein hydrolysates, based on the advantages that these processing techniques show (Cruz-Casas et al., 2021). Several bioactivities have been described for food-derived peptides, such as antioxidant (Ahmad et al., 2023; Montserrat-de la Paz et al., 2021), antihypertensive (Li et al., 2022; Villanueva-Lazo et al., 2021), antidiabetic (Nagaoka et al., 2021; Rivero-Pino et al., 2020) or anxiolytic (Santos-Sánchez et al., 2022). On top of that, there are peptides that have been described to exert antimicrobial activity that could be used for the prevention of food-borne diseases and food spoilage (Sharma et al., 2022). Several factors have to be taken into consideration in the choice of which proteins are used to generate bioactive peptides. The most relevant factor would be the protein composition, associated with certain sequences that, based on the protease employed, would release specific amino acid sequences. In this regard, parameters such as molecular weight, charge, and hydrophobicity determine the bioactivity of these peptides (Aguilar-Toalá et al., 2019). Beyond that, the environmental impact resulting from the production of the food or protein source as well as the socio-economic impact (new trends in diet habits, animal welfare (the growing trend towards plant-based diets instead of animal-based), worker safety, development of new technologies, etc., come also into play (Varela-Ortega et al., 2021). In this regard, plants are currently seen as an adequate alternative to be used in the food industry compared to other protein sources due to their advantages.

Plants have been demonstrated to be a rich source of antimicrobial bioactive peptides capable of extending the shelf life of products (Baindara & Mandal, 2022). In spite of that, no plant peptides are currently used in the food industry for these purposes (León Madrazo & Segura Campos, 2022). Furthermore, several proteins have shown to exert antimicrobial bioactivity, such as napins snakins, glycine-rich proteins, lipid transfer proteins, among others (Baindara & Mandal, 2022; Mignone et al., 2022). Antimicrobial peptides can be extracted from these plants, as natural sources, but can be also produced in other hosts by genetic recombination of genes. Recently, the synthetic analogues of these compounds are being proposed as more stable, less-toxic and bioactive alternatives. Several applications can be correlated with these peptides, such as in food, agriculture (Zakharchenko et al., 2021), environment, animal husbandry and/or pharmaceutical industries (Erdem Büyükkiraz & Kesmen, 2022). The use of plant-derived peptides within the food industry could help overcome the food spoilage concerns of fresh products such as bakery goods (e.g. bread) or meat products, also likely to suffer from lipid peroxidation. The interaction of these peptides with the different components of the food matrix would determine their stability and consequently, bioactivity, and whether this is maintained during the storage of the product (Rivero-Pino, 2023).

The aim of this review is to summarize recent studies (2019–2023) for the classification and production of antimicrobial peptides (including antibacterial, antifungal, and antiviral compounds), the current methodology employed for analysing these peptides, mechanisms underlying the inhibition of antimicrobial on the growth of microorganisms, and the applications of these peptides in the food industry, as functional ingredient, preservatives or agricultural aids

2. Production of peptides

2.1. Enzymatic hydrolysis

The peptides mixture produced by hydrolysing a protein is considered a protein hydrolysate. Proteins in their native state hide the functionality of their peptides, making it difficult for them to exert their bioactivity through association with other molecules. The release of peptide can be done by the action of (food-grade) proteases, which would act on the peptide bonds, specifically, based on their active site. However, due to the release of hydrophobic residues from enzymatic hydrolysis, some peptides can have a bitter taste (Rivero-Pino, 2023).

There are several proteases currently employed in the food industry, where subtilisin is the most employed one. Subtilisin (also referred as Alcalase) is an endoprotease capable of cleaving a wide range of peptide bonds, though the preference goes towards aromatic and methionine residues (Berraquero-García et al., 2022). Based on the mechanisms by which peptides would exert the antimicrobial activity, as it be discussed in the following section, it can be expected that the cleavage by trypsin would promote the release of antimicrobial peptides. This protease is specific towards arginine or lysine residues, releasing positively charged peptides into the medium. However, it must be noted that the reaction conditions, pH and temperature, would have an impact on the activity of the protease especially when hydrolysing proteins with more than one protease (simultaneously or sequentially). For instance, the activity of trypsin can be decreased above 47 °C in longer hydrolysis, and not release peptides effectively, whereas subtilisin presents higher resistance at high temperatures, and this has proved to have an effect on the release of bioactive peptides (Rivero-Pino et al., 2021).

Overall, the system protein-protease is key to release specific type of peptides, whereas considering all the relevant parameters in the hydrolysis reaction. There are studies aiming to model the release of bioactive peptides based on parameters such as temperature or pH, which can optimise the processing of proteins.

2.2. Fermentation

Bioactive peptides can also be released by fermentation (usually submerged fermentation or solid-state fermentation) carried out by several types of microorganisms. One advantage compared to enzymatic hydrolysis is the avoidance of the cost of production of the proteases employed in the manufacturing process, which are usually expensive (Nasri et al., 2022).

The basis of the fermentation process is the same as for the enzymatic hydrolysis with food-grade proteases, but in this case, proteins are cleaved by the action of microorganisms' activity and the proteases that these are able to secrete. Because of that, as for the specificity of the protease chosen in the enzymatic hydrolysis, it is the choice of microbial strain and proteolytic activity associated that will determine the optimal release of bioactive peptides (Nasri et al., 2022). Overall, lactic acid bacteria (*Lactobacillus* species) is currently the species mostly employed to produce protein hydrolysates, as it will be discussed in the following sections. The microorganism employed for the fermentation must be safe, or at least, its deactivation and removal from the final product be ensured, to avoid safety problems.

The main disadvantage for fermentation as a processing technique is the potential generation of other compounds (e.g. exopolysaccharides or bacteriocins) which should be also characterised and their biological properties and safety assessed. In addition, compared to enzymatic hydrolysis, fermentation is a less specific process, due to the poor reproducibility among batches, hampering the development of a product without batch-to-batch variation to be manufactured (Cruz-Casas et al., 2021).

3. Methods of analysis

The methodology employed by different researchers to analyse the antimicrobial properties of plant-derived peptides is widely varied. It is important to consider that unhydrolyzed proteins, carbohydrates and high molecular weight peptides favour microbial growth, thus impairing an accurate evaluation on the activity (Moscoso-Mujica et al., 2021). However, the final purpose of these protein hydrolysates or peptides is to be used in food systems, thus, the interactions between the peptides and the matrix are also a relevant factor when assessing the synergies or antagonism of different compounds in inhibiting microbial growth. The most commonly used techniques to analyse antimicrobial activity are disk-diffusion, well diffusion and broth or agar dilution (Balouiri et al., 2016). Harmonised methodologies, such as ISO 22196:2007 (which specifies a method of evaluating the antibacterial activity of antibacterial-treated plastic products, including intermediate products), are needed to facilitate the comparison among studies. Briefly, Agar disk-diffusion testing developed in 1940, is the official method used in many clinical microbiology laboratories for routine antimicrobial susceptibility testing. In this well-known procedure, agar plates are inoculated with a standardized inoculum of the test microorganism (Balouiri et al., 2016). Then, filter paper discs (about 6 mm in diameter), containing the test compound at a desired concentration, are placed on the agar surface. The Petri dishes are incubated under suitable conditions. Generally, antimicrobial agent diffuses into the agar and inhibits germination and growth of the test microorganism and then the diameters of inhibition growth zones are measured. Antibiogram provides qualitative results by categorizing bacteria as susceptible, intermediate or resistant. The antimicrobial gradient method is based on the possibility of creating a concentration gradient of the antimicrobial agent tested in the agar medium. This method is used for the minimum inhibitory concentration (MIC) determination of antibiotics, antifungals and antimycobacterials. MIC value is determined at the intersection of the strip and the growth inhibition ellipse. Finally, Agar well diffusion method is widely used to evaluate the antimicrobial activity of plants or microbial extracts. Similarly to the procedure used in disk-diffusion method, the agar plate surface is inoculated by spreading a volume of the microbial inoculum over the entire agar surface. Then, a hole with a diameter of 6–8 mm is punched aseptically with a sterile cork borer or a tip, and a volume (20–100 μL) of the antimicrobial agent or extract solution at desired concentration is introduced into the well. Then, agar plates are incubated under suitable conditions depending upon the test microorganism. The antimicrobial agent diffuses in the agar medium and inhibits the growth of the microbial strain tested.

4. Classification based on activity

4.1. Antibacterial peptides

The most common bacteria associated with food spoilage are Gram-positive bacteria such as *S. aureus, Bacillus* spp, *Clostridium* spp, Lactic acid bacteria, *Leuconostoc* spp, *Streptococcus* spp, *Brochothrix* spp, *Weissella* spp, and *Mycobacterium bovis.* These microorganisms can appear in many different foods and environments, and they are able to adapt to varying conditions (Cossettini et al., 2022). The growth of these microorganisms depends on several factors such as pH, temperature, water activity, relative humidity, nutrient content, oxidation-reduction potential, among others. The inclusion of antibacterial compounds would be an added factor in the equation, modifying the microbial population over the storage of the product. There are several low molecular antibacterial peptides identified from different plants, released by means of enzymatic hydrolysis or fermentation, proven to inhibit the growth of several bacteria. In Table 1, antibacterial peptides obtained by enzymatic hydrolysis are summarized, including sequence, structure, and minimum inhibitory concentrations - defined as the lowest concentration of an antimicrobial that will inhibit the growth of the target microorganism after incubation (see Table 2).

Hou et al. (2019) identified several antimicrobial peptides in Sichuan pepper (*Zanthoxylum bungeanum* Maxim) seeds, among which the one exerting the higher activity was AGDKKIKIGINGFGRIGRL. In addition, there are also studies reporting the bioactivity of Maillard compounds, resulting from the reaction of peptides with reducing sugar (Wang et al., 2019). This increased bioactivity has been reported for other properties of peptides such as antioxidant or antihypertensive but the identification of these compounds is sometimes not so easy. In this regard, Habinshuti et al. (2019) reported the antimicrobial properties exerted by Maillard

Table 2
Applications of plant-derived peptides, advantages and challenges.

Field	Application	Advantages	Challenges
Agriculture and livestock	Prevention of plant diseases and control the development of pathogens. Improvement of feed efficiency and prevention/ treatment of some animal diseases	Control of living organisms (plant and animals) without using antibiotics and chemicals.	Lack of *in vivo* studies L Isolation of peptides from plant at industrial scale
Functional ingredient	Extend the shelf life of products	Natural origin, considering animal welfare Environmentally friendly Lack of toxicity	High reactivity Isolation of peptides from plant at industrial scale Rapid diffusion from the surface to the mass of a product
Packaging			Isolation of peptides from plant at industrial scale The formulation of the packaging.

Table 1
Antibacterial peptides obtained by enzymatic hydrolysis and their characterisation.

Source	Enzyme	Sequence	Structure	Target bacteria	MIC	Reference
Sichuan pepper (*Zanthoxylum bungeanum* Maxim) seeds	Pepsin	AGDKKIKIGINGFGRIGRL	α-helix and random coil	*E. coli* and *S. aureus*	0.064 and 0.512 mg/ mL	Hou et al. (2019)
Chia seeds (*Salvia Hispanica* L.)	Alcalase + flavourzyme)	GDVIAIR	–	*E. coli, S. enterica,* and *L. monocytogenes* at fraction level	–	Aguilar-Toala et al., (2020)
Moringa oleifera seed protein	Alkaline proteinase	HVLDTPLL	Hydrophobic anionic peptide of the β-sheet structure.	*S. aureus*	1.99 mg/mL	Zhao et al. (2022)
Moringa oleifera seed	Subtilisin	MCNDCGA	anionic peptide rich in β-sheet structures	*S. aureus*	2 mg/mL	Wang et al. (2023)

reaction products obtained from sunflower, soybean and corn meal hydrolysates (hydrolysed with Alcalase and Flavourzyme) using xylose and cysteine at pH of 7.4, heated at 120 °C for 2 h. According to the authors, only the product derived from the reaction of sunflower protein hydrolysate exerted activity against the tested strains (i.e., *S. aureus* and *E. coli*, MIC being 70 and 80 mg/mL respectively). Authors attributed the different behaviour of samples based on the content of alcohols and carboxylic acids in the sample, which can positively contribute to the antimicrobial activity of peptides.

Other recent studies are also reporting improved extraction of peptides by combining the hydrolysis with non-thermal technologies, in order to release peptides in a more efficient way. Aguilar-Toala et al., (2020) reported the production of peptides with antimicrobial properties from chia seed (*Salvia hispanica* L.). These authors employed microwave-assisted hydrolysis with the proteases Alcalase and flavourzyme. In addition, fractionation according to molecular weight was carried out by ultrafiltration (3–10 and <3 kDa fractions). The assessment of the antibacterial potential was carried out towards *E. coli, S. enterica, and L. monocytogenes*. The 3 kDa showed higher bioactivity, inducing an increase in membrane permeability of *E. coli* and *L. monocytogenes*. From this fraction, the authors reported 16 sequences being cationic and hydrophobic peptides, where the most abundant peptide was found to be GDVIAIR. The remaining ones contained the amino acid K as either N- or C-terminal or both.

Song et al. (2020) evaluated the antibacterial activity and *in vitro* digestion stability of different fractions from cottonseed protein hydrolysates (hydrolysed with Alcalase), isolated by ion exchange resin system. Authors evaluated the content of amino acids, which different among fractions, as well as the size of the peptides. The hydrolysate and one of the fractions did not exert antibacterial activity, but instead, promoted the growth of the microorganism, *E. coli*. Authors reported a positive correlation between the bioactivity and the content of basic amino acid contents and a negative correlation with the acid amino acids, such as Glu or Asp. Highest inhibition (ca. 100%) at 2 mg/mL was reported for the fractions enriched in 1–2 kDa peptides. On the other hand, the *in vitro* digestion had slight effects on antibacterial activity of the samples analysed, indicating that peptides are not being degraded by the action of digestive proteases.

Osukoya et al. (2021) investigated the antibacterial activity of Calabash nutmeg (*Monodora myristica*) seed protein hydrolysates obtained using three digestive enzymes (pepsin, trypsin and pancreatin). Potent antibacterial activities of protein hydrolysates (against *E. coli, S. aureus, Staphylococcus epidermidis* and *Proteus vulgaris*) was reported for the peptides obtained following hydrolysis with pepsin. The differences obtained indicated that only peptides resulting from the specific-cleavage of pepsin would be able to exert the bioactivity, since no difference was found regarding the degree of hydrolysis. Osman et al. (2021) produced antibacterial peptides by hydrolysing cowpea seed proteins with Alcalase. The microorganisms inhibited by the peptides obtained were *L. monocytogenes, Listeria innocua, S. aureus, Streptococcus pyogenes, Klebsiella pnemoniae, P. aeroginosa, E. coli* and *Salmonella typhimurium* (MIC ranging from 25 to 150 μg/mL). The fraction exerting the highest activity was evaluated for the content of peptides, identifying 10 dipeptides with a molecular mass from 184 Da to 364 Da, in addition to one pentapeptide (659 Da). Transmission electron microscope showed that these peptides were capable of inducing changes in the bacterial cells affected. Moscoso-Mujica et al. (2021) obtained peptides by hydrolysing kanihua (*Chenopodium pallidicaule* Aellen) seed protein fractions with Alcalase and Pepsin-pancreatin sequentially and characterised them as antimicrobial agents. The target bacteria assessed were *E. coli, S. aureus,* and *C. albicans*. From the 216 hydrolysates obtained, only 28 presented a percentage of inhibition higher than ≥45%. Four peptides were subsequently purified by chromatography, confirming the antimicrobial activity of these compounds, among which one of them was anionic. Employing trypsin as protease, the release of antimicrobial peptides with potent activity was also reported from barley (*Hordeum vulgare* L.) grain proteins by *in vitro* and *in silico* analyses (Tok et al., 2021).

On top of characterising peptides with antimicrobial activity, aiming to optimise the release from natural sources, it is also important to evaluate their stability under different conditions. Several factors such as temperature, pH changes or the content of salt can also affect, as also described for Maillard products previously, the activity of the bioactive peptides. An appropriate analysis of the stability of peptides would help understand and manufacture products with these peptides in a way they can resist within the food matrix and the processing conditions applied.

Zhao et al. (2022) identified a novel peptide (HVLDTPLL) from *Moringa oleifera* seed protein hydrolysates (employing alkaline proteinase and ultrafiltered <3 kDa) capable of affecting the membrane of *S. aureus* (MIC: 2.204 mM). It is a hydrophobic anionic peptide having a β-sheet structure. Furthermore, the authors assessed the bioactivity under different conditions (i.e., temperature, pH, salt, and enzymes), and the major outcome were that the bioactivity was maintained under 5% salt (>90% of the antimicrobial activity) and at a high temperature of 115 °C for 30 min (78% of the activity). The mechanism causing the damage was attributed to an increased membrane permeability, resulting in the release of intracellular nucleotide pools. In addition, molecular docking revealed hydrogen bonding and hydrophobic interactions with dihydrofolate reductase and DNA gyrase through, which could be supporting this antimicrobial potential of the peptide described. Also, peptides from sorghum spent grain (the residue obtained after filtering the mash to obtain the sweet wort in the sorghum brewing process) hydrolysed with neutral protease-Purazyme and Flavourzyme exhibited antibacterial activity, against *L. monocytogenes* ATCC 7644, *Salmonella enterica* serotype Enteritidis SE86, *E. coli* ATCC 8739, *S. aureus* ATCC 1901, and *Bacillus cereus* ATCC 9634 (MIC = 24 mg/mL for the hydrolysate, lower values after fractionation). According to the authors, short peptides with positive charge and hydrophobic residues are those acting against the target bacteria (Garzón et al., 2022).

León Madrazo et al. (2022) assessed the stability to food processing conditions of antibacterial peptide fractions from chia seeds (*Salvia hispanica* L.). Hydrolysis was carried out employing pepsin and pancreatin, and the product was ultrafiltered to obtain fractions containing peptides with different molecular weight. The bacteria evaluated were *L. monocytogenes, Bacillus subtilis, Shigella fexneri, S. aureus, S. typhimurium, Salmonella typhi, Salmonella paratyphi, Salmonella enteritidis* and *E. coli*. Results showed that gram-positive bacteria were susceptible to the fraction of lower molecular weight peptides (below 1 kDa), where *L. monocytogenes* was highly inhibited (MIC = 635.4 ± 3.6 μg/mL). This fraction maintained the activity up to 80 °C and at different pHs, but the action of proteases (pepsin and trypsin) led to a loss of bioactivity. The authors suggested that KLKKNL could be the peptide displaying the antimicrobial activity, identified by a multicriteria analysis (following the "*Technique for Order of Preference by Similarity to Ideal Solution*"). In fact, the same authors also reported an *in silico* analysis where, employing computer-aided tools, identified a total of 1067 *de novo* sequences from the <1 kDa chia fraction, and proposed also the peptide YACLKVK to show high probability scores for antimicrobial activity (León Madrazo & Segura Campos, 2022). Similarly, Wang et al. (2023) recently isolated, identified and characterised the MCNDCGA peptide, from *Moringa oleifera* seed protein, as the one exerting the greatest inhibitory effect against *S. aureus* from a pool of five peptides. The MIC was found to be 2 mg/mL and as the peptide from the previous study, was identified as a hydrophobic anionic peptide composed of β-sheet structures. The bioactivity stability was also evaluated under different conditions, such as salt, pH and temperature, and it was observed a sensitivity towards temperatures higher than 100 °C.

In recent years, research on plant-derived antibacterial peptides has grown exponentially. In this review we present only some of the available examples, in which most of the studies reveal the high potential of these peptides as antibacterial agents. The research is generally focused on the extraction of these peptides, their identification and *in vitro* bioactivity analysis, and in some cases, the evaluation under different conditions in which, in a real system, the peptide could be found as a component. Based on the molecular features that these peptides usually present (mostly cationic and amphiphilic α-helical peptide molecules), the research should go towards processing proteins with proteases releasing sequences with positively charged amino acids at their terminal, such as trypsin. The identification of more sequences could allow to derive well-defined conclusions on the molecular features that these peptides. The evaluation of the efficacy of these peptides must also be evaluated as individual components (synthetic peptides), to reveal which peptides are the real ones responsible for the activity, and thus try to optimise the release process focused on this (by means of specific proteases, times certain reaction methods, or through the use of certain microorganisms).

4.2. Antifungal peptides

The two types of fungi that are important in food spoilage are yeasts and moulds. The most common fungi associated with food spoilage are *Penicillium* spp., *Aspergillus* spp. and *Fusarium* spp. (Arulrajah et al., 2022). The conditions that will determine the growth of fungi are similar to those affecting bacteria, and consequently, can be also very widely found in food.

One antifungal identified from tomato is called systemin, having a length of 18 amino acids. It has been shown that this medium-sized peptide is involved in inducing the synthesis of protease inhibitors in response to plant wounding and damage from herbivores. This peptide would move along the phloem of the plant, amplifying the signalling process to carry the response to distal leaves (Chiu et al., 2022).

Revalorisation of soybean by-products to produce bioactive peptides was investigated through the production of antifungal peptides (De Benedetti et al., 2021). These authors employed many proteases (bromelain, pancreatin, amano A2, amano S; Amano N, papain and trypsin) to optimise the content of antifungal peptides. The protein hydrolysate obtained with pancreatin was indicated by the authors as the most suitable one, inhibiting the growth of *Fusarium* and *R. solani* mycelia and *Pseudomonas* spp.

Arulrajah et al. (2021) produced antifungal peptides by fermentation (with *Lactobacillus pentosus* RK3) from kenaf seed protein, identifying eight cationic peptides having a molecular weight ranging from 840 to 1876 Da. The MIC and minimum fungicidal concentration (MFC) of KSPM against *Fusarium* sp. were 0.18 mg/mL and 0.70 mg/mL, respectively, while those for *Aspergillus niger* were 1.41 mg/mL and 2.81 mg/mL respectively. The test item exerted fungicidal effect, leading to a prolongation of the lag phase, with increased fungal membrane permeability. Peptides identified were LLESTPSKGLR, WYNFGLK, AHQVANWSGHSAK, AQFPHLK, MASQVSQMPSPSPLSSNK, AGNNILLISSGVNR, LNVSSTLVSKMR and VLALHSVPK. These same authors also reported antifungal peptides obtained from the fermentation of kenaf seed powder with *L. casei* and *L. pentosus* RK3. Sixteen low molecular weight cationic peptides ranging from 768 to 1416 Da were identified from the sample inhibiting *A. niger, Aspergillus flavus* and *Fusarium* sp., including nine of them being *de novo* peptides. The peptides exerted antifungal activity, with a MFC of 86 μg/mL against fungi. In addition, the authors reported that these bioactive peptides retained the efficacy under different conditions (temperatures up to 121 °C, 100 mM NaCl and in an acidic environment) whereas the bioactivity was partially lost in neutral conditions and the peptides are prone to be fully digested by gastrointestinal proteases (Arulrajah et al., 2022).

Research on plant-derived antifungal peptides is not as widely reported as on antimicrobial peptides. Despite this, most studies reveal the high potential of these peptides as antifungal agents, against different strains. Similar to antimicrobial peptides, research generally focuses on the extraction of these peptides, their identification, and *in vitro* assays for bioactivity. An evaluation of the mechanisms of peptide inhibition in the different phases of fungal growth is necessary to assess which protein substrates would be suitable for the release of peptides with said activity.

4.3. Antiviral peptides

Viruses can be transmitted through food and are implicated in zoonotic infections. Among foodborne viruses, the most common is Norovirus, followed by hepatitis A virus, transmitted by raw shellfish, vegetables, and berries (Bozkurt et al., 2021). On top of that, infection of pigs and rabbits with SARS-CoV-2 has been shown in some studies. Thus, the possibility of viral transmission through meat products suggests carry-through contamination (Yekta et al., 2021).

There are not many reports available in literature exploring specifically the antiviral activity of plant-derived peptides obtained by enzymatic hydrolysis or fermentation. However, it has been widely investigated the antiviral properties of proteins from plants such as cyclic peptides or Knottin, among others (Chia et al., 2023). Recent literature on antiviral peptides is focused mainly on investigating the potential of plant peptides to inhibit SARS-CoV-2, after the social situation caused by the pandemic (Galanakis, 2020).

Luo et al. (2020) evaluated *in silico* the potential of corn, sorghum, soybean, wheat, barley and oat peptides, by simulating gastrointestinal digestion employing the BIOPEP-UWM database (proteases being chymotrypsin, trypsin, pepsin). Peptides resulting from this analysis were subsequently docked to SARS-CoV-2 spike receptor-binding domain using HPEPDOCK. These authors proposed as most potent peptides, PISCR from wheat, VQVVN from oat and PQQQF from barley. Further analysis with synthetic peptides could help to elucidate the actual interaction between these peptides and the target receptor, to unravel the mechanisms behind it.

Similarly, Padhi et al. (2021) carried out a molecular docking study to characterize a soybean peptide (sequence ALPEEVIQHTFNLKSQ) obtained after fermentation by *Bacillus licheniformis* KN1G. Authors reported the ability of the peptide to inhibit SARS-CoV-2 S1 receptor binding domain, as well as the ability to modulate Toll Like Receptor 4. In fact, Shi et al. (2021) successfully demonstrated that several peptides derived from plants can bind to key targets of the COVID-19 virus and may thus exert antiviral activity. Among them, based on molecular docking and molecular dynamics simulation, these authors proposed as potent peptides the sequences (acting on specific targets) VSGAGRY from bitter melon seed, VMDKPQG from soybean, KDYRL from mung bean, NNNPFKF from peanut, DENSKF from *Terminalia chebula* tree. These peptides would act towards the virus-host binding process, virus protein replication process, virus genome replication, and transcription process.

There is little research available regarding plant-derived peptides obtained by enzymatic hydrolysis or fermentation as antiviral agents. The research on antiviral peptides, especially anti-COVID peptides, is gaining interest within the research community. However, the analysis carried out until now are mainly *in silico*, and consequently, of limited scientific relevance when extrapolating results to real applicability in society. The following step would imply real analysis of the effectiveness of these peptides in animal studies, to better understand the mechanisms by which these peptides are able (or not) to exert certain bioactivity. Finally, to clearly state the antiviral properties of these plant-derived peptides, well-designed human studies are needed.

5. Mechanisms

The greatest difficulty that the field of bioactive peptide research faces is, generally, the definition of the mechanism by which a given specific sequence of amino acids is bioactive. This topic is being investigated for other types of bioactive peptides, such as antihypertensive or antidiabetic drugs, in which the authors generally use very similar sequences, modify certain residues, and thus try to decipher which amino acid in which position is decisive for said bioactivity. In the case of antimicrobial peptides, the approach that can be used is similar, although there is some information that allows describing how peptides can inhibit the growth of bacteria, fungi and viruses.

The membrane of the microorganisms presents negative charge. As recently reviewed, most of these bioactive peptides exert their activity by interacting with bacterial membranes. There are at least 9 hypotheses of mechanisms of action: 1) electroporation; 2) carpet model, 3) membrane thinning or thickening, 4) non-lytic membrane depolarization, 5) toroidal pore, 6) oxidised lipid targeting, 7) barrel stave, 8) disordered toroidal pore, and 9) non-bilayer intermediate (Magana et al., 2020; Silveira et al., 2021). Briefly, antimicrobial peptides (AMPs) can exert its antimicrobial activity through membrane interactions that result in the disruption of the membrane and eventual death of a cell. The amphipathic and cationic structure of AMPs allow for electrostatic attraction to the negatively charged phospholipids, teichoic acids or lipopolysaccharides found in the membranes of microorganisms. This leads to the accumulation of the peptide on the membrane surface, after which, having reached a threshold concentration, the peptides begin to reorient in the lipid bilayer and the collapse of the membrane follows, mainly by the formation of pores or 2) through membrane disruption. The pore forming mechanisms include the "barrel-stave" and the "toroid pore" (wormhole) models. In the barrel-stave model, alignment of the hydrophobic regions of the peptide with the lipid tail occurs, leading to the formation of the inner surface of the pore with hydrophilic residues. The toroidal pore model is a result of the charged residues in the AMP causing an electrostatic shift in the hydrophilic heads of the lipids.

Some AMPs act via non-specific membrane permeabilization (membrane disruption), one of which is called the carpet model. Eventually, there is a destabilisation and collapse of the membrane structure into micelles. This final step is caused by the peptides saturating the surface of the membrane and, in a detergent-like manner, breaking the lipid bilayer into separate micelles.

In addition, AMPs can permeate the cell membrane. It is important to note that when this happens, intracellular mechanisms may be affected too for example, through the rapid efflux of ions and cytoplasmic membrane depolarization. In the case of the efflux of ions, it has been shown that membrane permeabilization can lead to the leakage of potassium ions from the cell, leading to cell death (Shwaiki et al., 2021). Plant AMPs have been found to induce apoptosis via the accumulation of reactive oxygen species (ROS) (Oyinloye et al., 2015). The overproduction and accumulation of ROS such as hydroxyl radicals, superoxide anions, hydrogen peroxide and peroxide radicals can ultimately lead to cell death (Ciociola et al., 2016). This generation of ROS can result in oxidative damage to macromolecules and ultimately lead to protein oxidation or fragmentation, or DNA damage. AMPs can promote the generation of these ROS, leading to such events (Oyinloye et al., 2015).

It has been reported that chia peptides caused lag phases to be prolonged, maximum growth and growth rate to be decreased, whereas multiple indentations (transmembrane tunnels) and membrane wrinkles were caused, and the integrity of bacterial cell membranes was severely impaired (Aguilar-Toalá et al., 2020).

In fact, mammalian cell membranes that are neutrally charged do not respond to these peptides, which should be positively-charged, thus, attracting the negatively charged membrane of the microorganisms. They can penetrate cell membranes and induce lysis due to their hydrophobic activity (Sharma et al., 2022). An increase in hydrophobicity of the amino acid sequence of AMPs correlates with low toxicity and selectivity toward mammalian cells. The hydrophobic residues penetrate the bacterial membranes, causing cytoplasmic leakage, membrane disruption, and eventually the death of the cells. Furthermore, cysteine residues in AMP are often connected by disulphide bridges, making them highly stable (Silveira et al., 2021).

For antifungal peptides, the mechanisms behind are also related to cell membrane disruption, implying subsequently cell leakage and cell death. Nonetheless, more complex mechanisms have been also reported for antifungal peptides, such as interaction with specific lipids, production of ROS, autophagy or programmed cell death (Struyfs et al., 2021). Another approach that has been reported in literature that could help to unravel the mechanisms of inhibition regarding antifungal peptides, is the development of a support vector machine-based model, aiming to design and predict therapeutic plant-derived peptides. According to the tool, the residue composition analysis indicated the relevance of the Cys, Gly, Lys, Arg and Ser amino acids. In addition, the residues would ideally be positioned in the N-terminal, specially for Gly, Lys, Arg and Ala. These conclusions are in line with the hypothesis previously reported on how trypsin, as Arg/Lys cleaver, would be useful in the release of peptides with antimicrobial activity. Similarly, residues Asn, Ser, Cys and Gly prefer the C-terminal (Tyagi et al., 2021).

Regarding the inhibition of growth of viruses, it is mainly through inhibition of transcription, viral attachment, and binding with key proteins that peptides exert their antiviral activity. However, the wide variety of viruses and their morphological differences is a challenge in the identification of antiviral peptides and especially, unravelling the mechanisms by which the interaction leads to the bioactivity.

The evidence until now allows suggesting the mechanisms by which peptides are able to inhibit microorganism growth, mostly by destroying or destabilising the membrane. In this field, *in silico* analysis can help elucidate and unravel the interactions between the compound and the targets. To examine the role of the peptide spatial structure on the bioactivity, research on-going aims to truncate forms of the peptide, modifying the structure, and evaluating the consequences towards the growth of the target microorganisms. Based on recent findings, secondary structural integrity, hydrophobic properties, and surface charge are all important for antimicrobial activities (Barashkova et al., 2022).

6. Use In the food industry

a. Agriculture and livestock

Agricultural production can be diminished by the appearance of several diseases caused by viruses, bacteria or fungi which would negatively affect the crop cultivation and consequently, decrease the amount of produced mass (crop yield) and would lead to economic losses (Erdem Büyükkiraz & Kesmen, 2022). Therefore, antimicrobial peptides could be employed, by expressing them in model plants (transformation of the plant by genes of AMP), to prevent plant diseases and control the development of these pathogens (Erdem Büyükkiraz & Kesmen, 2022). For instance, regarding systemin, the antifungal peptide from tomato plants, it has been reported that transgenic plants expressing prosystemin (the precursor of systemin) reduce lesions by at least 50% from Phytophthora infestans, a pathogen that causes late blight (Chiu et al., 2022). However, the use of antimicrobial peptides derived from plant proteins by enzymatic hydrolysis or fermentation are unlikely to be used in agriculture as antimicrobial, though it has been reported their use as biostimulants, aiming to reduce the use of agrochemicals and improve productivity parameters in a variety of cultivars (Rouphael et al., 2021).

On the other hand, the use of feed additives in animal nutrition has increased in recent years in order to improve nutrient utilisation, health parameters and animals' performance. It has been noted, however, that the use of antibiotics as feed additives has contributed to the emergence of antimicrobial resistance, which leads to higher rates of morbidity and mortality among diseases that would have previously been treated with antibiotics. A powerful method to prevent the development of resistant microorganisms is to use antimicrobial peptides, which have multiple biological activities. Because AMP increases feed efficiency and prevents/treats some animal diseases, AMP has been shown to be a potent alternative to antibiotics in animal nutrition, despite the limited number of studies conducted *in vivo* (Silveira et al., 2021).

b. Functional ingredient

One of the applications that peptide with antimicrobial activity could be used for is extension of shelf-life of foodstuffs. The growth of microorganisms -especially fungi-in fresh products such as bread limits its long-term conservation or requires the use of preservatives to increase its quality as a useful product. In this sense, antimicrobial peptides, preventing their growth, could be used to improve the functionality of these products.

Rizzello et al. (2017) aimed to extend the shelf life of wheat bread by fortifying it under pilot plant conditions with a hydrolysate from a mixture of legume (pea, lentil, and faba bean) flours with antifungal activity, obtained by hydrolysis with a proteolytic enzyme preparation obtained from *Aspergillus oryzae*. The indicator microorganism for antifungal assays was *Penicillium roqueforti* DPPMAF1. Authors reported inhibition towards several fungi, including *Aspergillus parasiticus* CBS971.97, *Penicillium carneum* CBS 112297, *Penicillium paneum* CBS 101032, *Penicillium polonicum* 112490, some of the most relevant spoilage fungi in baked goods. The bread fortified with the legume hydrolysate had a longer shelf-life than the control. Nine peptides were characterised from legume vicilins, lectins and chitinases as responsible for the antifungal activity. Asri et al., (2020) investigated the antifungal activity of low molecular weight peptides obtained through lacto-fermentation (*L. casei*) of palm kernel cake. Achieving a degree of hydrolysis of 43%, authors identified by LC/MS-MS 10 cationic peptides. The molecular weight of these peptides ranged from 932 to 1869 Da, in which seven of them were attributed to be derived from oil palm proteins and three of them were determined to be *de novo* peptides synthesised by the microorganism. Peptides exerted strong antifungal activity against *A. flavus, A. niger, Fusarium* sp., and *Penicillium* sp., and when added to the bread at 2 g/kg, led to a delay of fungal growth, implying an extension of shelf life for up to 10 days when added to the bread at 2000 mg/kg.

Heymich et al. (2021) evaluated the efficacy under food storage conditions of a chickpea-derived peptide (RIKTVTSFDLPALRFLKL). The antibacterial activity against *E. coli* was proved to be stable during at least 7 day at refrigerated and room temperature, and when used in meat, the MIC against *E. coli* was increased to 125 μM, but maintained the activity towards *B. subtilis*. Arulrajah et al. (2021) treated tomato puree with 1 g/kg of antifungal peptides from kenaf seed protein aiming to prolong the shelf-life of the product. Authors reported a delay in fungal growth for up to 14 and 23 days at 25 °C and 4 °C, respectively, significantly reducing *A. niger* and *Fusarium* sp. counts. These authors also reported the efficacy of peptides in prolonging the shelf-life of fruit juices (mango and pineapple) and bread systems, employing concentrations of 1000 and 3000 mg/kg. It was reported a ≥5-log10 reduction in the viable counts of *S. typhimurium, E. coli* and *L. monocytogenes* within 1–2 h during storage at 4 °C. Concerning the antifungal evaluation, at 3000 mg/kg the fortified bread at room temperature showed a reduction of *A. niger, A. flavus* and *Fusarium* sp. count by 3–4 log10. The results overall showed that the shelf-life of the fruit juices was extended around 5 months, and the bread for around 2 weeks, without involving physicochemical properties changes (Arulrajah et al., 2022).

Ghanbarinia et al. (2022) fortified hamburgers with sesame (*Sesamum indicum*) meal protein hydrolysates (obtained with alcalase and flavourzyme) at different concentration, ranging from 0 (control) to 3% of the peptide's mixture. During a period of 16 days, microbiological and chemical parameters were assessed in order to evaluate the quality of the products. As it was expected, physicochemical modifications were found in the fortified products, such as decrease in texture firmness, and also authors indicated a delay in the oxidation and microbial spoilage. Furthermore, evaluators carrying out the sensory analysis only approved the products containing up to 2% of protein hydrolysates in the burger, since higher concentration led to notable changes in odour and taste, and in the overall acceptance.

However, peptides are very reactive compounds, and they might produce different compounds after interacting with proteins or fats, potentially affecting their stability and subsequently, their bioactivity. Encapsulation is seen as an appropriate means to overcome these limitations. Along with this, the final product containing peptides would show a different behaviour (e.g. rheology, appearance, texture, etc.) (Cermeño et al., 2021). In depth-studies evaluating how the protein hydrolysates, and more specifically, the specific sequences responsible for the activity, could help to make a model of how this affects the growth of different microorganisms, and could elucidate the potential of these compounds as preservative agents. The inclusion of these antimicrobial peptides in combination with non-thermal processes opens new lines of research for innovation in the industry.

c. Packaging

As previously described, an improved food preservation can be achieved by adding antimicrobials directly into food products to combat microbial contamination (Becerril et al., 2020). However, in light of the rapid diffusion of antimicrobial substances from the surface to the mass of a product, this strategy proved to be of limited effectiveness, so the food industry sought new and innovative strategies. Through direct or indirect contact with the packaged food, antimicrobial packaging reduces, retards, or even inhibits microbial growth. Then, the antimicrobial properties of peptides can be also useful to create food packaging, with a similar purpose to adding the peptides into the food matrix to increase the shelf-life. Liu et al. (2021) recently reviewed about antimicrobial peptides and their application in food packaging. Active food packaging refers to that one carrying compounds with certain activity, such as antimicrobial peptides, that can appear encapsulated in nanocarriers to ensure a controlled release for food preservation during the storage (Becerril et al., 2020). Furthermore, food-derived peptides can also exert antioxidant activity that would also enhance the functionality of the food, for instance, by impeding lipid peroxidation (Jamróz et al., 2021). It has been reviewed about plant-based proteins as biodegradable polymers for green packaging materials, discussing about the crude protein content and the different production techniques as well as compositional parameters for the production of bioplastics (Senthilkumaran et al., 2022). Protein hydrolysates cannot be used as sole material for film formation by self-crosslinking due to low molecular mass (Wang et al., 2020).

A rapeseed protein hydrolysate-chitosan composite film was prepared and physico-chemically characterised and found to show better properties than the composite prepared with the protein isolate because hydrolysis contributed to making composite films denser. Regarding the antimicrobial properties, the composite film with 12% degree of hydrolysis showed better results compared with the chitosan film (Zhang et al., 2019). Similarly, it has also been reported the production of alginate films with cottonseed protein hydrolysates, aiming to evaluate several parameters including antioxidant and antimicrobial properties, as well as the release of peptides in two different alginate film food simulants. The addition of the cottonseed peptides (obtained with Alcalase) increased the thickness and water vapour permeability of the films. Concerning the antimicrobial properties of the peptides, the films including the protein hydrolysates showed an inhibitory effect against *S. aureus, Colletotrichum gloeosporioides and Rhizopus oligosporus*, but no effect was observed against *E. coli*. In addition, it was also reported by migration tests in aqueous media that in 0.5 h, more than 50% of the peptides were released from the active films, and also by simulating the activity with fatty foods, a controlled and gradual diffusion of the compounds embedded in the film was observed (Oliveira Filho et al., 2019). These results offer an insight on the mechanisms by which these peptides can exert their activity, not only by inhibiting the growth of microorganisms, but also in their interaction with food components. Similar results were also reported for soy and corn peptides when obtaining chitosan/peptide films (Li et al., 2020), improving the antibacterial activity of the film towards *E. coli* and *B. subtilis.*

The use of non-thermal technologies, as discussed to be useful in other areas, can be also a tool to be employed in the formulation of edible coatings. In this regard, Wang et al. (2020) reported that ultrasound was an adequate method to improve the properties of edible composite films, produced with chitosan and rice protein hydrolysate obtained with alcalase. The application of ultrasound (power up to 800 W, for 15 min at 40 kHz) led to a decrease of particle size and viscosity of the solutions, as well as a decrease on the peroxide value of oil (assessed for oxygen barrier capacity), associated with hydrogen bonds and covalent interactions between the peptides and the chitosan (enhanced protein-polysaccharide interaction).

Other studies focused on evaluating how the molecular weight of chitosan would affect on composite films prepared with rice protein hydrolysate and curcumin (Xie et al., 2022). According to the authors, the addition of the rice peptides improved the film solubility and extensibility of the manufactured composite film. Beyond that, the molecular weight of chitosan had also an impact on the antioxidant and antibacterial activity (assessed towards *E. coli* and *S. aureus*) not necessarily in a dose-dependent manner Curcumin also increased the bioactivity of the films, contributing to the antioxidant and antibacterial properties of the films.

Recently, also it has been studied how edible coatings formulated with peptides generated by lacto-fermentation of palm kernel cake fermented with *Lactobacillus plantarum* ATCC8014 and *Lactobacillus fermentum* ATCC9338 can control postharvest fungal spoilage of mango (*Mangifera indica* L.) fruit. The incorporation of the peptides was done with several polysaccharide polymers, and the antifungal activity, biodegradability and peptide releasing ability was assessed, showing promising results, i.e., the inhibition of the growth of fungi that commonly infest mangoes, such as *C. gloeosporioides and B. theobromae*. The sample employing chitosan and the peptides showed a high peptide release and biodegradability of the coating. However, according to the authors, research should also evaluate quality and sensory properties of treated mango (Ranjith et al., 2022). Similarly, Liu et al. (2022) evaluated carboxymethyl chitosan conjugated with hemp seed and maize peptides, to produce nanoemulsions to develop active films containing Camellia essential, which showed the ability to extend the shelf-life of blueberries.

The effect of different compounds in the formulation of edible films would affect different biological and technological properties, and consequently, the effect on the product intended to be packed. Beyond plants AMPs, other authors have also reported the employment of antimicrobial peptides (mostly synthetic ones) in packaging, for instance, on the shelf-life of fish burgers (*Coryphaena hippurus*) during refrigerated storage (Vuoso et al., 2022) or raw salmon fillets (Ambrosio et al., 2022). The number of parameters to be taken into consideration when formulating a bioactive edible packaging are several, and consequently, it represents a real challenge for the food industry currently.

7. Research gaps and future Perspectives

Considering the literature published until now, it can be concluded that antimicrobial peptides can be of diverse length and the composition of amino acids. The structure that these peptides would have been responsible for the mechanism by which they exert the antimicrobial activity, mostly by being in contact with membranes.

Concerning antimicrobial peptides obtained by enzymatic hydrolysis, literature has shown that most of the peptides have been obtained employing Alcalase, a non-specific endopeptidase, or Flavourzyme. However, there exists a wide variety of proteases with different specificities that could be assayed in order to explore the release of different peptides which could exert higher bioactivity. In addition, discovery and extraction of new proteases would always open new doors to optimization of the release of bioactive peptides (González-Velázquez et al., 2021). In terms of fermentation, recently the field of precision fermentation is gaining interest within the research community (Terefe, 2022). Precision fermentation consists of the use of genetically engineered microorganisms as starters for production of target molecules via fermentation.

In any case, a research field yet to be explored is the pre-treatment of the plant proteins in order to enhance the release of bioactive peptides. The application of non-thermal technologies such as high pressure homogenization, ultrasound, pulsed electric field, pulsed light, oscillating magnetic field, among others, could imply a benefit in the production of peptides, leading to higher concentration of bioactive peptides or a reduction in reaction time.

The assessment of the antimicrobial activity in real food system models is starting to be explored in recent years, increasing the weight of evidence that these peptides could serve as an alternative to antibiotics and preservatives. Once the peptide-containing product is formulated and its bioactivity is maintained, it must also be ensured that bioactivity is not lost during its life as a commercial product. Chemical reactions may occur during storage, depending on product formulation and storage temperature.

As widely reported in this review, plants contain compounds to protect themselves that can be extracted to be used with different purposes, especially in the food system. The exploitation of plant antimicrobial peptides naturally extracted to study their activity against plant, food, and human pathogens is increasing over the years and has opened the field of the chemical synthesis of these identified peptides for their application as potential food preservatives. Thus, it may be possible to combat food spoilage and food waste with synthetic peptides that are reduced in toxicity to human cells, more active, potent and stable. Nonetheless, the main drawback of this research field are the costs associated with the methods to synthesise peptides (Shwaiki et al., 2021). It can be expected an improvement in available technologies and development of new processes that can promote the full exploitation of synthetic peptides in the food sector.

Furthermore, the development of plant-derived peptides at industrial scale seems not to be feasible currently. In spite of being a promising alternative to change our food system towards natural components, which is sometimes consumer's preference, employing a natural extracted component from plant might imply safety concerns, depending on the effectiveness of the production process.

In addition, in this period where research is constantly evolving and the number of publications increases exponentially, it is important to create databases where the available information is summarized and accessible to users. A summary of the current AMP databases, a comparison of computational tools for predicting antimicrobial activity and mechanism of action, and new machine learning approaches to AMP activity to combat global antimicrobial resistance is presented elsewhere (Bin Hafeez et al., 2021). Focusing only of bioactive peptides derived, the database FermFooDb (https://webs.iiitd.edu.in/raghava/fermfoodb/) includes information of more than 2000 peptides, accounting for different bioactivities (including antimicrobial, 55 entries), as well as food source, functional activity, fermentation conditions, starter culture, testing conditions of sequences *in vitro* or *in vivo*, type of model and method of analysis (Chaudhary et al., 2021). A constant update of these databases, with reliable and comparable information could facilitate the revision of information, helping to draw global conclusions on food source and treatment to be applied, in order to obtain the desired pool of peptides.

Greater investment in projects related to green technologies, food safety, new production and analysis methodologies, and harmonization and communication between researchers is key to the proper development of this field of research. On top of the use of plant-derived biologically active peptides in the food industry, these bioactive molecules can play an important role as nutraceuticals, nutricosmetics, cosmeceuticals, etc. There is a wide interest in using natural-derived peptides for topical and oral treatments of skin-related pathogen (Apone et al., 2019; Sharma et al., 2022).

8. Conclusions

Research on the antimicrobial potential of peptides is broad, mostly *in vitro* studies. However, these studies mainly provide a characterization of peptides and consequently are not relevant for actual establishing the activity in food systems or in treatment of diseases.

This review offers an idea on the potential of plant protein-derived products to be incorporated into the food system, potentially avoiding the usage of compounds such as antibiotics. There is not solid evidence that these products could serve as alternatives to antibiotics or antiviral compounds, since further research is required to establish scientifically-based statements on their bioactive properties.

Improvement and harmonization of the analytical techniques employed to determine the efficacy of peptides would improve the research of these food-derived compounds, to elucidate the mechanisms and the most potent candidate sources.

In addition, several gaps have been identified in the manuscript, which could serve as a reference for the promotion of new lines of research and innovation in the near future.

Declaration of competing interest

The authors declare no conflicts of interest.

Data availability

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

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