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

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I. Abstract

The study showed that grains are a staple food in many cultures around the world and are considered a key component of the human diet. Grains vary, these are whole grains such as rice, wheat, barley, corn, and oats, and legumes such as beans and lentils. to legumes such as beans and lentils. Grains are of great importance in terms of their nutritional value, health benefits, and impact on ecological balance. Nutritionally, grains are important as they are a rich source of proteins, dietary fiber, carbohydrates, vitamins (such as B vitamins), and minerals (such as iron and magnesium). In addition, grains contain bioactive components such as phenols and flavonoids that support human health. The dietary fiber in grains helps improve digestion, regulate blood sugar levels, and reduce cholesterol levels. Consuming grains from a health perspective helps regulate and prevent many chronic diseases such as heart disease, obesity and diabetes. Whole grains contain antioxidants that reduce inflammation in the body and improve immune system function. Grains also contribute to preventing digestive problems such as constipation and help maintain a healthy weight due to their ability to promote a feeling of fullness for longer periods. The study indicated that grains and legumes play a vital role as an ideal environment for many microbes, such as bacteria and fungi. While some microbial species offer benefits, such as improving fiber digestion or producing beneficial substances for the body (like probiotics), there may be a risk of harmful microbes contaminating grains during storage or processing. Therefore, it is important to adhere to hygienic grain handling and storage methods to minimize the risk of microbial contamination. Grains have numerous applications in food. They are primarily used in the production of bread, baked goods, pasta, dry cereals, and snacks. They can also be used in soups, juices, and beverages, and serve as dietary supplements.

Grains contribute to the production of health supplements such as cereal powders and fortified meals. Within the framework of nutritional enhancement, grains are used in the production of innovative food products such as plant-based protein alternatives, including meat substitutes. Grains are not merely a source of energy; they play a significant role in improving overall health by providing essential nutrients. A balanced grain diet promotes healthy bodily functions and contributes to the prevention of many diseases.

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

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Summary

The study shows that grains are a staple food source in many cultures around the world and are considered one of the main elements of the human diet. Grains range from whole grains such as wheat, rice, barley, corn, and oats, and also include legumes such as beans and lentils. In terms of nutritional value, health benefits, and their impact on ecological balance, grains are nutritionally important as they are a rich source of carbohydrates, proteins, dietary fiber, vitamins (such as vitamin B), and minerals (such as iron and magnesium). In addition, grains contain bioactive components such as phenols and flavonoids that support human health. The dietary fiber in grains helps improve digestion, regulate blood sugar, and reduce cholesterol levels, while eating grains from a health standpoint regulates and prevents many chronic diseases, such as heart disease, diabetes, and obesity. Whole grains contain antioxidants that reduce inflammation in the body and improve immune system function. Grains also contribute to the prevention of gastrointestinal diseases such as constipation and help maintain an ideal weight thanks to their ability to provide a feeling of satiety for longer periods of time.

According to the study, grains and pulses play an active role as an ideal environment for many microbes, such as bacteria and fungi. While some microbial species bring benefits such as improved fiber digestion or the production of substances beneficial to the body (such as probiotics), there may be a risk of harmful microbes contaminating grains during storage or processing. Therefore, it is important to consider healthy grain processing and storage methods to minimize the risk of microbial contamination.

Grains have many applications in food. Grains are mainly used in the manufacture of bread, baked goods, pasta, dry cereals, and snacks. They can also be used in soups, juices, and beverages, and have a role as dietary supplements. Grains contribute to the manufacture of health supplements such as cereal powder and fortified meals. As part of nutritional enhancements, grains are used to create innovative food products such as plant-based protein alternatives, including meat substitutes. Grains are not just a source of energy, and they play an important role in improving overall health by providing essential nutrients. Eating grains in a balanced way promotes healthy functioning of the body and contributes to the prevention of many diseases.

II. Introduction

Most people's life 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

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order: Caesapiniaceae, 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 (NH_2) 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 claidin 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: such as beef, chicken, and lamb.

- Fish: such as salmon and tuna.

- Eggs: a rich source of protein.

- Dairy Products: such as 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.

1-1-4- Supplemental Proteins:

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

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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.

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.

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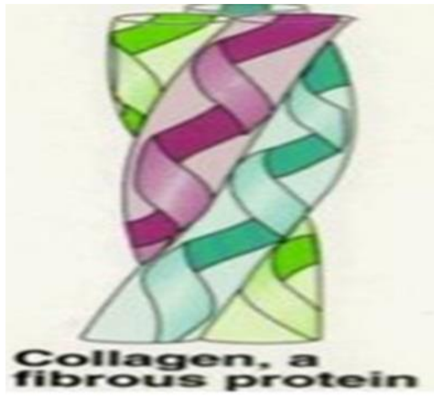

Figure (2) Fibrous proteins(Nelson, D. L., and Cox, M. M. (2021)

-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.

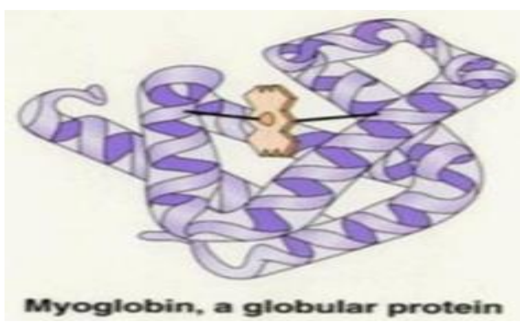

Figure (3) Conjugated Proteins(Voet, D *et al*.,2016)

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

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[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. [11] 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 [12].

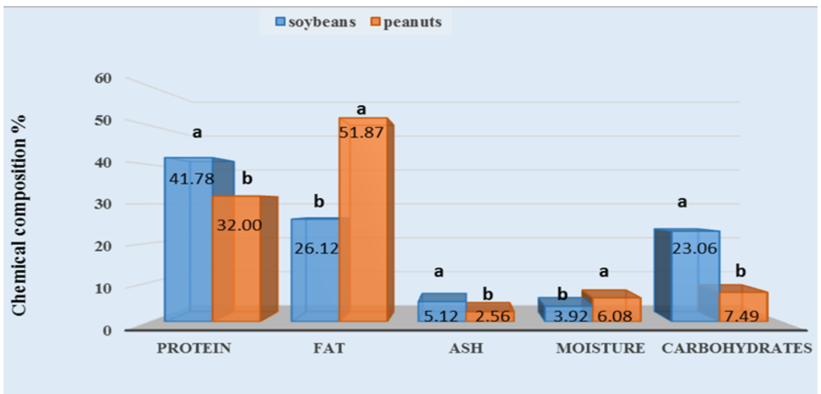

Figure (4) Chemical composition of peanuts and soybeans [16]

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 (glutilin). More necessary amino acids are found in albumins and globulins than in prolamins and glutilin. For instance, the amount of lysine in the bran proteins of rice and wheat is three times greater than that of the endosperm proteins [13]. 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.

• 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.

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• Lysine, threonine, and tryptophan: Essential amino acids, present in lower concentrations in some grains such as wheat [13].

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].

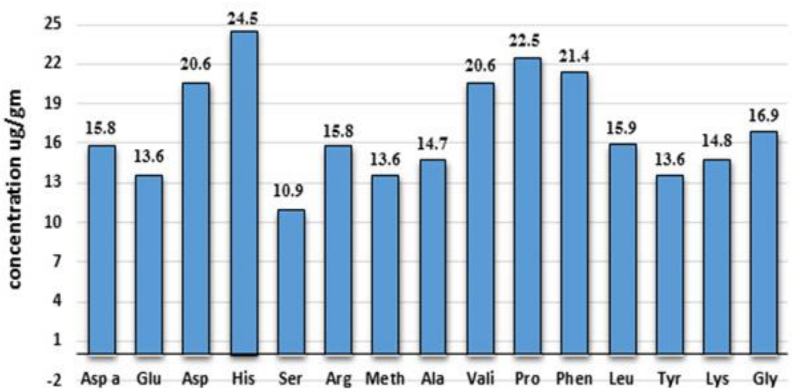

Figure (5) Amino acids in fenugreek seeds [17]

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

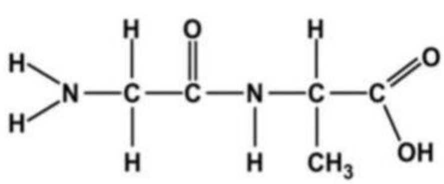
-Grain Peptides

Amino acid (g 100 g^{-1} protein)	Fortuna	Sasanka	Danica	Somborka	Kremna	Kosmajka	Šumadija	Morava	KG 56 S	Ljubičevka	Mean 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

Peptides are bonds between amino acids, specifically between the carboxyl group of one amino acid and the amino group of another, with the loss of a water molecule. The bond between them is called a peptide bond. When two amino acids are linked by a peptide bond, it is called a dipeptide; when three amino acids are linked, it is called a tripeptide; and when a large number of amino acids are linked, it 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.

-**Types of Peptides:**

*Dipeptide: Links between two amino acids



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

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***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 defined as compounds capable of preventing or delaying food spoilage resulting from oxidation. This is achieved through mechanisms such as binding to free radicals [18]. The role of antioxidants is not limited to maintaining food safety and quality; it extends to extending shelf life and reducing nutritional value loss. Furthermore, they inhibit the oxidation of fats, which is a major cause of food spoilage and the deterioration of sensory properties and product quality during manufacturing and storage [19]. The process of preparing grain protein peptides is an important and good way to utilize these proteins. Grain protein peptides have good application potential as antibacterial, antioxidant, anti-inflammatory, and anticancer agents, and in lowering blood pressure, controlling blood sugar levels, and preventing blood clots [20]. Excessive production of free radicals damages essential macromolecules such as proteins and lipids, including DNA. Oxidative stress occurs when the formation of free radicals, such as reactive oxygen species, exceeds the capacity of available antioxidants to control them, 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 were significantly increased the antioxidant potential of DPPH, and ABTS lysates in fermented flour 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 cleared 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 greater antioxidant potential due to 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.

However, while the biological activity of peptides is related to protein, there appears to be no direct correlation between protein content and biological activity, or protein production. In a study investigating the

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antioxidant properties of sorghum peptides, a series of enzymes were used for hydrolysis. It was found that one enzyme with the lowest yield among those used in the study had a better ability to remove free radicals (DPPH) compared to other enzymes with higher yields [31]. The type of enzyme and the degree of hydrolysis may also play important roles in the efficacy of peptides, as the duration of hydrolysis may determine the size of the peptides produced. It is also interesting to note that while albumin generally had higher efficacy in the ORAC assay, globulin 11S was the best sample for OH• analysis, which may indicate that different mechanisms. [32, 33] Previous studies indicated the importance of amino acid sequence in peptides relative to the C-terminus. Since one of the antioxidant mechanisms in free radical scavenging is their ability to donate hydrogen, due to the hydroxyl group, it has been observed that the presence of aromatic amino acids such as tyrosine at the C-terminus of peptides gives the peptide a higher free radical scavenging capacity, which can readily donate hydrogen [34, 35].

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*.

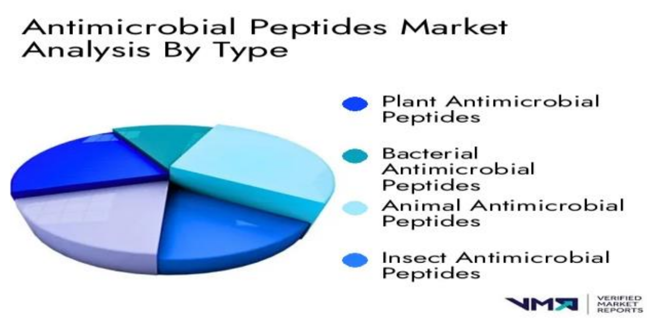

Figure (7) shows the size, growth, and industry trends of the antimicrobial peptide market.(Wang, G . .(2023)

In general, antimicrobial peptides are short-chain molecules composed of 8–50 amino acids with a molecular weight of less than 10 kDa. They carry a positive charge due to the presence of 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 [36].

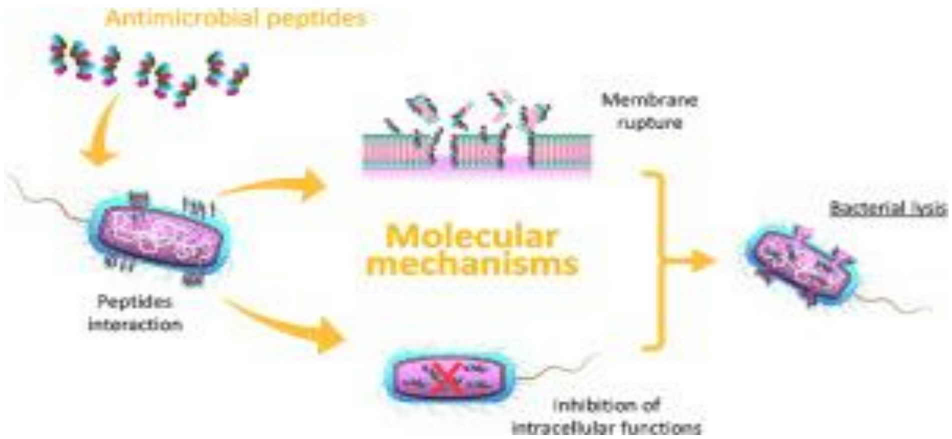

Figure (8) Mechanism of action of peptides as an antimicrobial(Zhang, Q. Y.,2021)

Cationic AMPs exert antibacterial activity by interacting with the negatively charged bacterial membrane, increasing membrane permeability and leading to cell membrane lysis and release of cellular contents. Upon approaching the cytoplasmic membrane via electrostatic interaction with the microbial membrane, AMPs bind to the microbial membrane and interact with the anionic components of the plasma membrane. Prior to this, AMPs must pass through polysaccharides and other cell wall components, such as LPS for Gram-negative bacteria and lipotic acid and peptidoglycan for Gram-positive bacteria[37].

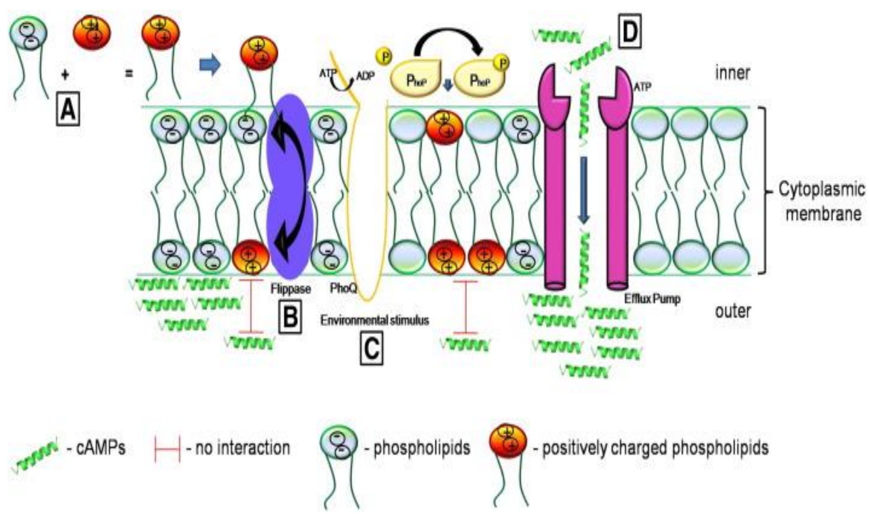

Figure (9) Cell wall structure of Gram-positive and Gram-negative bacteria (Madigan, M. T et al.,2023)

Studies have shown that α-helical AMPs bind to the anionic lipid membrane and convert their disordered structure in aqueous solution to a water-soluble α-helical structure to facilitate interaction with the membrane. Beta-sheet peptides differ from α-helical peptides and do not undergo a major phase transition upon

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interaction with the membrane due to the stable disulfide bond bridge [38]. The peptide-to-lipid ratio is another key factor affecting the interaction of AMPs with the cell membrane. A low peptide-to-lipid ratio plays a role in balancing AMPs on the plasma membrane surface [39]. As the peptide-to-lipid ratio increases, AMPs are oriented vertically and settle at the hydrophobic center of the membrane. Ultimately, membrane penetration leads to ion leakage and intracellular biosynthesis, resulting in cell death [40].

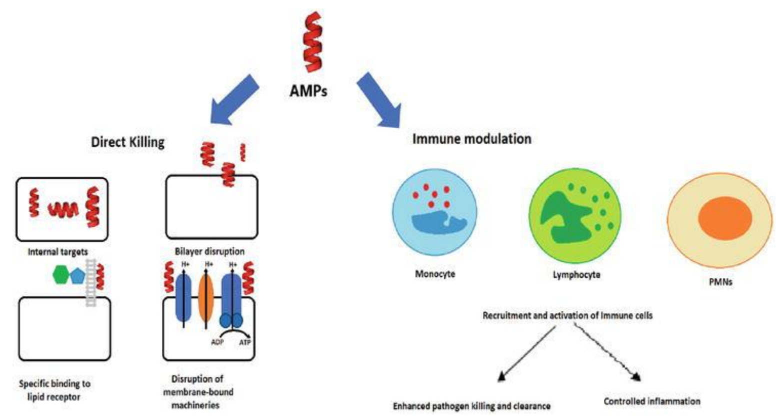

Figure (10) The lethal effect of AMPs on microbial DNA(Lei, J *et al*.,2019)

Application of Peptides in Dietary Systems:

Food-derived functional peptides were first discovered in dairy products, and studies have demonstrated their potential to promote human health. These studies have shown that food-derived functional peptides have the potential to enhance human health. There are many types of food-derived functional peptides, which can be categorized into plant protein peptides, animal protein peptides, and microbial protein peptides based on their sources. According to specific raw material types, common plant protein peptides include wheat peptides, buckwheat peptides, corn peptides, soybean peptides, and chickpea peptides. Animal protein peptides have been studied more extensively and include milk peptides, insect peptides, meat peptides, egg peptides, fish peptides, and various marine peptides [41].

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