

Grain peptides and their role as antimicrobial agents, and their applications in food

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I. Abstract

Grains are a staple food in many cultures and play a crucial role in the human diet. They include whole grains such as rice, wheat, and oats, as well as legumes like beans and lentils. Grains are rich in proteins, dietary fiber, carbohydrates, vitamins (including B vitamins), and minerals (such as iron and magnesium). They also contain bioactive components that enhance health, promote digestion, regulate blood sugar, and lower cholesterol levels. Consuming grains can help prevent chronic diseases like heart disease, obesity, and diabetes, while also reducing inflammation and supporting a healthy weight by promoting a feeling of fullness. Research indicates that grains and legumes create an ideal environment for various microbes, including beneficial bacteria and fungi, which can enhance fiber digestion and produce helpful substances like probiotics. However, there is a risk of harmful microbes contaminating grains during storage or processing, making hygienic handling essential. Grains are widely used in food production for items such as bread, baked goods, pasta, cereals, and snacks. They can also be added to soups, beverages, and dietary supplements. Additionally, grains contribute to health supplements like cereal powders and innovative products like plant-based protein alternatives. Overall, grains are vital sources of energy and essential nutrients, supporting healthy bodily functions and aiding in disease prevention.

Keywords: Grain peptides, prevention, antimicrobial, antioxidant, health.

II. Introduction

Most people's lives revolve around grains and pulses, particularly in Third World emerging nations. Grains and their derivatives are the staple food for these populations and occupy a prominent place in both human and animal nutrition due to their nutritional importance. They provide the body with essential carbohydrates, proteins, fiber, vitamins, and minerals. Their small size and low moisture content (around 15%) facilitate easy transport and long-term storage without spoilage. They are rich in nutrients, with a dry matter content of 85%. Protein comprises approximately 7-12%, lipids 2-5%, and carbohydrates 85% in the form of starches [1, 2].

Grain crops, such as barley, wheat, millet, sorghum, maize, oats, and other crops, belong to the grass family (Gramineae or Poaceae) [3]. Legumes belong to the legume family, Leguminosae, also known as the Bean Family or Pulse Crop. These are seeded food crops cultivated for their seeds. They are called the Fabaceae family because their seeds are contained within a pod, or the Prunaceae family because of the shape of their flowers. The Leguminosae family is one of the largest plant families, comprising 690 genera and



approximately 1800 species. The botanist Hutchinson identified three subfamilies within the Leguminae order: Caesalpinaceae, Papilionaceae, and Mimosaceae. This family includes many vegetables and field crops. Peas, for example, were discovered in Switzerland more than 4500 years BC and are highly nutritious, containing high levels of nitrogen [1, 2].

Legumes belong to the family Leguminosae, specifically the subfamily Papilionaceae, and the genus Pisum. Peas (*Pisum sativum*) are the fourth most important legume crop and a staple crop for several reasons. They are rich in nutrients and contain a high amount of protein, making them an ideal protein source, especially in areas where meat and dairy products are unaffordable or difficult to obtain [4, 5]. Additionally, legumes are high in soluble fiber and low in fat, which can help control blood sugar and decrease cholesterol. Health groups advise using them to fight non-communicable diseases like diabetes and heart disease because of these qualities. It has been demonstrated that legumes can combat obesity. Proteins are complex organic compounds, specifically polymers, chemically composed of 51% carbon, 23% oxygen, 16% nitrogen, 7% hydrogen, 3% sulfur, and 1% phosphorus. They consist of amino acid units, some containing a single polypeptide chain, while others contain multiple polypeptide chains [6]. Proteins are chains of amino acids linked by peptide bonds, where the amino group (NH₂) of one amino acid is bonded to a carboxyl group (COOH) of another. Essential amino acids are abundant in proteins of animal origin, such as milk, egg, and meat proteins. Plant proteins contain fewer essential amino acids. The storage proteins in flour are responsible for the dough's resistance and elasticity, which are related to loaf size, dough stretch resistance, and dough formation time. The claudin protein is responsible for the elasticity and extensibility of the dough. In contrast, the glutenin protein is responsible for the resistance and cohesion of the dough, which is due to its polymeric nature. This difference in rheological properties, efficiency, and manufacturability depends on the quantity and quality of wheat proteins [7, 8].

1-1: Sources of Protein

1-1-2: Animal Proteins:

- Meat includes beef, chicken, and lamb. Fish includes salmon and tuna. Eggs are a rich source of protein. Dairy products include cheese, milk, and yogurt.

1-1-3- Plant Proteins:

- Legumes: such as lentils, chickpeas, and beans.
- Nuts and Seeds: such as almonds, walnuts, and chia seeds.
- Grains: such as quinoa, rice, and oats.
- Vegetables: Some vegetables, such as spinach and broccoli, contain good amounts of protein [9].

1-1-4- Supplemental Proteins:

- Protein Powders: such as whey protein, soy protein, and pea protein.

1-2: Functions of Proteins

Proteins are divided into:



-Structural proteins: These provide support and cohesion to cells and tissues. Examples include collagen and keratin.

-Enzymatic proteins: These act as catalysts in chemical reactions within the body. Examples include enzymes such as amylase and pepsin.

-Transport proteins: These help transport substances within the body. An example is hemoglobin, which carries oxygen in the blood.

- Immunological proteins: These play a role in the immune system to fight infection. Examples include antibodies.

-Hormonal proteins: These act as chemical messengers to regulate bodily functions. Examples include insulin and adrenaline.

- Storage proteins: These store amino acids or nutrients. An example is casein in milk.[9,10].

1-3: Protein Classification

Proteins are generally classified based on their chemical structure or their combination with other organic and inorganic substances into:

❖ Simple proteins

❖ Conjugated proteins

❖ Derived proteins

1-3-1: Simple proteins: These are the simplest type of protein and are composed of peptides and chains made up of amino acids only. These are divided into:

-Fibrous proteins: These include proteins that are insoluble or resistant to solvents and form the supporting parts of animal organs. They are called albuminoids. Examples of these proteins include collagen and keratin.



Figure (2) Fibrous proteins[11]

-Globular proteins: These are soluble proteins that have a spherical shape due to the coiling of their constituent molecules and the formation of disulfide and other bonds between them. Examples include albumins, globulins, glutalins, prolamins, protamines, and histones.

1-3-2: Conjugated proteins:

Conjugated proteins are proteins composed of a protein part and a non-protein part, called a prosthetic group, such as carbohydrates, fats, or nucleic acids. These proteins include: nucleic proteins, carbohydrate proteins, phosphorus, chromosome proteins, lipoproteins, and mineral proteins.

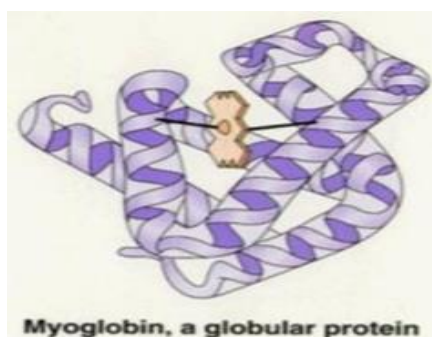


Figure (3) Conjugated Proteins [12]

1-3-3: Derived Proteins:

These are the result of protein hydrolysis and are composed of peptide chains such as peptones and peptides, as well as heat-treated and naturally altered (denatured) proteins, and coagulated proteins. Examples of derived proteins include meta proteins, peptones, and proteolysis [9].

Cereals: In the world, cereals constitute the main source of plant proteins, carbs, and energy. Currently, up to 35% of grains are used as animal feed, and only 41% are consumed by humans. The two crops that are produced in the greatest amounts each year are rice (755 million metric tons) and wheat (766 and 1,148 million metric tons, respectively). Other globally significant cereal crops include oats, barley, and sorghum [10]. Cereals may be crucial to the shift to a more sustainable and healthful diet, but they have been disregarded as a source of plant proteins that are both environmentally and healthily sustainable. Promoting biodiversity, using living soils, employing integrated pest control, reducing greenhouse gas emissions, and concurrently providing high-quality food and preserving food security are all components of sustainable grain production systems. [13] Compared to many animal-based raw material production systems, grain production has a smaller environmental impact and yields reasonably priced goods that can be utilized in a variety of local contexts. Depending on the product type, grain-based foods might have different health impacts. Increased consumption of whole-grain meals is justified and promoted by official dietary guidelines in many countries because it is consistently linked to health advantages at the population level [14].

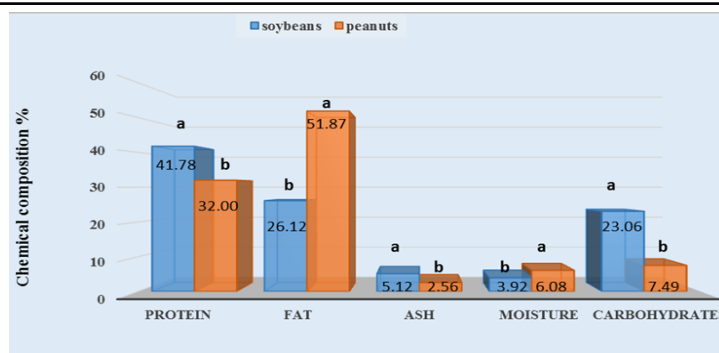


Figure (4) Chemical composition of peanuts and soybeans [16]

Cereal proteins: Depending on the species and variation, cereals can contain anywhere from 18% to 7% of dry matter. The endosperm contains the majority of cereal proteins, whereas the bran's aleurone and subaleurone layers contain the most protein. Cereal proteins are often categorized according to how soluble they are in water (albumin), salt (globulin), aqueous alcohols (prolamin), or acidic/basic solutions (glutinin). More necessary amino acids are found in albumins and globulins than in prolamins and glutinin. For instance, the amount of lysine in the bran proteins of rice and wheat is three times greater than that of the endosperm proteins [16]. These proteins include:

- Gliadin: A major protein in wheat.
- Gluten: A combination of gliadin and glutenin, considered a major protein in gluten-containing grains such as wheat and barley [17].
- Albumins: Water-soluble proteins found in all grains.
- Zein: A protein found in corn.
- Avenins: Proteins found in oats.
- Sitin: A protein found in rice.
- Arginine: An amino acid found in many grains.
- Lysine, threonine, and tryptophan: Essential amino acids, present in lower concentrations in some grains such as wheat [18].

1-4: Amino Acids in Grains

Amino acids are among the essential components of all foods; however, there is considerable variation in their content. They are a fundamental building block of plant proteins and are organic compounds with low molecular weights ranging from -100 to -200. They contain at least one carboxyl group (COOH) and one amino group (NH). The differences between various amino acids are due to the nature of their side chains (R-), which are essential and distinguish each amino acid from the others, Humans and animals can synthesize nine of the twenty essential amino acids. In this study, conducted on 10 wheat varieties, the amino acid

content varied according to the variety. The amino acids alanine, aspartic acid, cysteine, glutamic acid, proline, glycine, serine, tyrosine, and arginine were found, as shown in Table 1, Amino acids are the basic building blocks of proteins. Fifteen amino acids were found in fenugreek seeds: aspartic acid, glutamic acid, asparagine, histidine, serine, arginine, methionine, alanine, valine, proline, phenylalanine, leucine, tyrosine, lysine, and glycine. These were analyzed using an amino acid analyzer [14, 15].

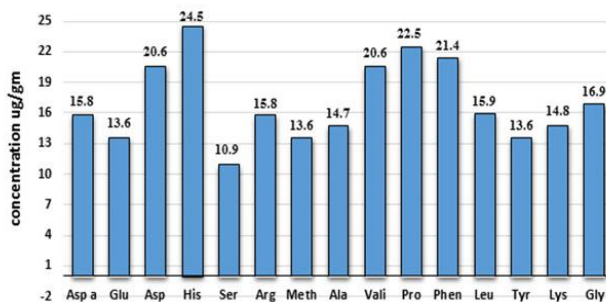


Figure (5) Amino acids in fenugreek seeds [17]

Table (1) Amino Acids in Different Wheat Varieties (grams/100 grams)

-Grain Peptides

Peptides are bonds between amino acids, specifically between the carboxyl groups of one amino acid bonds with the amino group of another amino acid, resulting in the loss of a water molecule. This type of bond is known as a peptide bond. When two amino acids are linked by a peptide bond, the resulting molecule is

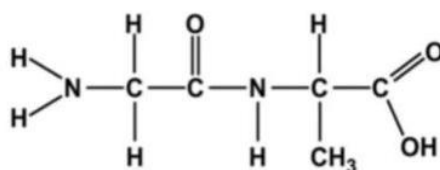
Amino acid (g/100 protein)	Fortun	Sasanka	Dznca	Somborka	Kremna	Kosmajka	Stanija	Morava	KG 56 S	Ljubičevka	Mein AA	Min	Max	CV (%)
Alanine	3.83	3.89	4.02	3.94	3.88	4.00	4.13	4.24	4.14	4.32	4.038	3.83	4.32	4.05
Aspartic acid	5.41	5.28	5.54	5.28	5.30	5.30	5.43	5.64	5.35	5.72	5.425	5.28	5.72	2.92
Cysteine	1.62	1.98	2.11	2.18	1.95	1.98	1.85	2.12	2.08	2.17	2.004	1.62	2.18	8.55
Glutamic acid	16.74	17.82	17.79	17.93	17.33	18.59	19.51	19.72	19.41	20.22	18.506	16.74	20.22	6.26
Glycine	4.07	3.95	4.18	3.98	3.94	4.17	4.36	4.36	4.25	4.45	4.171	3.94	4.45	4.41
Proline	12.95	12.8	12.04	11.49	12.35	11.70	11.88	12.44	11.00	12.82	12.147	11.00	12.95	5.26
Serine	4.04	4.14	4.11	4.05	3.94	4.03	4.00	4.4	5.17	4.43	4.231	3.94	5.17	8.70
Tyrosine	2.99	2.13	2.16	2.60	2.93	2.02	2.46	2.59	2.75	2.61	2.524	2.02	2.99	13.18
Arginine	5.32	4.86	4.92	5.00	4.58	4.61	4.90	5.34	5.42	5.22	5.017	4.58	5.42	5.96
Histidine*	2.70	2.76	2.82	2.77	2.86	2.65	2.53	2.73	2.73	2.81	2.736	2.53	2.86	3.46
Isoleucine*	3.95	3.93	3.83	3.90	4.31	3.68	3.65	3.87	3.51	4.06	3.869	3.51	4.31	5.82
Leucine*	5.50	5.63	5.62	5.52	5.90	5.57	5.46	5.92	7.55	5.98	5.865	5.46	7.55	10.60
Lysine*	2.89	2.76	3.03	2.80	2.76	2.90	2.92	3.02	2.97	3.06	2.911	2.76	3.06	3.80
Methionine*	1.28	1.20	1.23	1.34	1.40	1.13	1.14	1.33	1.36	1.36	1.277	1.13	1.4	7.57
Phenylalanine*	3.70	4.10	4.64	4.34	4.03	4.93	5.84	5.22	3.95	5.45	4.620	3.70	5.84	15.52
Threonine*	2.87	2.82	2.89	2.82	2.74	2.79	2.76	3.00	2.89	3.06	2.864	2.74	3.06	3.57
Valine*	4.37	4.51	4.51	4.47	4.65	4.47	4.41	4.69	4.34	4.78	4.520	4.34	4.78	3.18
Tryptophan*	1.12	1.32	1.29	1.05	0.99	1.27	1.17	1.27	1.25	1.32	1.205	0.99	1.32	9.70



called a dipeptide. When three amino acids are linked, it is referred to as a tripeptide. Additionally, when many amino acids are linked together, the structure is called a polypeptide. The chain has a specific orientation; the terminal amino group does not bond to a new amino acid, while the carboxyl group can bond to another amino acid [19].

-Types of Peptides:

***Dipeptide:** Links between two amino acids



(Figure (6) Linking of two amino acids to dipeptides (Berg, J. M *et al.*,2019)

***Tripeptides:** These consist of three amino acids linked together by peptide bonds.

***Polypeptides:** These are composed of more than 10 amino acid units linked by peptide bonds. Most proteins are polypeptides. Peptides also exhibit basic and acidic properties, most notably high melting points. This allows them to crystallize from neutral solutions in an ionic form. The basic and acidic properties of peptides are due to the greater number of free carboxyl groups compared to the amino acid group. This results in weaker electrochemical interactions between them, leading to higher dissociation constants for the alpha-carboxylate groups compared to the carboxyl groups themselves in the amino acids, since these bonds are the fundamental building blocks of proteins [9].

-Peptides as Antioxidants:

Antioxidants are compounds that can prevent or delay food spoilage caused by oxidation. They achieve this by binding to free radicals. The function of antioxidants goes beyond just maintaining food safety and quality; they also help extend shelf life and reduce the loss of nutritional value. Additionally, antioxidants inhibit the oxidation of fats, which is a primary cause of food spoilage and can lead to a decline in sensory properties and overall product quality during manufacturing and storage. Preparing grain protein peptides is an effective way to utilize these proteins, which have potential applications as antibacterial, antioxidant, anti-inflammatory, and anticancer agents. They can also aid in lowering blood pressure, controlling blood sugar, and preventing blood clots. Excessive free radical production damages vital macromolecules like proteins, lipids, and DNA, leading to oxidative stress when antioxidants can't keep up, leading to various diseases, including cancer [21, 22]. Peptides have been shown in numerous *in vitro* and *in vivo* investigations to function as antioxidants, reducing the build-up of free radicals and the ensuing oxidative damage [23, 24]. Peptides are thought to have antioxidant properties through free electron donation, free radical scavenging, and metal chelation. Two millet enzymes, catalase and trypsin, were examined using millet proteins to determine their antioxidant activity (DPPH). The catalase hydrolysate exhibited more antioxidant activity than the trypsin hydrolysate, according to the data. This could be because catalase hydrolyzes smaller peptides than trypsin, which leads to a larger degree of trypsin hydrolysis [25, 26, 27]. Ortiz-Martinez *et al.*

reported that protein type (albumin, globulin, prolamin, and glutelin), grain type (e.g., ordinary corn versus high-quality protein corn), and extraction method (catalase hydrolysis) have a clear effect on the antioxidant properties and oxygen radical capacity (ORAC) of proteins. It has been reported that glutathione fractions from chickpeas (germinated in the presence of selenium) exhibited higher cellular antioxidant capacity compared to albumin fractions [28]. In a study [29,30], conducted on corn, quinoa, and wheat fermentation, the antioxidant potential of DPPH and ABTS lysates in fermented flour was compared to the control sample, except for the wheat sample, where there was only a marginal increase for all *Bifidobacteria* spp. used. The study also showed that there is no direct relationship between fermentation duration (0, 24, 48, and 72 hours) and antioxidant potential for all DPPH assays except for corn, where there is greater antioxidant potential due to the increased fermentation period. It appears that the different processing techniques used and the nature of the original protein will result in the production of BAPs (bioactive peptides) with amino acid residues.

Although the biological activity of peptides is related to proteins, there appears to be no direct correlation between protein content and biological activity or protein production. In a study investigating the antioxidant properties of sorghum peptides, various enzymes were used for hydrolysis. It was discovered that one enzyme, which yielded the lowest amount of peptides, had a superior ability to reduce free radicals (DPPH) compared to other enzymes that produced higher yields [31]. The type of enzyme and the extent of hydrolysis can significantly affect the effectiveness of the peptides, as the duration of hydrolysis influences the size of the peptides produced.

Interestingly, although albumin generally showed higher efficacy in the ORAC assay, globulin 11S was the best sample for hydroxyl radical ($\bullet\text{OH}$) analysis, suggesting the involvement of different mechanisms [32, 33]. Previous studies have emphasized the importance of amino acid sequences in peptides, particularly at the C-terminus. Because one antioxidant mechanism for free radical scavenging is the ability to donate hydrogen from a hydroxyl group, the presence of aromatic amino acids, such as tyrosine, at the C-terminus of peptides has been observed to enhance their free radical scavenging capacity, enabling them to readily donate hydrogen.

Antimicrobial Peptides

Peptides are small molecules that play a significant role against a wide range of microorganisms, including bacteria, fungi, parasites, and viruses. Specifically, they are classified as 431 bacteria, 7 protozoa, 6 fungi, 824 plants, and 2519 animals. They also possess a variety of biological functions, such as immune regulation, wound healing, and antitumor activity. The sources of antimicrobial peptides are diverse, including plant, animal, and microbial origins. More than 500 types of antimicrobial peptides have been isolated from a wide range of organisms, and the first antimicrobial peptide was isolated from the moth *Hyatophora cecropia*. [34,35].



Antimicrobial Peptides Market Analysis By Type

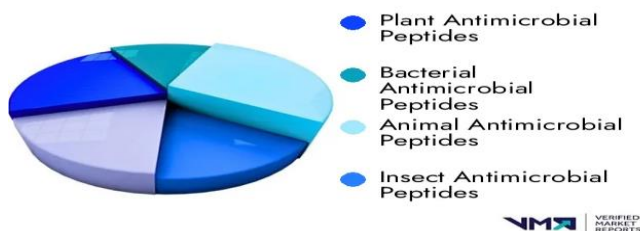


Figure (7) shows the size, growth, and industry trends of the antimicrobial peptide market [36]

Antimicrobial peptides are short molecules of 8–50 amino acids, weighing less than 10 kDa, and carry a positive charge from lysine, arginine, and histidine. Antimicrobial peptides contain approximately 50% hydrophobic amino acids, which selectively interact with the negative charge on the bacterial surface through direct electrophoresis with microbial cell membranes, causing an increase in membrane permeability [37].

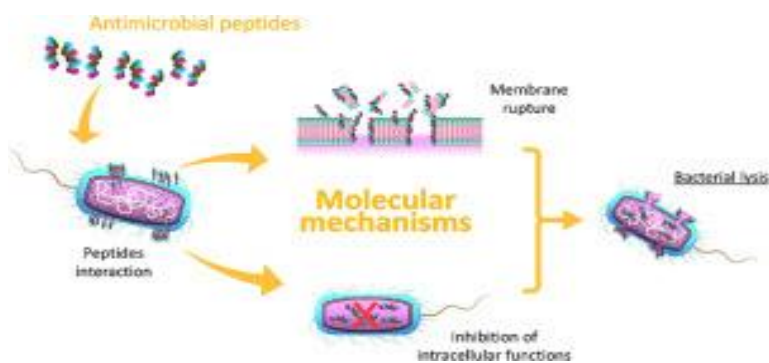


Figure (8) Mechanism of action of peptides as an antimicrobial [38]

Cationic antimicrobial peptides (AMPs) kill bacteria by binding to their negatively charged membranes, increasing permeability and causing cell lysis, and the subsequent release of cellular contents. As AMPs approach the cytoplasmic membrane, they engage in electrostatic interactions with the microbial membrane, allowing them to bind to the membrane and interact with its anionic components. Before this can occur, AMPs must first penetrate the cell wall, which includes polysaccharides and other components. For Gram-negative bacteria, this includes lipopolysaccharides (LPS), while for Gram-positive bacteria, the constituents are lipoteichoic acid and peptidoglycan [39].

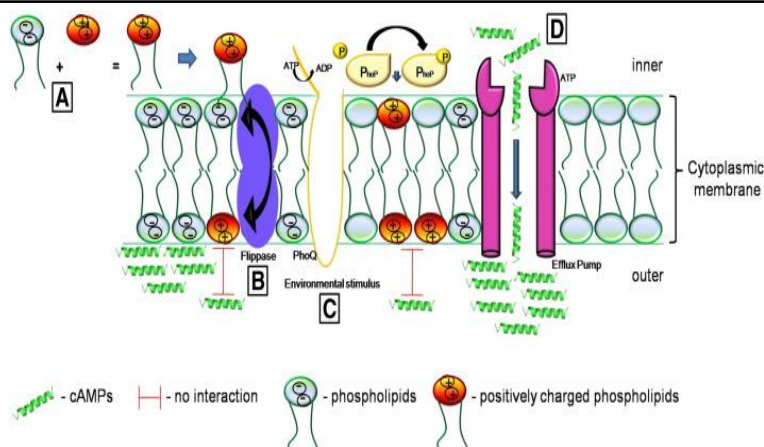


Figure (9) Cell wall structure of Gram-positive and Gram-negative bacteria [40]

Studies have shown that α -helical antimicrobial peptides (AMPs) bind to anionic lipid membranes, transitioning from a disordered structure in aqueous solutions to a water-soluble α -helical structure, which facilitates their interaction with the membrane. In contrast, beta-sheet peptides differ from α -helical peptides as they do not undergo a significant phase transition when interacting with membranes due to the presence of stable disulfide bond bridges [41]. The peptide-to-lipid ratio is another key factor influencing the interaction of AMPs with cell membranes. A low peptide-to-lipid ratio helps maintain a balance of AMPs on the surface of the plasma membrane [42]. However, as the peptide-to-lipid ratio increases, AMPs become oriented vertically and penetrate the hydrophobic core of the membrane. This penetration ultimately leads to ion leakage and interferes with intracellular biosynthesis, resulting in cell death [43]

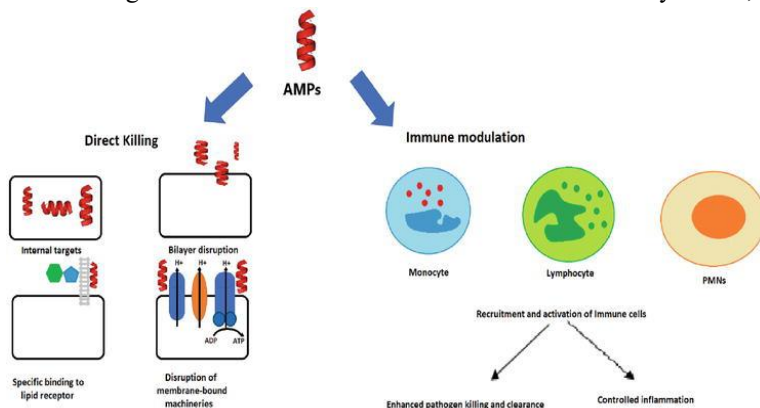


Figure (10) The lethal effect of AMPs on microbial DNA [43]

Application of Peptides in Dietary Systems:

Food-derived functional peptides, initially discovered in dairy products, have shown potential to enhance human health. They can be categorized into plant, animal, and microbial protein peptides. Common plant protein peptides include those from wheat, buckwheat, corn, soybean, and chickpeas. Animal protein

peptides, which have been more extensively studied, include milk, insect, meat, egg, fish, and various marine peptides [44]

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