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Title	Future of antimicrobial peptides derived from plants in food application- a focus on synthetic peptides
Authors	Shwaiki, Laila N.;Lynch, Kieran M.;Arendt, Elke K.
Publication date	2021-06-01
Original Citation	Shwaiki, L. N., Lynch, K. M., Arendt, E. K. (2021) 'Future of antimicrobial peptides derived from plants in food application- A focus on synthetic peptides', Trends In Food Science and Technology, 112, pp. 312-324. doi: 10.1016/j.tifs.2021.04.010
Type of publication	Article (peer-reviewed)
Link to publisher's version	10.1016/j.tifs.2021.04.010
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Download date	2026-11-05 19:51:34
Item downloaded from	https://hdl.handle.net/10468/12692

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Trends in Food Science & Technology 112 (2021) 312–324

Contents lists available at ScienceDirect

Trends in Food Science & Technology

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

Future of antimicrobial peptides derived from plants in food application – A focus on synthetic peptides

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ARTICLE INFO

Keywords:
Synthetic peptides
Plant antimicrobial peptides
Food waste
Spoilage
Peptide design

ABSTRACT

Background: Food spoilage is caused by the undesirable growth of spoilage microorganisms in food products. This spoilage can lead to the global loss and waste of food. It is estimated that 1.3 billion tons of edible food produced for human consumption is either lost or wasted each year. Different preservation techniques have been developed to prevent this spoilage; however, the problem still occurs. Plants have the ability to produce compounds to protect themselves from the harsh environment. This has led to the exploitation of plant antimicrobial peptides (AMPs) which have been naturally extracted to study their activity against plant, food, and human pathogens. The chemical synthesis of such peptides has also grown in popularity over the years.

Scope and approach: This review will focus on the different techniques that can be applied to generate peptide sequences, produce them through various chemical and biological methods, and predict their activity. These approaches are reviewed for their ability to develop synthetic peptides (based on plant AMPs) with greater antimicrobial activity and characteristics essential for their application as potential food preservatives. The development of these synthetic peptides with reduced toxicity to human cells, improved activity and stability can be a possible solution to the continuous fight against food spoilage and food waste.

Key findings and conclusions: Synthetic antimicrobial peptides are being developed and modified in such a way to encompass potent and safe characteristics. This knowledge can be exploited for the potential application of such peptides in foods as preservative agents. A deeper understanding of their structure, function and mechanisms of action can be used to integrate them into food for the reduction of food spoilage, and consequently, of food waste. Although their development and production through the methods reviewed can generate peptides with suitable characteristics for reducing food spoilage, the cost of synthesis can be a drawback to such methods. Nevertheless, as the technologies improve and develop over time, the development of these synthetic AMPs can be fully exploited for their potential role in the food sector.

1. Introduction

Food waste is increasingly prevalent in modern society and can be attributed both to consumer behaviour and the undesirable growth of spoilage microorganisms (bacteria, fungi (moulds) and yeast that cause food spoilage). Food waste and food loss are terms that the Food and Agriculture Organization (FAO) define as the "decrease in quantity or quality of food along the food supply chain" at different stages (FAO, 2019). In addition, a clear distinction between them can be made by identifying food waste as occurring "at the retail and consumption level", while food loss is predicated to occur "along the food supply chain from harvest/slaughter/catch, up to, but not including, the retail level". These distinctions are important to further understand the role of spoilage microorganisms in the food production chain. An estimated 1.3 billion tons of edible food produced for human consumption is either lost or wasted each year. In Europe and North America, 280–300 kg of food is lost or wasted per capita every year – a high figure compared to the 120–170 kg/year of food lost in sub-Saharan Africa and South/-Southeast Asia. Due to consumer behaviour, per capita food waste can be as high as 115 kg/year in developed countries, compared to 6–11 kg/year in developing countries (FAO, 2019).

Spoilage microorganisms play a large role in determining the degree of food waste and food loss worldwide. Different food groups can be affected by microorganisms such as bacteria, fungi (mould) and yeast.

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https://doi.org/10.1016/j.tifs.2021.04.010
Received 2 September 2020; Received in revised form 19 November 2020; Accepted 7 April 2021
Available online 14 April 2021
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Table 1
Peptides in the different families of plant AMPs that can have an application in food as potential preservative agents.

Type of AMP	Name of AMP	Source	Chemical and structural Properties	Antimicrobial Properties	Potential in food applications	Reference
Defensin	Rs-AFP1 Rs-AFP2	*Raphanus sativus*	Highly basic peptides, rich in cysteine residues; molecular weights of 5 kDa; Contain net charge of +4 and + 6, respectively. Cysteine-stabilized αβ-motif typical of plant defensins	Antifungal and antiyeast activity	Synthetic Rs-AFP1 and Rs-AFP2 contain antiyeast activity against food spoilage yeast (*Zygosaccharomyces bailii)* in various beverages	(Shwaiki et al., 2020a; Terras et al., 1992)
	IbAMP1	*Impatiens balsamina*	Highly basic peptide. Contains four cysteine residues which form two intramolecular disulfide bonds. Contains a net charge of +5	Antifungal and antibacterial activity	Antimicrobial activity was found against common enteric foodborne pathogens (*Escherichia coli, Salmonella enteric, Pseudomonas aeruginosa)*	(Tailor et al., 1997; Wu et al., 2013)
	Cp-thionin II	*Vigna unguiculata*	Conformation is stabilized by four disulfide bonds between cysteine residues. Contains a net charge of +8	Antifungal and antibacterial activity	Prevention of the contamination of wheat grains during storage (against the fungal spoilage pathogens *Fusarium culmorum, Aspergillus* niger, *Penecillium expansum)*	Schmidt et al. (2019)
	MsDef1 MtDef4	*Medicago sativa*	A conserved region of two antiparallel beta strands with an interposed loop containing a cationic charge that participates in one to four disulfide bonds. The net charge for MsDef1 is +3 and + 6 for MtDef4	Antifungal activity	Antifungal activity against a common wheat and barley pathogen (*Fusarium graminearum*)	Sagaram et al. (2011)
Thionins	Tu-AMP 1 Tu-AMP 2	*Tulipa gesneriana*	Both peptides are rich in cysteine and basic amino acids (arginine and lysine). Tu-AMP 1 is a single-chain peptide which contains four disulfide bonds. Tu-AMP 2 had a unique structure; it was a heterodimer consisting of α-chain and β-chain	Antifungal and antibacterial activity	Antifungal and antimicrobial activity against plant pathogens (e.g. *Fusarium oxysporum* and *Curtobacterium flaccumfacien*) known to cause spoilage of important food crops (potatoes, tomatoes, legumes)	Fujimura et al. (2004)
	Wheat β-Purothionins	*Triticum aestivum*	8 cysteine and 10 basic residues are distributed throughout the peptide. 4 disulfide bonds are present	Antibacterial activity	Antibacterial activity against common food pathogens that cause major spoilage in commercially significant plants such as tomatoes and peppers (*Corynebacterium michiganense*, *Xanthomonas campestris*)	(Fernandez de Caleya et al., 1972; Mak & Jones, 1976)
	Thionin 2.4	*Arabidopsis thaliana*	A 5 kDa peptide with 3 disulfide bonds present within its sequence. It contains a net charge of +1	Antifungal activity	Activity against the destructive crop fungal pathogen *F. graminearum* (targeting barley, wheat and corn crops)	Asano et al. (2013)
Snakin/ GASA	St-SN1	*Solanum tuberosum*	St-SN1 is highly basic and has a short, central hydrophobic stretch. It contains a net charge of +9	Antifungal, antiyeast and antibacterial activity	Antipathogenic properties of the Snakin peptide revealed the potential to inhibit growth of potato and wine grape pathogens. The synthetic form of St-SN1 reduced yeast spoilage in various beverages. *Listeria monocytogenes*, a major pathogen in ready-to-eat foods was also found sensitive to this peptide	(López-Solanilla et al., 2003; Segura et al., 1999; Shwaiki et al., 2020c)
	MsSN1	*Medicago sativa*	MsSN1 contains 12 cysteine residues within a conserved C-terminal region	Antifungal and antibacterial activity	Antifungal activity of this peptide reduced the growth of pathogenic fungal species effecting the alfalfa crop (*Phytophthora medicaginis*). The same fungal specie is also a common pathogen of the chickpea and lentil plant	García et al. (2014)
	Snakin-Z	*Ziziphus jujuba*	A 31 amino acid peptide with a molecular weight of 3.318 kDa	Antifungal and antibacterial activity	Strong antibacterial action against the pathogen *Staphylococcus aureus* that can be transmitted through contaminated/ spoiled food	Daneshmand et al. (2013)
Cyclotides	Cycloviolacin O2	*Viola odorata*	Peptides consists of a circular backbone and 3 disulfide bridges which are arranged in a knotted fashion. Peptides contains net charge of +2	Antifungal and antibacterial activity	Antipathogenic properties against common food spoilers (*Salmonella, E. coli)* and antifungal activity against fungal pathogens known to target rice and legume crops	(Pränting et al., 2010; Zarrabi et al., 2013)
	Cycloviolacin O8			Antifungal activity	Antifungal activity against a common wheat and barley pathogen (*F. graminearum*)	Parsley et al. (2018)
Knottin-type	PAFP-S	*Phytolacca americana*	Cationic and highly Basic peptide containing three disulfide bridges in its structure (6 cysteine residues responsible for	Antifungal activity	Common crop pathogens of legumes, rice and barley (*F. oxysporum* and *Pyricularia oryzae*) are susceptible to the peptide's strong antifungal activity	Shao et al. (1999)

(*continued on next page*)

Table 1 (*continued*)

Type of AMP	Name of AMP	Source	Chemical and structural Properties	Antimicrobial Properties	Potential in food applications	Reference
			disulfide bridges). Contains a net charge of +4			
	Mj-AMP1 Mj-AMP2	*Mirabilis jalapa*	Peptides are highly basic and contain 3 disulfide bridges within their structures. Both peptides contain a net charge of +3	Antifungal activity	Major pathogenic fungal species (*F. oxysporum)* known to target agriculturally significant crop such as rice were sensitive to the antifungal properties of these knottin-type peptides	Cammue et al. (1992)
Hevein-type	Ee-CBP	*Euonymus europaeus*	Peptide contains 5 disulfide bridges (linked by 10 cysteine residues present in its structure) and a net charge of +5	Antifungal activity	Strong antifungal properties against *F. culmorum* and *Alternaria brassicicola*, pathogens of wheat and cabbage crops	Van Den Bergh et al. (2002)
	SmAMP3	*Stellaria media*	A basic and cysteine-rich peptide containing a net charge of +2 and 3 disulphide bridges in its structure	Antifungal activity	The growth of fungal fruit (grapes and apricot) pathogen *Aspergillus niger*, and legume pathogen *Fusarium solani* were inhibited by the antifungal activity of the havein-type peptide	Rogozhin et al. (2015)
	EAFP1 EAFP2	*Eucommia ulmoides*	Both sequences contain 41 amino acid residues. Both contain 10 cysteines, cross-linked to form five disulfide bridges	Antifungal activity	Pathogenic fungal species that cause spoilage to tomato, wheat and potato crops (A*culops lycopersici)* are susceptible to the antifungal activity of both peptides	Huang et al. (2002)
Lipid Transfer Protein (LTP)	Ha-AP10	*Helianthus annuus*	A basic 10 kDa peptide containing 4 disulfide bridges	Antifungal activity	Fungal pathogen *Fusarium solani* that effects major economically significant food crops was susceptible to the antifungal properties of the peptide	Regente and De La Canal (2000)
	Ca-LTP1	*Capsicum annuum*	A 9 kDa peptide with 4 α-helices within its structure	Antifungal activity	The growth of the common bean plant pathogen *Colletotrichum lindemunthianum* was inhibited by the peptide's antifungal activity	Diz et al. (2011)
	Mung bean nsLTP	*Phaseolus mungo*	Peptide consists of four α-helices stabilized by four disulfide bonds and a net charge of +7	Antifungal and antibacterial activity	Peptide's antifungal activity resulted in strong inhibitory effects against major crop fungal pathogens (*F. solani, Fusarium oxysporum).* Antibacterial action against *S. aureus,* a major spoiler and contaminant of foods such as meat and dairy products	(Lin et al., 2005; S. Y.; Wang et al., 2004)
2S albumin proteins	Pe-AFP1	*Passiflora edulis*	The peptide consists of 2 disulfide bonds in its sequence formed by 2 conserved cysteine residues	Antifungal and antiyeast activity	The peptide was successful in causing inhibition to the fungal pathogen *F. oxysporum*, a spoiler of agriculturally significant crops	Pelegrini et al. (2006)
	CW-1	*Malva parviflora*	A 15 amino acid peptide with a molecular weight of 1.783 kDa	Antifungal activity	Antifungal activity against a common grain crop pathogen *Fusarium graminearum*	(X. Wang & Bunkers, 2000)

The contributing factors for their growth in food are dependent on the type of substate; with particular focus on the water, sugar, salt, oxygen and nutrient content (Petruzzi et al., 2017). Just like pathogenic spoilage bacteria (that can cause disease and the spoilage of food), fungal spoilage pathogens (yeast and moulds) are also likely to grow and cause outbreaks. The production of mycotoxins by some fungal species can result in the contamination of major crops such as maize (aflatoxins, fumonisins and zearalenone mycotoxins (Tarazona et al., 2020)), rice (aflatoxin, citrinin and deoxynivalenol mycotoxins (Tanaka et al., 2007)) and wheat (deoxynivalenol and zearalenone mycotoxins (Tibola et al., 2015)). These toxins can go on to cause food-borne illnesses (Benedict et al., 2016). Food spoilage by fungal pathogens is nevertheless a factor that contributes to the global food waste and food loss concern and should be regarded as part of the problem.

Although foodborne illnesses are a devastating result of pathogenic spoilage microorganisms, the loss of food at different stages of production through to consumption due to spoilage can be economically destructive. The exact figure for the total economic loss caused by food spoilage is unknown, however it is estimated that 25% of food produced globally is lost due to spoilage microorganisms (Bondi et al., 2014).

The use of food preservation techniques, in the form of temperature control, food additives (chemical and natural), modified packaging, thermal and ionisation processes are all applied with the aim to reduce spoilage (Bhat et al., 2012). However, the loss of food in this way continues to be a problem. Consumer perceptions of food and their best-by date increases the challenges faced in the battle to reduce food loss. Consumers begin to pass judgment on the consumability of a food product that may be safe for consumption due to the 'best-by' or 'best-before' dates. In addition, consumer behaviour related to the amount of food purchased and the sale of increasing large serving or pack sizes for products, has led to an ever increasing amount of food being discarded, either because it was not consumed before it deteriorated or because it was simply forgotten about; such behaviour further contributes to food loss. In the EU, it was reported that household waste was one of the highest contributor to food waste with approximately 47 million tonnes ±4 million tonnes reported in 2012 (Stenmarck et al., 2016).

Increasing preservative levels in foods may, in theory, be a way to tackle this problem. However, additives like chemical preservatives that are linked to increasing shelf-life are perceived as negative by consumers; increasingly there is a demand for different and more natural forms of preservatives (Bedale et al., 2016).

Antimicrobial peptides (AMPs) are compounds found in animals, plants and even microorganisms, that function to protect against microbial pathogens. In plants, this mechanism is crucial for survival. (Adem Bahar & Ren, 2013; Nawrot et al., 2014). Plant AMPs have been researched for their potential to inhibit different pathogens, including food spoilage pathogens, from bacterial to mould and yeast species. A

new and increasingly recognised avenue for the study of plant AMPs for such applications is their synthesis through various approaches. This review will look at these novel approaches and at the methods employed for the design of plant AMPs and their functional prediction; furthermore, the status of these novel AMPs in the future of food preservation is discussed. The focus of this review is to develop an understanding of plant AMPs and how they can be exploited for their antimicrobial activity for the prevention of food loss and food waste. AMPs from other sources (e.g, animals and humans) will also be considered in areas where their knowledge can be applied for plant AMPs.

2. Plant antimicrobial peptides (AMPs)

Plants are stationary entities whose ability to photosynthesise and produce the oxygen that we need to survive make them one of the most remarkable organisms on earth. Nevertheless, they can find themselves vulnerable to environmental factors, herbivores, and like other organisms, to microbial (bacteria and fungi) infections. Plants have evolved and adapt to such stresses by developing physical or chemical defences. Physical traits may include the production of thorns or spines to deter herbivores, or the production of bark or waxy cuticles to deter microorganisms; while chemical defences are within the plant system (Moles et al., 2013). Amongst these is the production of AMPs, whose synthesis occurs in nearly all parts of a plant during any stage of its life (Campos et al., 2018). For example, the seeds of the radish plant continuously produce AMPs belonging to the defensin family. During seed germination, the production of these defensins are significantly increased to aid in protecting the seedlings against pathogens (Stotz et al., 2009). Just like defensins, there are many other AMPs that plants can produce; the major ones belong to the families of thionins, cyclotides, snakin, hevein (and hevein-type peptides), knottin (and knottin type peptides), lipid transfer proteins (LTP) and 2S albumin proteins (Nawrot et al., 2014). These AMPs have been the focus of essential research to understand their relationship and interaction with plant pathogens (Benko-Iseppon et al., 2010), food spoilage pathogens (Hintz et al., 2015) and even human health (De Souza Cândido et al., 2014).

AMPs from different families have been studied for their effect against plant pathogens and food spoilage pathogens with the aim to reduce spoilage (and ultimately loss) throughout the food chain. Since the discovery of these AMPs, most research has been performed to investigate the microbial inhibition potential of many of these peptides. Plant pathogens such as *Pythium graminicola, Botrytis cinerea, Verticillium dahlia* and *Aspergillus niger* are significant crop spoilage organisms, which have been successfully inhibited by AMPs extracted from rice (thionin) (Ji et al., 2015), potato tubers (snakins) (Segura et al., 1999), amaranth (hevein-type peptide) (Broekaert et al., 1992) and peas (defensins) (Almeida et al., 2000), respectively. A thionin peptide produced by *Arabidopsis thaliana* was found to be inhibitory against common food spoilage pathogens *Staphylococcus aureus* and *Escherichia coli* (Loeza-Ángeles et al., 2008), while *Klebsiella pneumoniae* was inhibited by the presence of a cyclotide peptide from the plant *Viola odorata* (Pränting et al., 2010). Table 1 gives a list of peptides from various families of plant AMPs. Their structure, source, and their antimicrobial properties against common food/crop spoilage pathogens are included in this table.

These AMPs can be extracted from plants using different extraction and purification methods and variations of these methods have also proven successful. One approach for the purification of a plant peptide is to perform an extraction procedure using the plant of interest and a buffer of a specific pH. This solution can undergo several centrifugation and precipitation (ammonium sulfate) stages. Ion exchange chromatography is used to separate the target peptide by charge (anion and cation exchange chromatography) and an extra chromatography step can be applied (reverse-phase high performance liquid chromatography (RP-HPLC)) for its further purification. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) is performed to approximate its molecular weight and an amino acid analysis can determine a portion or the majority amino acid sequence of the isolated peptide. Its potential name and origin can be further assessed using a protein BLAST search on an online database (Tang et al., 2018). This approach of natural extraction can be time consuming and the yield concentration of peptide is typically low, therefore a large-scale extraction procedure is required. In addition to this, AMPs extracted and purified from a plant host can display antimicrobial activity at higher concentrations compared to their synthetic form (i.e. they can contain lower antimicrobial properties than the synthetic form). Some studies have shown higher antimicrobial activity by the synthetic version of an AMP that has been originally purified from the host plant. A prime example of this is the synthetic linear analogue of the native Cp-thionin II peptide from the seeds of the cowpea plant. Minimum inhibitory concentrations (MIC) of 20 and 200 μg/mL were observed against the food spoilage fungi *Fusarium culmorum* and *Penicillium expansum,* respectively (Thery & Arendt, 2018). This synthetic AMP resulted in more potent antimicrobial activity compared to the AMP from which its sequence was synthesised from. The extracted and purified peptide from the seeds of the cowpea plant displayed higher MIC values against the same fungal spoilers in the study by Schmidt et al., 2019. MICs of 50 μg/mL against *F. culmorum* and >500 μg/mL against *Penicillium expansum* were observed, both levels higher than what the synthetic cowpea peptide required to cause inhibition. These studies demonstrate the lower antimicrobial properties that naturally extracted AMPs can have compared to their synthetic counterparts.

The synthesis of AMPs via various chemical processes are being employed as an alternative method to produce AMPs such as those mentioned above, but also more potent derivatives, or even totally novel peptides, based on best 'design' and structural and functional characteristics of the most potent natural plant AMPs. These methods can be exploited to increase the safety of food as well as reduce food waste, as their directed design could provide a potency and stability surpassing that of naturally extracted AMPs from plants.

3. Synthetic AMPs from plants

3.1. Approaches to develop synthetic peptides

Plant AMPs have been successfully extracted and purified from different sources and their antimicrobial activity has been characterised. The chemical synthesis of AMPs is being recognised as an alternative to natural extraction for its potential in reducing production time. An entire plant peptide sequence may be analysed for the synthetic development of novel peptides with even more potent antimicrobial activities. This has been explored through the natural cleavage or *in-vitro* proteolysis of AMPs to acquire short natural fragments with improved characteristics (Thery et al., 2019). Although not well studied in plant AMPs, this method of peptide production has been performed on the human milk protein, Lactoferrin, producing analogs (Lf (1–11) and Lfcin H17–31) with positive antifungal activity against the human fungal pathogens *Aspergillus fumigatus* and *Candida albicans,* respectively (Fernandes & Carter, 2017; Håversen et al., 2010). Another study that explored this approach looked at the analogs developed from the amphibian cationic α-helical peptide Temporin-SHf. These peptide analogs showed antimicrobial and antifungal activity against various Gram positive and Gram negative bacteria, and yeasts, including *Staphylococcus aureus, Escherichia coli,* and *Candida albicans.* In addition to this, at antimicrobial concentrations, cytotoxic effects were not observed (André et al., 2015). Peptides obtained via this approach not only display improved activity, but also reduced cytotoxicity to human cells, increasing their value as they are both potent and safe.

A different approach for the generation of synthetic peptides is through *de novo* synthesis using combinatorial libraries. This method bases the assembly of new peptides on already established AMPs that may have strong antimicrobial properties but encompass negative

characteristics such as cytotoxicity to human cells. Such peptides can be modified to fit desired characteristics for safety and improve their potential for future applications. The Antimicrobial Peptide Database (APD_3) (G. Wang et al., 2015) is amongst one of the numerous databases available. The input of sequences into these databases calculates the hydrophobicity, structure, total net charge – amongst other properties – of a peptide, as well as correlate its sequence to known AMPs already on the database. This method has proved successful in generating potent peptides and will be discussed in more detail in the section that explores predicting peptide activity. In one particular study, three *de novo* cationic peptides were designed and found to exhibit strong activity against several pathogenic spoilage yeast and fungal species, including *C. albicans,* and *Aspergillus* and *Fusarium* species (Maurya et al., 2013). This ability to modify peptide sequences and alter their properties is valuable for the production of more stable and active peptides. A study by Chu et al., 2013 found the substitution of tryptophan or histidine residues with D-β-naphthylalanines resulted in a peptide that was less sensitive to high salt concentrations. This resistance to high salt concentrations signifies the peptide's stability. Its ability to retain its antimicrobial activity in a high salt solution denotes the peptide's potent antimicrobial properties. A peptide's ability to resist high salt concentrations is a good indicator of its potential as a food preservative. The salt concentrations encountered in various food products can cause the deactivation of AMPs, therefore a higher resistance to such salt concentrations is optimal for their potential application as food preservatives.

These studies highlight what can be achieved through the modification of known peptide sequences. Whether through natural cleavage, *in vitro* proteolysis, or the generation of a novel peptide based on already established peptides, plant AMPs can be used to generate more potent, stable peptides that can be further exploited for their use in food preservation.

3.2. Production of synthetic peptides

Following the design of a peptide where its functional properties will be maximised through targeted design and consideration of its sequence, it will be synthesised (Fig. 2 summarises the various methods of peptide synthesis). Chemical synthesis is preferred over the biological synthesis of peptides due to the possibility of incorporating non-natural components into a sequence (for example alpha, beta-didehydrophenylalanine) (da Costa et al., 2015; Maurya et al., 2013). Solid-phase peptide synthesis (SPPS) and solution-phase synthesis (SPS) are commonly practised forms of chemical synthesis employed in the production of peptides. SPPS was introduced after the development of SPS for its simplicity, efficacy, and compatibility with automated synthesis. This made it possible to produce and develop peptides on a much larger scale than SPS, whose long and laborious production process was a disadvantage for clinical use. Hybrid synthesis is a combination of the two methods, combining the benefits and advantages of both procedures (Goodwin et al., 2012). To better understand these methods and the processes involved, the articles by Raibaut et al., 2015 and Tsuda & Okada, 2011 explore, in more depth, the steps required for peptide production using these techniques.

Biological synthesis refers to the use of recombinant organisms for the production of peptides or proteins. The early 1980s brought about the production of insulin using this type recombinant technology. Two small genes containing nucleotide sequences coding for human insulin was inserted into *E. coli* cells, allowing for the production of large quantities of the peptide (Riggs, 1981). This discovery made it possible for human insulin to be manufactured for large scale therapeutic use. The production of AMPs by recombinant methods has since been recognised as an efficient system to generate larger peptides – such as defensins, that typically contain 30-50 amino acid residues (in comparison to the peptides discussed already, which may have as little as 11 residues in their sequences). Although proven effective, some disadvantages exist such as low production yield and the necessity to overcome the potential toxic effect of the peptide on the host microorganism. In addition, the introduction of non-biological components cannot be accomplished using this method. To overcome this, these AMPs may be fused with other proteins such as thioredoxin, which not only prevents cell lysis but also aids in the formation of disulfide bonds. Various cell vectors have been used for this function, including the most well-known, *E. coli*, the fungal species *Aspergillus*, yeast cells belonging to *Saccharomyces cerevisiae,* and even chloroplast expression systems (Matejuk et al., 2010). Potato AMPs, Snakin-1 (sn1) and Defensin-1 (pth1) were successfully generated through biological synthesis via expression in *E. coli* cells. The peptides were found to be inhibitory against bacterial and fungal potato pathogens including *Clavibacter michiganensis* subsp. *sepedonicus* and *Colletotrichum coccoides,* and the wine grape pathogen *Botrytis cinerea* (Kovalskaya & Hammond, 2009). A separate study found similar results when expressing genes for the AMP Pg-AMP1, found in the seeds of the guava (*Psidium guajava*) plant, into *E. coli* cells. This AMP inhibited various gram-negative and gram-positive bacterial food spoilage pathogens (Tavares et al., 2012). The production of synthetic AMPs by this cell vector method further demonstrates the potential of synthesising such peptides to reduce the growth of food spoilage pathogens in order to develop them further for their use as potential food preservatives.

A different method for obtaining peptides from plants is through the process of enzymatic hydrolysis. During this process, the use of one or more enzymes are applied to hydrolyse a specific protein (in this case, a plant protein) to produce short peptide sequences in the form of hydrolysates (Daliri et al., 2017). A study by Aguilar-Toalá et al., 2020 gives insights into this method to produce hydrolysates derived from the seeds of the *Salvia hispanica* plant. The study successfully generated peptides with antimicrobial activity against food spoilage microorganisms *Escherichia coli* and *Listeria monocytogenes*. They accomplished this through the hydrolysing actions of enzymes alcalase and flavourzyme on the proteins present in the seeds of the plant. A separate study by Sornwatana et al., 2013 used a similar approach to generate antibacterial peptides from the fruit protein of *Fructus Bruceae* by peptin hydrolysis. This method is often preferred over the process of employing microorganisms to perform this hydrolysing mechanism. This approach (referred to as microbial fermentation) involves hydrolysing a target protein through the action of enzymes that a microorganism releases as it grows. This aids the proteins to break down into peptides that can be screened for their antimicrobial activities. In addition to the short reaction time, the ease of scalability and predictability, the enzymatic hydrolysis approach renders it the preferred method over microbial fermentation (Daliri et al., 2017).

These approaches (summarised in Fig. 2) have been proven effective for the synthetic production of AMPs. Although some of these methods may have drawbacks, the design of potent peptides with optimal characteristics can be taken one step further. Modifying a peptide sequence can lead to the improvement of its antimicrobial activity, stability, and cytotoxic properties (safety).

3.3. Designing synthetic AMPs

For AMPs to be considered for food applications, it is crucial to identify any negative effect that they may have on human cells. A peptide's toxic effect, as well as its haemolytic activity and susceptibility to proteolysis are important factors that need to be considered when applying AMPs in foods that will be consumed. In AMPs from natural plant sources, these factors can pose a challenge. However, in synthetic assembly, a peptide may be designed in such a way that can exclude these negative effects as much as possible. Designing AMPs is a sensitive process as one modification, while positive from the point of reducing cytotoxicity and increasing safety, may have a detrimental effect on the overall antimicrobial characteristics and potency of the peptide. Factors including cationicity, hydrophobicity, amphipathicity, secondary

Table 2
Factors to consider when designing synthetic AMPs.

Designing synthetic antimicrobial peptides			
Factors to consider	Summary	Examples	Reference
Cationity & distribution of residues	Positive charged AMPs facilitate the interaction with microbial membrane. Lys, Arg, and His strongly interact with membrane lipid layer, enabling strong antimicrobial activity. An AMP incorporating too many Arg residues can cause the haemolytic activity to rise. Insertion of Arg or Lys in the hydrophobic face of a helix peptide can reduce haemolysis.	Addition of Arg residues in the temporin-Shf analogs increased activity against *Saccharomyces cerevisiae* – MIC as low as 12.5 μM was observed for some analogs.	André et al. (2015)
Hydrophobicity	Hydrophobic residues can facilitate insertion into microbial membrane. Increasing hydrophobicity above a certain point can result in loss of activity and increase in toxicity.	Single amino acid substitution increased hydrophobicity of HPRP-A1 analogs. Good antimicrobial and antifungal activity were observed, however accompanied by strong haemolytic activity (>120% difference in haemolysis between HPRP-A1 and some of its analogs with similar activity).	Zhao et al. (2013)
Amphipathicity	A peptide that contains both hydrophobic and hydrophilic residues in its sequence. An amphipathic peptide can have strong antimicrobial activity, however above a certain threshold, it can have haemolytic/toxic effects.	A significant increase in the affinity to red blood cells was observed in a modified peptide (with the same hydrophobicity to the parent peptide) consisting of Tyr and Leu instead of two Lys residues. Haemolysis at 55 μM increased from 8 ± 2 (parent peptide) to 85 ± 12 (modified peptide).	Hollmann et al. (2016)
Length	A peptide must be of at least 7–8 amino acid residues in length for good amphipathic structure to develop. Shorter AMPs are known for their strong antimicrobial activity and reduced toxicity/haemolysis and salt sensitivity.	The 18-mer peptide (RI18) derived from the 36-residue parent peptide (PMAP-36) showed strong inhibitory activity – 99.9% clearance – against *E. coli* in the presence of 25 and 50% serum.	Lyu et al. (2016)

structure formation (for example α-helical and β-sheet) and sequence length all contribute to the formation of a stable peptide with potent activity (Thery et al., 2019). Table 2 summarises these different factors and their significance for the generation of a synthetic AMP.

3.3.1. Cationicity and distribution of residues

The activity of AMPs against microorganisms is a consequence of a peptide's overall positive net charge. The cationicity of a peptide refers to its positive charge that allows it to interact with negatively charged microbial membranes. The potency of a peptide can be explained by its interaction with negatively charged biological membranes. Basic amino acid residues arginine (Arg) and lysine (Lys) cause initial interaction with the anionic surface membrane, facilitating the residues' insertion into the membrane lipid bilayer (Thery et al., 2019). Furthermore, these positively charged residues can participate in covalent and non-covalent interactions with other residues of the membrane bilayer (such as valine (Val), tryptophan (Trp) leucine (Leu), alanine (Ala), tyrosine (Tyr) and phenylalanine (Phe)) (Li et al., 2013). The incorporation of Arg residues has been found to generate stronger antimicrobial activity than Lys; however, a downside to this is the high haemolytic activity that accompanies it. The stable interactions observed between the side chains in an Arg residue and those found in the membrane bilayer can make it easier for it to navigate past the hydrophobic membrane core of microorganisms (Schibli et al., 2006). This was observed in the study by André et al., 2015, that found that the addition of Arg residues in the temporin-Shf analogs increased their activity against yeast spoilage strain of *Saccharomyces cerevisiae* (spoiler of alcoholic beverages).

The distribution or location of these residues in a sequence can determine activity or potency. Lowering the haemolytic activity of an α-helical peptide was found to be promoted via the insertion of Arg or Lys residues in the hydrophobic face of the helix – causing a decrease in the hydrophobic moment (measure of amphipathicity) and ultimately in the haemolytic activity of the peptide (Jin et al., 2016). Furthermore, the amino acid residue histidine (His) can be used as an alternative to Arg and Lys, due to the lower isoelectric point and its net charge increasing under acidic conditions. If the amphipathicity of a peptide is negatively affected by the presence of an Arg or Lys residue, the more neutral His residue can be used instead (Sharma et al., 2010).

3.3.2. Hydrophobicity

Hydrophobic residues are just as important in a peptide sequence. The hydrophobicity of a peptide defines the overall charge distribution as non-polar and refers to the presence of hydrophobic residues in a sequence. The nature of the side chains found on amino acids determine their hydrophobicity (glycine (Gly), valine (Val), and proline (Pro) are examples of such residues). The binding of peptides to the membranes of microorganisms is facilitated by these residues, making them crucial for activity. Increasing the hydrophobicity of peptides can reduce their sensitivity to high salt conditions which can result in the loss of membrane-disrupting effects (Saravanan et al., 2014). However, the hydrophobicity of a peptide can only be moderately increased before losing selectivity and potency and increasing its cytotoxic effects (Ong et al., 2014). This is due to the capacity of peptides with high hydrophobicity to readily enter the inner mammalian bilayer and cause haemolysis. Zhao et al., 2013 demonstrated this in their study that observed the effect of single amino acid substitution on the hydrophobicity of the bacterial (*Helicobacter pylori*) peptide HPRP-A1. The study found these analogs to exert good antimicrobial and antifungal activity, but also developed strong haemolytic activity. This corroborates the assumption that hydrophobic characters in a peptide sequence may be linked to high haemolytic activity due to the high hydrophobicity of a peptide enabling interaction with various types of lipid bilayers. It is therefore important to understand the balance of hydrophobic residues in a peptide sequence to reduce the likelihood of toxic effects towards mammalian cells.

3.3.3. Amphipathicity

Amphipathicity is the state in which a peptide contains both hydrophilic and hydrophobic residues. This attribute permits the hydrophobic elements of a peptide to further permeate microbial membranes after initial attachment to the phospholipid head groups. It does this through its insertion and interaction with the phospholipids present (Takahashi et al., 2010). The hydrophobic moment is used to measure the amphipathicity of a peptide in an alpha-helical conformation. The

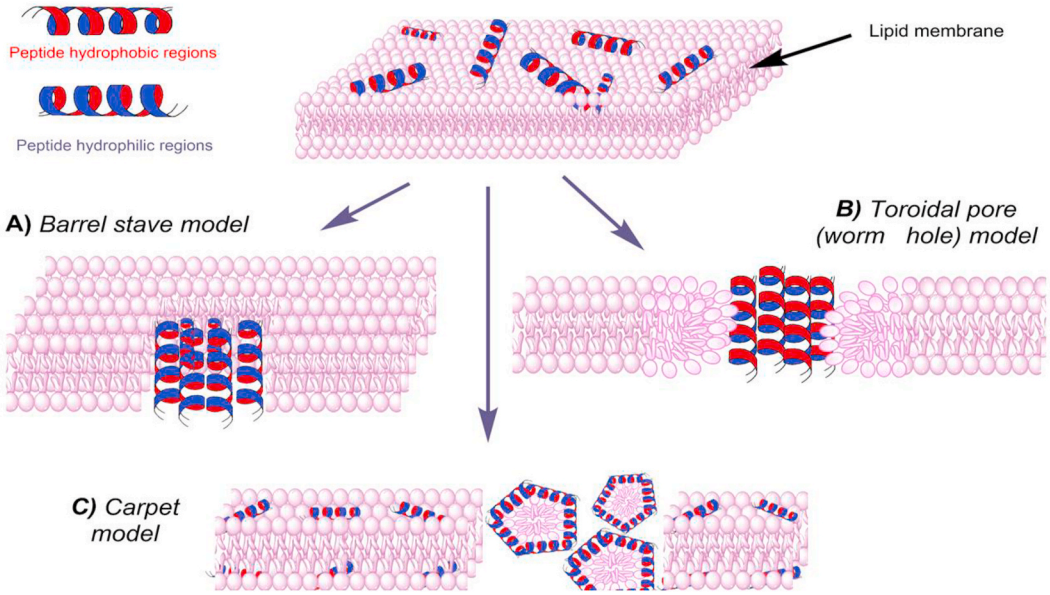


Fig. 1. Illustration of the mechanism of action of AMPs through the formation of pores and the disruption of the membrane. The barrel-stave model (A) and the toroidal pore (worm hole) model (B) signify a peptide's pore forming abilities that can result in the further disruption of a cell's normal function. The carpet model (C) is illustrative of the disruptive mechanism that AMPs can act on to form micelles from the membrane structure.

amphipathicity of a peptide is typically associated with helix motifs; as such hydrophobic and hydrophilic residues are located between the opposite faces of a helix, resulting in a fitting distribution for membrane binding (Drin & Antonny, 2010). Alpha helical peptides are one of the most encountered peptides in research, due to their reputation as highly potent AMPs. Alpha-purothionin from wheat (Han et al., 1996) and Ah-AMP1 from horse chestnut (Fant et al., 1999) both contain helical structure in their overall form.

Similar to the property of hydrophobicity, studies have demonstrated that modulating the amphipathicity can result in different outcomes with respect to antimicrobial activity and cytotoxic effects of a peptide. Hollmann et al., 2016 demonstrated this when cationic peptides based on a previously studied AMP were designed and modified to have the same hydrophobicity and different hydrophobic moments. A significant increase in the affinity to red blood cells was observed in the modified peptide consisting of Tyr and Leu instead of two Lys residues, due to the presence of a higher hydrophobic moment. This higher hydrophobic moment caused the peptide to become highly amphipathic and develop a secure helical structure, thus explaining the increase in the haemolytic activity.

A similar outcome was observed by Zhang et al., 2016, where a dramatic decrease in the amphipathicity of their peptide resulted in lower haemolytic effects for some of the analogs originated, while also preserving good antimicrobial activity. The minimum inhibitory concentration (MIC) for the majority of bacterial species tested was double that of the original, native peptide; 12.5 μM vs 6.25 μM. Although the activity was not fully preserved, the resulting activity is still deemed effective. The slight increase in the MIC was a resulting consequence for the lower haemolytic activity. This was accomplished through the substitution of residues on the non-polar face of the peptide, resulting in the lower haemolytic effect. This effect has been suggested to be due to the disruption of the alpha-helical amphipathic structure that is linked to enhanced haemolytic activity.

3.3.4. Length

As observed with the cationicity, hydrophobicity and amphipathicity, the length of a peptide bares great importance on its activity. The connection between an amphipathic peptide and its length is ensured through the presence of at least 7–8 amino acids residues in the sequence. This is also true when considering the structure of a peptide. For example, an effective alpha-helical peptide requires at least 22 amino acids to traverse the lipid bilayer, while a beta-sheet requires at least 8 amino acids (Adem Bahar & Ren, 2013). Shortening a peptide sequence can, however, result in the increase in activity and reduction in toxicity, haemolysis and in some cases, salt/serum sensitivity. In a study by Mohanram & Bhattacharjya, 2016, various 12-residue cationic, amphipathic *de novo* peptides were designed and tested for their bactericidal activity in both a serum and salt solution, with their activity being retained. Likewise, shorter analogs of the porcine cathelicidin peptide (PMAP-36) were designed into an 18-mer peptide (RI18) with better fungicidal activity and salt resistance (Lyu et al., 2016). This further supports the concept of employing shorter antimicrobial peptides for increased activity. In addition, shortening the length of a peptide reduces production costs, making it more economically viable to manufacture.

The effect of each of the discussed parameters or properties on their own is not well established, as the activity and selectivity of peptides are not dependant on one factor alone; instead, they are all dependant on one another. The studies mentioned highlight the necessity for a better understanding of the structure-function relationship of peptides, and how modifying a sequence for enhanced activity may be accompanied by increased negative effects. A balance between good antimicrobial activity and reduced cytotoxicity is therefore critical for the development of these peptides. However, the more that is understood about relationship and interdependency of the different parameters in a peptide, the more efficient it can be to the development and synthesis of effective, enhanced and safe peptides. Adopting an integrated thinking on peptide design could be revolutionary in designing synthetic AMPs that are potent, safe and economical to combat the effects of crop and food spoilage pathogens right across the food chain.

3.4. Mechanism of action

3.4.1. Membrane interactions

A cationic AMP can exert its antimicrobial activity through membrane interactions that result in the disruption of the membrane and eventual death of a cell. Unlike conventional antibiotics, AMPs act rapidly. What AMPs can do in a matter of minutes, antibiotics may take hours to accomplish (Fantner et al., 2010). This fast response is not only a result of membrane interaction with peptides, but it is also the effect of

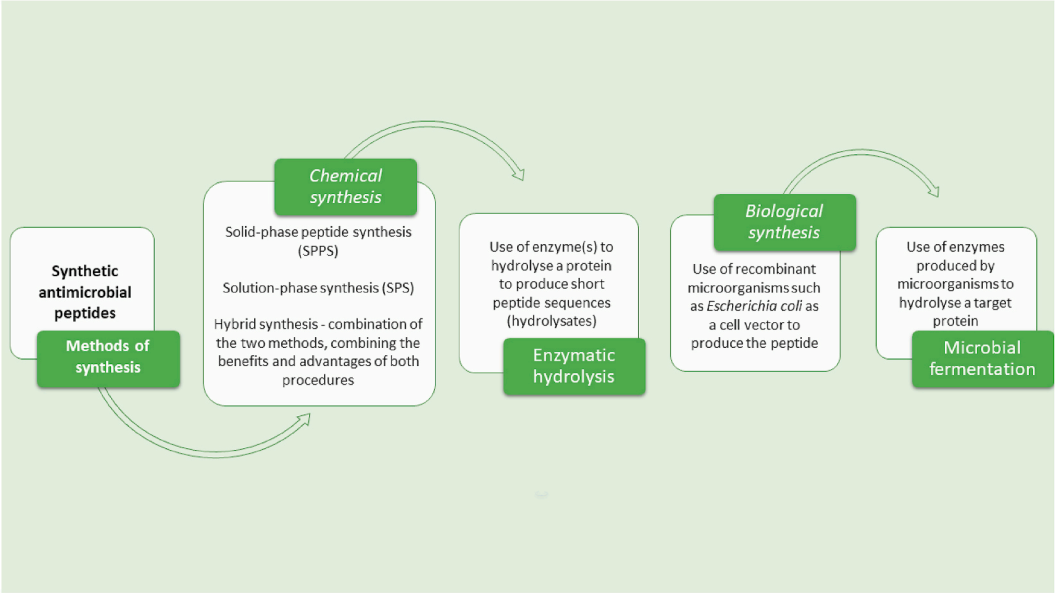


Fig. 2. A summary of the different approaches that peptides can be synthesised through chemical and biological synthesis.

their interference with metabolic activities and cytoplasmic components through the interference of various signalling processes such as wound repair, chemotaxis or cell migration (Splith & Neundorf, 2011).

Plant AMPs have been extensively studied for their mechanism of action that targets the cell membranes of microorganisms (Shwaiki et al., 2020c). This has led to different hypothetical model systems being proposed for their interaction with and effects on cells. 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. The manner by which this happens has been proposed through different mechanisms which can further be classified into different groups based on their primary mode of action; 1) 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. This causes the membrane to bend back on itself, causing the top and bottom monolayers to be continuous (Nawrot et al., 2014). Some AMPs act via non-specific membrane permeabilisation (membrane disruption), one of which is called the carpet model. It gets its name from the way that the peptide begins to cover the surface of the membrane once it has reached a critical concentration, in a "carpet-like" fashion. 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 (Lee et al., 2015). Fig. 1 illustrates these 3 mechanisms.

3.4.2. *Intracellular mechanisms*

As discussed above, at high concentrations plant 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 permeabilisation can lead to the leakage of potassium ions from the cell, leading to cell death (Kim & Lee, 2019; Shwaiki et al., 2020b).

Reactive oxygen species (ROS) are potent oxygen-containing molecules that can lead to the oxidative stress of a cell if present in excess. Plant AMPs have been found to induce apoptosis via the accumulation of these ROS (Oyinloye et al., 2015). Microorganisms are reliant on these species for various processes including their metabolism and stress response. However, 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. Superoxide anions can be converted to hydrogen peroxide via the mitochondrial respiratory chain of a cell. This ROS can then react to form the reactive and more potent hydroxyl radical, whose presence will subsequently lead to the induction of apoptosis (Cho & Lee, 2011). AMPs can promote the generation of these ROS, leading to such events (Oyinloye et al., 2015).

3.5. *Safety of AMPs*

The safety of plant AMPs is an important factor to consider for their application in food. Just like the therapeutic application of drugs, it is essential that AMPs in food applications are deemed safe for human consumption. The cationic nature of AMPs makes them attracted to the negatively charged membranes of microorganisms, making them the ideal antimicrobial agents. Due to this selectivity, AMPs are less likely to target human cells that are neutral or slightly positively charged. In addition, the component of a mammalian membrane bares differences to the membrane of microorganisms. An example of this is the higher content of cholesterol in human cell membrane, thus providing protection against AMPs. Nevertheless, weak hydrophobic interactions can occur, making cytotoxicity a potential risk (Chen et al., 2016).

In the synthetic production of peptides, the cytotoxic effects described can be minimised, through targeted design. Some ways that this can be achieved is through the disruption of alpha-helical structures or modification of the peptide backbone. The ability of a peptide to bind and form a helical configuration upon interaction with neutral membranes has been found to cause enhanced toxicity (Mihajlovic & Lazaridis, 2012; Zhang et al., 2016). Modification of the peptide backbone has resulted in improved biological properties of peptides, including reduced toxic effects. This has been accomplished through the

substitution of residues with un-natural amino acids, for example, the replacement of the naturally occurring L-amino acid for the unnatural D-form (Shah et al., 2020). Furthermore, this modification has been shown to improve sensitivity to proteases of such modified peptides (Manabe & Kawasaki, 2017). However, it is speculated that improved stability in the presence of proteases can also occur from such modifications. This could be due to the longer *in vivo* half-lives that peptides containing D-amino acids possess, in comparison to their natural counterparts (Wade et al., 1990). Using the full D-enantiomeric structure of a peptide by substituting all L-amino acids with their D-counterparts is known to create more resistant peptides to proteolytic digestion (Melchionna et al., 2016). Resistance to proteolytic digestion is important when considering plant AMPs for their application in foods, as a peptide must also be capable of being broken down by enzymatic activity in the gut, where its action is no longer desired.

For the systemic application of these AMPs, a low haemolytic activity against red blood cells must also be considered. Plant AMPs are known to rupture human erythrocytes, an indicator sign of cytotoxicity, making them unsuitable for food applications. As discussed in the previous section, the length, hydrophobicity and amphipathicity are all factors that can be considered when designing synthetic AMPs, for reducing the haemolytic (cytotoxic) activity of the peptide and increasing overall safety.

3.6. Predicting and modulating activity of novel AMPs

There are various methods that can facilitate the synthetic design of potent, stable, and safe AMPs. An effective peptide may be designed on the basis of a natural peptide or number of peptides that can be used as a template. By selecting the characteristics and properties of desirable peptides (as described in section 3.3), such derivative, chimeric peptides can be designed, incorporating the desirable characteristics of the original natural peptide(s), for greater antimicrobial activity, better stability, and reduced cytotoxicity. In addition, this can be achieved through single amino acid substitutions to help identify the significance of certain residues and understand their role in the peptide sequence and the link to the peptide properties (Fjell et al., 2012). This form of modification is known as post-translational modification (PTM), and can also involve disulfide bonds, C-terminal amidation, bromination, chlorination, or the presence of D-amino acids (Falanga et al., 2016). In the study by Carroll et al., 2010, an AMP derived from bacteria was used as the template for three enhanced peptides called nisin S, T and C. These derivatives were based on the parent peptide and through the incorporation of newly introduced residues, resulted in the enhancement of antimicrobial activity over the native peptide. Panteleev et al., 2015 used the template-based approach to reduce the cytotoxic activities of analogs from the AMP, arenicin. This was done by identifying the amino acid residues responsible for this toxicity and performing a single amino acid substitution with hydrophilic residues on the non-polar face of the peptide. This method of prediction relies heavily on the clear understanding of the structure and function of each residue present in the template peptide (as mentioned in section 3.3).

A different method to the template-design approach is the use of biophysical techniques to examine the structural features of AMPs as they interact with a target membrane. This is of importance as some peptides can adopt a different structure upon interaction or binding with a membrane, and understanding their structure and mode of interaction can be of benefit when developing AMPs (Avci et al., 2018).

Virtual screening is a more complete but also more complex approach for the prediction of peptide activity. Quantitative structure-activity relationships (QSAR) is a common technique based on molecular modelling of small AMPs. QSAR takes into account the numerous descriptors of a peptide, such as size, charge, hydrophobicity, etc., and generates an overview of the predicted biological activity of the peptide (Barley et al., 2018). Machine learning algorithms and statistical analysis techniques can also be used for this aim. Techniques such as logistic regression (Veltri et al., 2017), artificial neural networks (ANN) (Torrent et al., 2011), support vector machines (SVM) (Meher et al., 2017) and random forests (RF) (Bhadra et al., 2018) have all been exploited for the prediction of AMP activities. These approaches are being increasingly exploited for their high speed, low cost, and high efficiency. The relationship between the antimicrobial activity and the biochemical attributes of a peptide can be interpreted using these machine learning systems. Several AMP databases that adopt these techniques are available and contain an extensive list of AMPs. Some databases may focus on AMPs derived from specific sources, such as PhytAMP and PlantPepDB that focus on plant AMPs, or ones that deal with AMPs derived from all sources of organisms ($CAMPR_3$, APD3, DBAASP). These databases can be used as prediction tools and in the case of many of these, peptide sequence assembly and functionality are frequently based on machine learning algorithms. The CAMP database, for example, employs SVM, ANN and RF (Su et al., 2019).

This method of prediction is a powerful and efficient way of understanding and developing a potent, stable and safe peptide. Not only is it economically viable and time-efficient, but virtual screening of AMPs or potential AMPs can serve as a guiding tool for the time-consuming work that wet-researchers perform (in the laboratory, but also to the *in silico* research involved). Nevertheless, the complexity of these computational tools for the development of novel AMPs may be intimidating and sometimes difficult to interpret. This downside of virtual screening can result in researchers avoiding this approach and relying more on template-based or biophysical approaches.

3.7. Benefits and challenges of synthesising AMPs in the aim to reduce food waste

The virtual screening and chemical synthesis of AMPs are promising methods that can aid in the production of peptides with potent antimicrobial properties. The ability to modify a peptide to achieve better antimicrobial properties and improved stability makes them prospective candidates that can be applied to food to reduce spoilage. Exploiting these methods can therefore generate peptides with enhanced properties. A peptide with a greater tolerance to high salt concentrations generally found in foods, with greater sensitivity to proteolytic degradation to allow for their breakdown in the human gut, and reduced toxicity against human cells, can be developed. The knowledge required to develop an AMP with suitable characteristics for its application in food to reduce the growth of spoilage microorganisms can be acquired through the understanding of how AMPs can be generated and synthesised. The basis of these synthetic peptide sequences is derived from plants, a source from which AMPs are constantly being discovered. The vast number of peptides already known and the potential for more to be discovered is a benefit for the development of synthetic peptide sequences derived from this source.

Consumer perception of current preservation techniques, especially chemical preservatives, has increased the desire for more natural forms of preservatives in foods. The approach of synthesising peptides based on plant peptide sequences can be seen as a possible solution to this. Various studies have been successful in providing evidence for the inhibition of spoilage microorganisms with AMPs and, with future developments in peptide understanding, production and synthesis, the further reduction of food spoilage can be envisaged. All of the techniques and approaches presented in this review can be further developed and built upon for the production of effective peptides whose sequences, configuration and properties are based on natural plant AMPs.

The challenge that synthetic AMPs face in the application of food waste reduction is its cost of synthesis. The low market cost of current preservative agents (chemical preservatives such as sodium benzoate for example) gives these forms of preservatives the advantage. The cost of chemical synthesis processes is currently expensive, thus, the production of large quantities of peptide required for application could be currently cost prohibitive. Regardless of these challenges, as the technologies



Fig. 3. A SWOT analysis demonstrating the Strengths, Weaknesses, Opportunities and Threats of utilising synthetic AMPs derived from plants to reduce food spoilage and food waste.

improve and develop over time, the development of synthetic AMPs could be more fully exploited. The research reviewed here exemplifies their potential to be considered as a real solution to the growing problem of food waste. Fig. 3 illustrates a SWOT analysis demonstrating the Strengths, Weaknesses, Opportunities and Threats of utilising synthetic AMPs derived from plants to reduce food spoilage and food waste.

4. Summary and conclusion

Food loss and waste caused by pathogenic spoilage microorganisms has led to an increasing amount of food being lost globally. Although food preservation using traditional techniques has assisted in reducing the burden of food waste due to the growth of spoilage microorganisms, it is still an issue that presents a challenge. This review examined the use of plant AMPs as a possible solution to food waste, and more specifically their synthetic counterparts and derivatives. To fully exploit synthetic peptides, there needs to be an awareness on how to develop, design and predict such peptides and their antimicrobial activity. Developing an understanding of the structure-function relationship of a peptide sequence can serve as a starting point for the improvement of plant AMPs, whose inhibitory activity may be ideal but whose safety towards human cells may not be optimal. Generating synthetic AMPs has proven successful via natural and in-vitro proteolysis or even *de novo* synthesis using combinational libraries. Various methods have been adopted to predict the activity and functionality of novel AMPs. Virtual screening provides the most beneficial technique thus far for this aim, as it combines the use of machine learning with already well-established databases available. Both chemical and biological methods of peptide synthesis can be used; however, chemical production via SPPS is currently the more common method due to its time efficient, flexible and direct production processes.

As the purpose of these synthetic AMPs is for the prevention of the growth of spoilage microorganisms in food systems, their safety is of utmost importance. The probability of developing a peptide with cytotoxic effects can be avoided by generating a peptide with certain desirable sequence characteristics, as described in this review. It is therefore important to find a balance between the level of antimicrobial activity and the level of cytotoxicity that a peptide may demonstrate. However, this negative trait cannot be disregarded when discussing the drawbacks of the incorporation of, either natural or synthetic, AMPs into foods.

The research presented in this review illustrates the potential for the application of synthetic AMPs derived from plants to reduce food/crops spoilage. Synthetic AMPs are reviewed for their consideration as possible solutions to the continuous existence of food waste.

Acknowledgements

This work was supported by the Department of Agriculture, Food and the Marine (F.I.R.M.), Project Reference 15/F/731.

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