Antioxidant Peptides from Proteins: Separation, Identification, Mechanisms, and Applications in Food Systems

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***Abstract*—With an emphasis on their identification, separation, modes of action, and possible uses in food systems, this work offers a thorough investigation of antioxidant peptides generated from bioactive proteins. Antioxidant peptides are essential for combatting free radicals and averting oxidative damage. They are often generated by the enzymatic degradation of proteins from diverse sources, including plants, animal, and marine sources. High-performance liquid chromatography (HPLC), ultrafiltration, membrane filtration, and mass spectrometry are some of the sophisticated techniques used in the separation and identification of these peptides. These techniques enable accurate isolation and characterization. It's important to comprehend the processes by which these peptides exercise their antioxidant benefits; research suggests that their main modes of action include suppression of lipid peroxidation, metal ion chelation, and free radical scavenging. These bioactive peptides have a great deal of promise to improve food items' functional and nutritional qualities. Their integration into food systems can enhance their nutritional content, self-life, and health advantages, making them important components in the creation of functional meals. While the study highlights the promising potential of bioactive peptides, further research is essential to evaluate their stability, bioavailability, and safety under real-world conditions. Factors such as gastrointestinal degradation, absorption efficiency, and potential toxicity must be thoroughly assessed to ensure practical applicability. In addition, this work addresses the difficulties pertaining to these peptides' stability and bioavailability in food matrices and identifies areas for future investigation to maximize their application in the food sector.**

Keywords*—Antioxidant; Peptides; Bioactive; Proteins; Separation; Identification; Method; Food System

I. Introduction

Antioxidant peptides produced from bioactive proteins have attracted a lot of attention lately because of their potential health benefits and applications in the food business. Preventing oxidative stress and improving food stability are two benefits of these peptides, which are generally obtained by the enzymatic degradation of proteins from plants, animals, and marine organisms [1]. They also possess strong antioxidant contents. A surplus of free radicals relative to antioxidants in the body leads to oxidative stress, which is associated with several chronic illnesses, including cancer, neurological disorders, and heart disease. The potential of antioxidant peptides as agents in the development of functional foods for disease prevention and health promotion stems from their capacity to counteract free radicals and oxidative processes [2]. To fully realize the potential of antioxidant peptides, isolation and identification are essential first steps. These peptides have been isolated and characterized using sophisticated techniques such membrane filtration, ultrafiltration, high-performance liquid chromatography (HPLC), and mass spectrometry. This has allowed for the investigation of their structure and functional characteristics [3]. Antioxidant peptides must first be isolated and identified before they can be used effectively in the food and health sectors. A variety of sophisticated procedures are used to do this, such as membrane separation techniques like ultrafiltration, which enable the initial fractionation of protein hydrolysates according to molecular weight. The active peptide fractions are then further purified using high-performance liquid chromatography (HPLC), which allows for accurate separation depending on hydrophobicity or polarity. Lastly, the structural makeup and sequence of the bioactive peptides are ascertained by mass spectrometry, which is frequently combined with tandem methods (MS/MS), and amino acid sequencing. These analytical approaches not only facilitate the identification of peptides with potent antioxidant activity but also provide insights into structure–activity relationships, which are crucial for optimizing their functional performance in food, nutraceutical, and pharmaceutical applications.

It is similarly vital to comprehend the mechanisms via which antioxidant peptides work, since this sheds light on how they interact with lipid peroxidation processes, metal ions, and free radicals. Their ability to extend the shelf life and improve the nutritional value of food goods, as well as their protective function in biological systems, are largely dependent on these mechanisms. Bioactive proteins are an essential part of the pharmacological toolkit that plants have been using for generations as a rich supply of therapeutic chemicals [4]. These proteins are essential to plant defense processes and have been demonstrated to provide a range of medicinal benefits. To fully use these proteins' potential in medication research and dietary applications, it is imperative to comprehend their structural and functional properties [5]. But there are several obstacles facing scientists, including the variety of plant species and the intricacy of protein structures.

The study of bioactive proteins has undergone a revolutionary change with the introduction of high-throughput sequencing and proteomic technology. Massive genomic data sets produced by large-scale sequencing efforts are a useful tool for finding and describing bioactive proteins.

Proteomic methods allow for the deep investigation of protein expression, alteration, and interaction networks, such as mass spectrometry [6]. These datasets are integrated by bioinformatics, which provides strong tools for predicting protein function, clarifying methods of action, and evaluating possible toxicity. Antioxidant peptides have use in food systems that go beyond just promoting health. By stopping oxidation, a primary factor in spoiling and quality degradation, their integration into food matrices can greatly increase the stability and quality of products. Optimizing their stability, bioavailability, and effectiveness within intricate food matrices is still a challenge, though. Exploring antioxidant peptides' potential to satisfy consumers' increased desire for natural, health-promoting food components is becoming more and more popular as this field of study develops. With an emphasis on their separation, identification, mechanisms of action, and uses in food systems, this study attempts to give a comprehensive analysis of antioxidant peptides produced from bioactive proteins. Through investigating these facets, the research aims to further knowledge regarding the efficient application of these peptides to augment the nutritional content and the durability of food items, thereby facilitating the creation of novel functional foods that correspond with contemporary health and wellness fads.

II. Methodology

This study's process for producing antioxidant peptides from bioactive proteins includes multiple crucial steps, including choosing a protein source, producing the peptides, separating and purifying them, identifying and characterizing them, and measuring their antioxidant activity. The purpose of each step is to methodically investigate the characteristics and possible uses of antioxidant peptides in food systems [9]. First, protein sources high in bioactive peptides are chosen for the investigation. Plant proteins (soy, wheat, legumes), marine proteins (fish, algae), and animal proteins (milk, eggs, meat) are some examples of sources. The source is selected based on the antioxidant peptides' known or prospective yield. Peptides are released from proteins through enzymatic hydrolysis. Under carefully monitored circumstances, enzymes like alkaline, pepsin, and trypsin are utilized to break down protein molecules into smaller peptide fragments. To improve the yield of antioxidant peptides, variables such as pH, temperature, and enzyme concentration are adjusted during the hydrolysis process [10]. To separate peptides according to their molecular weight, the crude hydrolysate is first passed through membrane filtration and ultrafiltration processes. This stage aids in the concentration of the desired peptides while eliminating smaller, non-peptide molecules and larger, inactive proteins. High-performance liquid chromatography (HPLC), in particular reverse-phase HPLC, is used to isolate individual antioxidant peptides and achieve further purification. The peptides' hydrophobicity and polarity serve as the basis for the separation, enabling the collection of fractions enriched with peptide sequences [11].

Using mass spectrometry techniques such as electrospray ionization (ESI) and matrix-assisted laser desorption/ionization (MALDI), purified peptide fractions are analyzed to determine their molecular characteristics. These soft ionization methods are particularly well-suited for studying biomolecules, as they allow for the accurate measurement of peptide molecular weights without extensive fragmentation. Furthermore, tandem mass spectrometry (MS/MS) enables the sequencing of peptides by fragmenting them into smaller ions and analyzing their mass-to-charge (m/z) ratios. This approach facilitates the identification of specific amino acid sequences responsible for bioactivity, thereby providing critical insights into structure–function relationships. The application of ESI and MALDI thus plays a central role in confirming the identity and integrity of antioxidant peptides, supporting their further development for functional and therapeutic uses.

Techniques like Edman degradation and tandem mass spectrometry (MS/MS) are used to further identify the samples, giving comprehensive details about the peptide sequences. Understanding the relationship between the antioxidant peptides' structure and activity requires completion of this stage [12]. This methodology covers the whole process from peptide manufacture to application in food systems, offering a thorough approach to the research of antioxidant peptides produced from bioactive proteins. This study intends to unleash the potential of antioxidant peptides as functional additives in the food industry by fusing cutting-edge analytical techniques with real-world culinary applications.

III. Bioactive Proteins

"Bioactive proteins" are specialized proteins that have a significant effect on biological processes and live organisms [13]. These proteins are necessary for preserving wellbeing, averting illness, and delivering medicinal interventions [14]. Fig. 1 shows the summary of functions related to bioactive peptides released from feed protein sources under protease treatment.

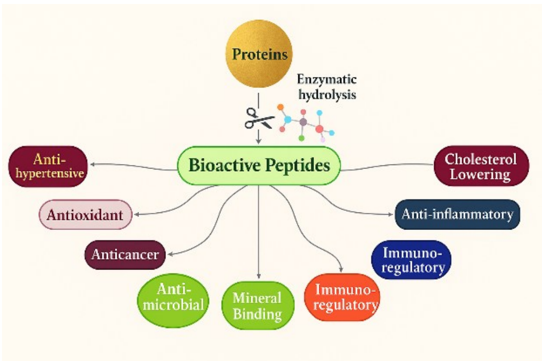

Fig. 1. The summary of functions related to bioactive peptides released from feed protein sources under protease treatment

They are involved in several physiological processes. Enzymes, which catalyze vital biochemical reactions; hormones, which function as chemical messengers controlling a range of physiological activities; and antibodies, which the immune system produces to detect and eliminate infections, are examples of bioactive proteins. Additionally, growth factors promote cell division, development, and proliferation, while cytokines tiny proteins involved in cell signaling have a significant influence on inflammation and immunological responses. Another type of bioactive protein that kills viruses, bacteria, and fungi is called an antimicrobial peptide. Nutraceutical proteins, found in foods like casein and whey, offer beneficial health effects [15]. In the fields of nutrition, biotechnology, and medicine, bioactive proteins which can be generated synthetically or naturally are being studied for their potential to treat illnesses, strengthen the immune system, and improve general health.

Because of their significant impacts on biological processes and human health, bioactive proteins are essential [16]. These proteins are necessary for preserving physiological equilibrium and enhancing general well-being. Enzymes are bioactive proteins that play a crucial role in biochemical reactions supporting various cellular functions, including metabolism. Hormones control essential biological functions that affect metabolism, development, and reproductive health. Through their ability to recognize and eliminate dangerous pathogens, antibodies are essential for immune protection [17]. Important facets of cytokine activity include immunological responses and inflammation, which aid in the body's defense processes. Growth factors stimulate cell division, proliferation, and tissue repair, all of which are essential for development and healing. Antimicrobial peptides work by eliminating bacteria, fungi, and viruses to offer a natural defense against illnesses [18]. Nutraceutical proteins, which are present in foods like whey and casein, improve immune function and general health in addition to providing nutritional advantages. Bioactive proteins have several uses in medicine, biotechnology, and nutrition, which highlights their significance since they may lead to better disease prevention, innovative treatments, and an enhanced standard

Although bioactive proteins have many benefits, there are several barriers that keep them from being utilized extensively and effectively. One major challenge is stability; many bioactive proteins can be degraded by enzymatic breakdown, pH fluctuations, and temperature variations, all of which can reduce their efficacy [19]. Ensuring the stability of these proteins during transit, storage, and administration is crucial but difficult. Another challenge is getting them out of natural resources and purifying them, which sometimes requires pricy and intricate techniques. Additionally, the generation of neutralizing antibodies because of immunological reactions to bioactive proteins may cause allergic reactions or, over time, a decline in efficacy. Because complex delivery techniques are required to ensure that the proteins reach their targets without degrading or stopping, it is highly challenging to distribute these proteins to body parts or cells. In addition, there are obstacles to be addressed in terms of technology and legislation when producing bioactive proteins on a big scale, particularly when utilizing recombinant DNA technology [20]. Further research and innovation are needed to overcome these problems and provide bioactive protein-based medications and products that are more stable, safer, and efficacious.

IV. Antioxidant Peptides

Small bioactive fragments called antioxidant peptides (see Fig. 2), which are produced from proteins, are essential for reducing oxidative stress and neutralizing free radicals in both biological systems and food products. Usually, the enzymatic breakdown of proteins from different sources plants, animals, and marine organisms produce these peptides. Antioxidant peptides can be found, for example, in soy proteins, fish collagen, and milk proteins like whey and casein. When these proteins are broken down by digestive enzymes such as pepsin, trypsin, or alkalis, peptides with strong antioxidant properties are released [21]. These peptides scavenge free radicals, chelate pro-oxidant metal ions, and prevent lipid peroxidation as mechanisms for their antioxidant actions. Antioxidant peptides are useful in prolonging the shelf life of food goods because they inhibit oxidation, which can cause rancidity and spoiling. Furthermore, their inclusion in nutraceuticals and functional foods may improve health by lowering oxidative stress, which is connected to a number of chronic illnesses. Despite their potential, problems such as maintaining their stability during food processing and ensuring their bioavailability in the human body must be addressed if antioxidant peptides made from proteins are to be properly utilized. In the context of food systems, antioxidant peptides are especially intriguing because they offer a natural alternative to synthetic antioxidants, which are increasingly being scrutinized for potential health risks. By adding these peptides to food items, it is possible to improve the oxidative stability of dairy, meat, and oil products, increasing their shelf life and preserving their nutritional and sensory value. Furthermore, the utilization of antioxidant peptides is in line with the rising desire from consumers for clean-label goods those devoid of artificial ingredients [22]. Antioxidant peptides are particularly interesting in the context of food systems because they provide a natural substitute for synthetic antioxidants, which are coming under increased scrutiny due to possible health hazards. By adding these peptides to food items, it is possible to improve the oxidative stability of dairy, meat, and oil products, increasing their shelf life and preserving their nutritional and sensory value. Furthermore, the utilization of antioxidant peptides is in line with the rising desire from consumers for clean-label goods those devoid of artificial ingredients [23].

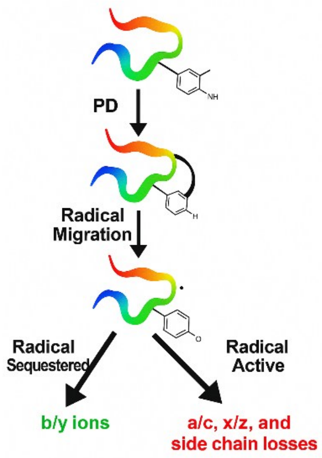

Fig. 2. Using RDD to identify antioxidant peptides

V. Separation of Antioxidant Peptides

An essential stage in the synthesis of antioxidant peptides is the separation of protein-derived peptides from complicated mixtures of hydrolyzed protein fragments, ensuring the isolation of peptides with the necessary bioactivity. Several methods are used in this procedure to concentrate and purify antioxidant peptides according to their size, charge, hydrophobicity, and other physicochemical characteristics. Enzymatic hydrolysis of proteins, which reduces big protein molecules into smaller peptides, is the initial stage in the separation process. The selection of an enzyme (such as alkalize, trypsin, or pepsin) and the hydrolysis conditions (such as pH, temperature, and the ratio of enzyme to substrate) have a major impact on the size and makeup of the peptides that are produced [24]. To separate peptides according to size, ultrafiltration membranes with certain molecular weight cut-offs (MWCO) are frequently utilized. Peptides, for example, can be separated into different ranges of molecular weight (<1 kDa, 1-5 kDa, >5 kDa), and smaller peptides frequently show stronger antioxidant activity because of their superior capacity to interact with free radicals [25]. By separating peptides according to their size and charge, nanofiltration can further refine the peptide fractions obtained from ultrafiltration and enable the concentration of peptides with characteristics that support antioxidant activity.

Peptides are separated using this method according to their charge. Positively charged peptides are bound by cation-exchange chromatography, whereas negatively charged peptides are bound by anion-exchange chromatography. Peptides can be eluted in order of charge by progressively altering the pH or salt concentration [26]. This process enables the isolation of peptides with certain charge properties that may be correlated with antioxidant activity. This technique is quite useful for dividing peptides according to how hydrophobic they are. More hydrophobic peptides elute later, separating the peptides as they flow through a column filled with hydrophobic material. Antioxidant peptides can be precisely isolated with this method, especially those having hydrophobic residues that improve their interaction with lipid radicals. This method, which is also referred to as gel permeation chromatography, divides peptides according to size. Smaller peptides take longer to travel through the column's porous beads than larger peptides, which elute first.

GPC can be used to fractionate peptides into more uniform size ranges for the purpose of analyzing their antioxidant activity. CE separates peptides based on their size-to-charge ratio in an electric field. This very sensitive and efficient technique can isolate very small amounts of peptides. Antioxidant peptides from complicated combinations can be analyzed and purified with its help [27].

VI. Identification of Antioxidant Peptides

It takes a multifaceted approach to isolate, characterize, and validate the bioactivity of antioxidant peptides generated from proteins. Larger protein molecules are broken down into smaller peptides by the process known as enzymatic hydrolysis, which usually starts the process. The peptides in this mixture are then separated according to their chemical characteristics using chromatographic techniques like high-performance liquid chromatography (HPLC) or ultrahigh-performance liquid chromatography (UHPLC) [28]. After separation, the molecular weight and sequence of the peptides are ascertained by mass spectrometry (MS), wherein methods such as Electrospray Ionization (ESI) and Matrix-Assisted Laser Desorption/Ionization (MALDI) offer comprehensive structural details. The amino acid sequence is further clarified by fragmenting the peptides and examining the resultant patterns using tandem mass spectrometry (MS/MS) [29]. To verify that the discovered peptides have antioxidant qualities, bioactivity tests are essential.

The ability of the peptides to neutralize free radicals and suppress oxidative processes is measured by techniques like the ORAC (Oxygen Radical Absorbance Capacity) assay, ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) assay, and DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay [30]. Further information about the conformation and stability of the peptide can be obtained by structural characterization methods such as circular dichroism (CD) and nuclear magnetic resonance (NMR) spectroscopy [31]. Lastly, the integration of data from diverse analyses is facilitated by bioinformatics tools, which assist forecast peptide activity and possible uses. This thorough method guarantees the precise identification of peptides that exhibit strong antioxidant activity, opening the door for their use in nutritional supplements and food systems [32]. Antioxidant peptide identification from proteins is a difficult but necessary procedure that includes mass spectrometry, enzymatic hydrolysis, sophisticated separation methods, bioactivity testing, and structural analysis [33]. Researchers can identify and characterize peptides with strong antioxidant activity by meticulously optimizing each stage, opening the door for their application in nutritional supplements and food systems. Peptide identification will become more accurate and efficient as analytical methods and bioinformatics continue to progress, opening new and more focused application areas [34].

VII. Method of Action of Antioxidant Peptides

Protein-derived antioxidant peptides work to reduce oxidative stress through several important processes. These peptides neutralize reactive oxygen species (ROS) such hydrogen peroxide, superoxide anions, and hydroxyl radicals by acting as scavengers of free radicals. Antioxidant peptides stop these free radicals from causing damage to lipids, proteins, and DNA by giving them an electron [35]. These peptides also can bind metal ions, such copper and iron, which act as catalysts in the Fenton and Haber-Weiss processes that produce ROS. Because of this chelation, there are fewer of these metal ions available, which lowers the production of free radicals.

Additionally, antioxidant peptides prevent lipid peroxidation, a process that damages and malfunctions cells by allowing free radicals to target polyunsaturated fatty acids in cell membranes [36]. By stopping this chain reaction, these peptides help to maintain the integrity and functionality of cells. Additionally, some antioxidant peptides may change oxidative stress-sensitive signaling pathways, affecting cellular defenses and repair mechanisms. Together, these effects support the peptides' capacity to shield cells from oxidative stress, lessen inflammation, and possibly even minimize the risk of chronic illnesses linked to oxidative stress [37].

VIII. Discussion

The study of protein-derived antioxidant peptides provides important new information on their possible uses in food systems and health promotion. These bioactive peptides are produced by the enzymatic degradation of proteins derived from various sources. They exhibit noteworthy antioxidant activity, which is essential for the preservation of food as well as the reduction of health issues associated with oxidative stress [38]. Understanding antioxidant peptides' action and possible uses requires first understanding their separation and identification. High-performance liquid chromatography (HPLC), membrane filtration, and ultrafiltration are some of the techniques that are essential for isolating peptides with the appropriate molecular weight and bioactivity. These methods enable the identification of peptide sequences with strong antioxidant qualities. Mass spectrometry (MS), which includes techniques like MALDI and ESI, helps further in the molecular characterization of the peptides by giving specific details about their composition and structure [39]. But there are difficulties in the separation process, especially when it comes to guaranteeing the peptides' production and quality. A variety of peptides with differing levels of antioxidant activity might result from the diversity of protein sources and the unpredictability of enzymatic hydrolysis. Antioxidant peptides have the power to bind metal ions and neutralize free radicals, making them useful tools in the fight against oxidative stress, a major cause of chronic disease development. Their function in preventing lipid peroxidation is especially important for food preservation because oxidation is a primary factor in food product deterioration and quality reduction. The amino acid sequence and content of the peptide, which can change depending on the hydrolysis circumstances and protein supply, determines how effective these mechanisms are. This emphasizes how crucial it is to pinpoint certain peptides that, in addition to having potent antioxidant action, also show stability and bioavailability in intricate dietary matrices and biological systems.

The integration of antioxidant peptides into food systems presents encouraging advantages, such as prolonged shelf life, improved nutritional content, and the possibility of promoting health via functional meals. These peptides' natural source meets consumer demand for clean-label goods devoid of artificial additives. By using them in food products like dairy, meat, and emulsions, they can greatly increase the oxidative stability of the product, maintaining its nutritional value and sensory appeal [40]. The integration of multi-omics data, including proteomics and genomics, improves the analysis's depth by revealing patterns of protein expression and post-translational changes that affect safety profiles and biological activity. Going forward, the full therapeutic potential of plant-derived bioactive proteins can only be possible with sustained progress in bioinformatics. To improve evidence-based applications in healthcare, agriculture, and other fields, this entails developing bioinformatics databases, improving predictive models, and incorporating cutting-edge technologies. The significance of responsible research and development in utilizing these natural substances for global health and environmental sustainability is further highlighted by ethical considerations related to sustainable sourcing procedures and fair distribution of biotechnological advantages. Antioxidant peptides offer an exciting potential for synergistic effects in functional foods when combined with other bioactive substances like vitamins and polyphenols. It is probable that antioxidant peptides will become more significant as this field of study develops, contributing to the creation of natural, health-promoting food products that meet consumer needs and address issues related to public health. The substantial potential of antioxidant peptides derived from proteins in food systems and health promotion is highlighted by research. Even if there are still obstacles to overcome, further research into these bioactive peptides should open new avenues for their use in the food sector and other fields.

The combination of antioxidant peptides with other bioactive compounds, such as polyphenols, vitamins, or minerals, holds great promise for enhancing functional efficacy through synergistic interactions. Such combinations may lead to improved antioxidant capacity, increased stability, and complementary health benefits, thereby broadening the scope of applications in functional foods and nutraceuticals. This integrated approach aligns with consumer preferences for natural, multi-functional ingredients and supports global efforts toward sustainable, health-promoting dietary solutions. As research continues to unravel the mechanisms of synergy and optimize formulation strategies, bioactive peptides are poised to play an increasingly vital role in advancing public health and meeting the demands of a growing market for clean-label and evidence-based functional food products

IX. Conclusion

This extensive investigation of protein-derived antioxidant peptides highlights their important potential as natural food preservation and health-promoting agents. These peptides may be efficiently isolated, recognized, and described by enzymatic hydrolysis of proteins derived from diverse sources. This process reveals their ability to celebrate metal ions, suppress lipid peroxidation, and neutralize free radicals. Because of these methods of action, antioxidant peptides are useful for reducing oxidative stress, which is a major contributing factor to the onset of chronic diseases, as well as for increasing the shelf life of food goods. The study emphasizes how crucial it is to refine methods of separation and identification in order to separate out the peptides that have the most antioxidant properties. Antioxidant peptides are a natural substitute for artificial preservatives in practical applications, meeting the increasing demand from consumers for clean-label goods. Their integration into food systems can boost sensory attributes, increase oxidative stability, and aid in the creation of functional meals that promote health. To reach their full potential, though, issues including peptide stability during processing, bioavailability, and regulatory approval need to be resolved. Antioxidant peptide uses may grow in the future thanks to ongoing research into new protein sources and the creation of creative peptide engineering and delivery methods. These bioactive substances are expected to become more significant in the food and health sectors as research advances since they provide safe, natural solutions for both food preservation and wellbeing enhancement.

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