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Bioactive Peptides from Fermented Foods as Natural Antimicrobials and Antioxidants

Harshita

Department of Pharmaceutical Sciences, Gurugram University, Gurugram, Samaspur, Haryana 122003-India

ABSTRACT

Fermented foods represent a rich source of bioactive peptides with significant antimicrobial and antioxidant properties, offering promising alternatives to synthetic preservatives and therapeutic agents. During fermentation, proteolytic enzymes from lactic acid bacteria, yeasts, and other microorganisms cleave food proteins into smaller peptide fragments that exhibit enhanced biological activity. These bioactive peptides demonstrate broad-spectrum antimicrobial efficacy against pathogenic bacteria, fungi, and viruses through multiple mechanisms, including membrane disruption, enzyme inhibition, and interference with cellular processes. Common fermented foods such as yogurt, kefir, cheese, kimchi, miso, and fermented fish products contain peptides with potent antimicrobial activity against foodborne pathogens like Escherichia coli, Salmonella, and Listeria monocytogenes. The antioxidant properties of these peptides contribute to their protective effects against oxidative stress, which is implicated in aging, cardiovascular disease, and cancer. Peptides from fermented dairy products, particularly those derived from casein and whey proteins, exhibit strong radical scavenging activity and metal chelation properties. Fermented plant-based foods also yield peptides with notable antioxidant capacity, including those from fermented soybeans and legumes. The dual functionality of these naturally occurring peptides makes them attractive candidates for food preservation, nutraceutical development, and therapeutic applications. Their biodegradability, safety profile, and consumer acceptance as natural food components position bioactive peptides from fermented foods as sustainable alternatives to conventional antimicrobial and antioxidant compounds. Future research should focus on optimizing fermentation conditions, characterizing peptide structures, and developing scalable extraction methods to fully exploit their commercial potential.

Keywords: *Kefir, Cheese, Kimchi, Miso, Escherichia coli, Salmonella, and Listeria Monocytogenes.*

Introduction

Fermented foods have emerged as a promising source of bioactive peptides (BAPs) that demonstrate significant antimicrobial and antioxidant properties, offering sustainable alternatives to synthetic preservatives and therapeutic agents [1]. BAPs are peptides consisting of 2 to 20 amino acids originating from precursor proteins following an activation process involving multiple reactions such as chemical hydrolysis, enzymatic hydrolysis operated by proteolytic enzymes, or microbial fermentation by proteolytic bacteria [2]. These small oligopeptides, typically containing 1-100 amino acid residues with amphipathic structures, are initially encrypted and inactive within parent proteins but become physiologically active upon release through various hydrolytic processes during fermentation [3]. The fermentation process represents a unique and natural method for producing bioactive peptides with specialized biological activities. During fermentation, proteolytic microorganisms, including lactic acid bacteria, yeasts, and filamentous fungi, break down proteins in food matrices, releasing peptide sequences that exhibit diverse health-promoting properties [4]. BAPs offer numerous health benefits, including antimicrobial, antioxidant, antihypertensive, and anti-inflammatory properties, making them valuable functional food ingredients with therapeutic potential [5].

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*Corresponding Author: **Harshita**
Email Address: **harshitasaini978@gmail.com**

The antimicrobial properties of fermented food-derived bioactive peptides stem from their ability to disrupt microbial cell membranes and interfere with essential cellular processes [6]. These peptides have a broad spectrum of action, showing an inhibitory effect on microorganisms such as bacteria, molds, yeasts, and parasites, offering natural preservation mechanisms for food products [7]. Fermented dairy products, particularly kefir and yogurt, have been extensively studied for their antimicrobial bioactive peptides [8]. Kefir exhibits antimicrobial properties due to being loaded with probiotics, organic acids, and bioactive compounds, which work together to inhibit harmful bacteria, fungi, and viruses [9].

The antimicrobial activity, bioactive peptides from fermented foods demonstrate significant antioxidant properties, protecting against oxidative stress and cellular damage [10]. These peptides can scavenge free radicals, chelate metal ions, and inhibit lipid peroxidation, contributing to their potential role in preventing chronic diseases associated with oxidative damage [11]. BAPs can possess antioxidant, antimicrobial, and antiviral properties, making them multifunctional bioactive compounds [12]. The production of bioactive peptides during fermentation is influenced by various factors, including microbial strain selection, fermentation conditions, substrate composition, and processing parameters [13]. Different fermentation approaches can yield peptides with varying degrees of bioactivity, emphasizing the importance of optimizing production conditions to maximize therapeutic potential [14]. The primary functions of fermented food-derived BAPs are antihypertensive, antioxidant, and antibacterial [15]. Recent research has expanded beyond traditional fermented dairy products to explore bioactive peptides from fermented cereals, legumes, meat products, and plant-based sources [16]. This diversification offers opportunities to develop novel functional foods and nutraceuticals targeting specific health conditions [17]. The growing interest in natural antimicrobials and antioxidants, driven by consumer demand for clean-label products and concerns about synthetic additives, positions bioactive peptides from fermented foods as promising alternatives for food preservation and health enhancement [18].

Production of Bioactive Peptides in Fermented Foods

Bioactive peptides (BAPs) are unique, low molecular weight peptide sequences generally consisting of 2–20 amino acid residues that exhibit physiological activities beyond basic nutrition [19]. These peptides remain inactive within parent protein molecules but become biologically active once released through hydrolysis processes, particularly during microbial fermentation [20]. Fermented foods have emerged as a significant source of BAPs with diverse health benefits, including antimicrobial, antioxidant, antihypertensive, and immunomodulatory properties.

Production of Bioactive Peptides in Fermented Foods

Microbial Fermentation as a Source of BAPs

Microbial fermentation represents a novel and efficient method for producing specialized bioactive peptides from various dietary matrices. During fermentation, proteolytic microorganisms secrete enzymes that cleave specific peptide bonds in food proteins, releasing bioactive sequences that were previously encrypted within the parent protein structure [21]. The fermentation process not only preserves food but also enhances its nutritional value by generating peptides with therapeutic potential. The proteolytic activity of fermentation microorganisms is crucial for BAP production. Lactic acid bacteria (LAB), particularly Lactobacillus and Streptococcus species, are primary contributors to peptide liberation through their complex proteolytic systems [22]. These microorganisms possess cell wall-associated proteinases and intracellular peptidases that work synergistically to break down proteins into smaller, bioactive fragments.

Influence of Microbial Strains and Fermentation Conditions

The production of BAPs is significantly influenced by the specific microbial strains used and the fermentation conditions employed. Different bacterial strains exhibit varying proteolytic capabilities, resulting in distinct peptide profiles [23]. For instance, *Lactobacillus helveticus* has been extensively studied for its ability to produce ACE-inhibitory peptides, while Lactobacillus plantarum strains are recognized for generating antimicrobial peptides. Environmental factors such as temperature, pH, fermentation time, and substrate composition significantly impact peptide production. Optimal fermentation conditions typically involve temperatures between 30-45°C, with pH values ranging from 4.5 to 6.5, depending on the specific microorganism and substrate used [24]. Extended fermentation periods generally increase peptide yields, though excessive fermentation may lead to peptide degradation or the formation of bitter compounds.

Common Food Sources of Fermentation-Derived BAPs

Dairy Products: Fermented dairy products represent the most extensively studied source of BAPs. Yogurt, kefir, and fermented milk products contain numerous bioactive peptides derived from casein and whey proteins [25]. The proteolytic activity of starter cultures, particularly Lactobacillus bulgaricus and Streptococcus thermophilus, releases peptides with ACE-inhibitory, antimicrobial, and antioxidant properties.

Plant-Based Foods: Fermented legumes, cereals, and soy products are rich sources of plant-derived BAPs. Soy sauce, miso, and tempeh contain peptides with antihypertensive and antioxidant activities [26]. Fermented bean pastes and fermented cereals also contribute significant amounts of bioactive peptides with diverse physiological functions.

Meat Products: Fermented meat products such as dry-cured ham, salami, and fermented sausages contain peptides derived from muscle proteins. These peptides exhibit antimicrobial, ACE-inhibitory, and antioxidant properties [27].

Mechanisms of Bioactivity

Structural Features and Bioactivity Relationship

The bioactivity of fermentation-derived peptides is closely related to their structural characteristics. Peptide length, amino acid composition, and sequence significantly influence biological activity. Generally, peptides with 2-20 amino acids exhibit optimal bioactivity, with shorter peptides often showing higher stability and bioavailability [28]. The presence of specific amino acids at terminal positions often determines peptide activity. For antimicrobial peptides, the presence of cationic amino acids (lysine, arginine) and hydrophobic residues contributes to membrane-disrupting capabilities. The amphipathic nature of these peptides allows them to interact with bacterial cell membranes, leading to antimicrobial effects [29].

Peptide Release and Stability During Fermentation

The release of bioactive peptides during fermentation is a complex process involving multiple proteolytic enzymes working in sequential steps. Cell wall-associated proteinases initiate protein hydrolysis, while intracellular peptidases further cleave the resulting peptides into smaller, bioactive fragments [30]. The stability of these peptides depends on their resistance to further proteolytic degradation and their ability to maintain structural integrity under fermentation conditions. Peptide stability is influenced by factors such as pH, temperature, and the presence of other peptides or proteins. Some peptides may undergo further modifications during fermentation, potentially altering their bioactivity profile [31].

Antimicrobial Properties of Fermentation-Derived BAPs

Mechanisms of Antimicrobial Action

Fermentation-derived antimicrobial peptides exhibit diverse mechanisms of action against pathogenic microorganisms. The primary mechanism involves disruption of microbial cell membranes through electrostatic interactions and membrane permeabilization [32]. These peptides typically possess cationic charges that allow them to interact with negatively charged bacterial cell surfaces, leading to membrane destabilization and cell death. Additional mechanisms include interference with DNA replication, inhibition of protein synthesis, and disruption of enzymatic processes essential for microbial survival.

Some peptides also exhibit antifungal properties by interfering with fungal cell wall synthesis or membrane integrity [33].

Examples of Antimicrobial BAPs from Various Fermented Foods

Dairy-Derived Peptides: Lactoferricin, derived from lactoferrin during fermentation, exhibits broad-spectrum antimicrobial activity against both Gram-positive and Gram-negative bacteria. Nisin, produced by Lactococcus lactis, is a well-characterized bacteriocin with potent antimicrobial properties against various foodborne pathogens [34].

Plant-Derived Peptides: Fermented soy products contain peptides with antimicrobial activity against Escherichia coli, Staphylococcus aureus, and various fungi. These peptides contribute to the natural preservation of fermented foods and provide health benefits to consumers [5].

Meat-Derived Peptides: Fermented meat products yield peptides with antimicrobial properties, particularly against Listeria monocytogenes and other food borne pathogens. These peptides contribute to the safety and shelf-life extension of fermented meat products [6].

Potential Applications as Natural Food Preservatives

The antimicrobial properties of fermentation-derived BAPs offer significant potential for natural food preservation. These peptides can serve as alternatives to synthetic preservatives, addressing consumer demands for natural and clean-label products. Their incorporation into food systems can extend shelf life, maintain food safety, and provide additional health benefits [17]. The application of antimicrobial peptides in food preservation requires careful consideration of factors such as peptide stability, interaction with food components, and maintenance of organoleptic properties. Research continues to explore optimal delivery systems and formulations to maximize the preservative efficacy of these natural compounds.

Antioxidant Activities of Fermentation-Derived BAPs

Mechanisms of Antioxidant Action

Antioxidative peptides prevent reactive oxygen species from causing damage by acting as direct radical scavengers, and metal chelators, and by removing radical compound precursors [12]. The antioxidant mechanisms of fermentation-derived bioactive peptides operate through multiple pathways that collectively protect against oxidative damage. Figure 1 shows the antioxidant mechanism of fermentation-derived peptides.

Free Radical Scavenging: Antioxidants are known to scavenge various free radicals like hydroxyl radicals and peroxyl radicals, and also inhibit lipid peroxidation. Fermentation-derived peptides demonstrate significant radical scavenging activity through their ability to donate electrons or hydrogen atoms to neutralize free radicals [19]. The amino acid composition, particularly the presence of aromatic amino acids such as tyrosine, tryptophan, and phenylalanine, contributes to the electron-donating capacity of these peptides.

Metal Chelation: Many antioxidant peptides exhibit metal-chelating properties, binding to transition metals like iron and copper that catalyze oxidative reactions. The presence of histidine, cysteine, and methionine residues in peptide sequences enhances their metal-chelating capabilities, preventing metal-catalyzed oxidation [31].

Lipid Peroxidation Inhibition: Fermentation-derived peptides can interrupt lipid peroxidation chain reactions by scavenging lipid peroxyl radicals and preventing the formation of secondary oxidation products. This mechanism is particularly important in food systems where lipid oxidation affects both nutritional quality and sensory properties [32].

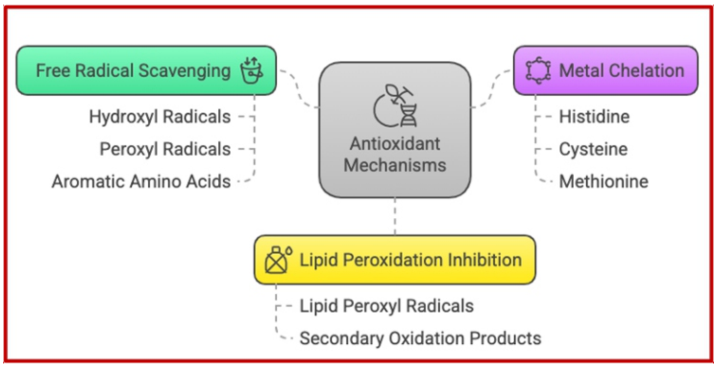

Fig 1. Antioxidant mechanism of fermentation-derived peptides

Notable Antioxidant Peptides and Their Sources

Dairy-Derived Peptides: Dairy products such as milk, yogurt, fermented milk, and cheese have been found to have antioxidant effects, possibly due to the functional activity of their protein components (casein and whey proteins) and/or peptide fragments from different protein fractions in the matrix. Specific peptides like VAPFPEVFGK and LLVYPFPGPLH have been identified in fermented dairy products with notable antioxidant properties [33].

Plant-Based Sources: An increasing number of peptides and hydrolysates possessing antioxidant capacity have been characterized by soybean and other soy-based products. Fermented soy products, including tempeh, miso, and fermented soy sauce, contain peptides with significant antioxidant activities. The peptide LLPHH from soy protein hydrolysates has shown potent radical scavenging activity [7].

Mushroom-Derived Peptides: Oxidative stress has been linked with the pathogenesis of many human diseases, including cancer, aging, and atherosclerosis. The present study investigates the antioxidant activities of peptides isolated from the medicinal mushroom, Ganoderma lucidum. Fermented mushroom products yield peptides with unique antioxidant properties that contribute to their therapeutic potential.

Marine-Derived Peptides: Fermented fish products and marine protein hydrolysates contain peptides with strong antioxidant activities. These peptides often demonstrate superior stability and bioactivity compared to their terrestrial counterparts [19].

Enhancement of Antioxidant Capacity Through Fermentation

Fermentation significantly enhances the antioxidant capacity of food matrices through several mechanisms. The proteolytic activity of fermentation microorganisms releases encrypted antioxidant peptides from parent proteins, increasing their bioavailability. Following fermentation, the total polyphenol content only increased slightly; however, the flavonoid content increased by 24.3%. The radical scavenging activity increased from 22.4 to 27.5% and the XO inhibitory activity increased from 20.2 to 62.4% at 500 μg/ml, demonstrating the effectiveness of fermentation in enhancing antioxidant properties.

The fermentation process also facilitates the conversion of bound antioxidants into free forms, increasing their accessibility and biological activity. Specific microbial strains, particularly lactic acid bacteria, produce enzymes that selectively cleave peptide bonds to release bioactive sequences with enhanced antioxidant potential [32].

Factors Influencing Yield and Bioactivity

Impact of Fermentation Parameters

Temperature: Fermentation temperature significantly affects peptide production and bioactivity. Optimal temperatures typically range between 30-45°C, with higher temperatures potentially enhancing proteolytic activity but risking peptide degradation. The optimal process for preparing angiotensin-converting enzyme (ACE)-inhibitory peptides by EH in sunflower seed meals through single-factor and orthogonal experiments was determined to be: solution pH of 8, enzyme addition at 7%, water bath temperature at 55°C, and hydrolysis time of 2 h, indicating the critical role of temperature optimization.

pH: The pH of fermentation media influences both microbial growth and proteolytic enzyme activity. Most lactic acid bacteria produce optimal proteolytic activity at pH values between 5.5-6.5, though specific strains may have different pH optima. pH also affects peptide stability and bioactivity, with extreme pH values potentially causing peptide degradation [3].

Time: Fermentation time directly correlates with peptide yield and bioactivity. Extended fermentation periods generally increase peptide production but may lead to over-hydrolysis and loss of bioactivity. The optimal fermentation time varies depending on the substrate and microorganisms used, typically ranging from 12-72 hours [12].

Role of Microbial Species and Substrate Selection

Microbial Species: Different microbial strains exhibit varying proteolytic capabilities and produce distinct peptide profiles. Lactobacillus helveticus, L. plantarum, and L. casei are particularly effective at producing antioxidant peptides. The choice of microbial strain significantly influences both peptide yield and bioactivity [13].

Substrate Selection: The protein source and its composition determine the potential for bioactive peptide production. Substrates rich in specific amino acids (histidine, tyrosine, tryptophan) tend to yield peptides with enhanced antioxidant properties. The degree of protein denaturation and accessibility also affects peptide release during fermentation [14].

Technological Innovations to Enhance BAP Production

Encapsulation Technologies: Encapsulation techniques protect bioactive peptides from degradation during processing and storage while enhancing their bioavailability. Microencapsulation using polysaccharides or proteins can improve peptide stability and controlled release [15].

Ultrasound-Assisted Fermentation: Ultrasonic treatment can enhance cell wall permeability and enzyme activity, leading to increased peptide production. The mechanical effects of ultrasound facilitate protein unfolding and improve substrate accessibility to proteolytic enzymes [17].

Enzymatic Pre-treatment: A Combination of enzymatic hydrolysis with fermentation can enhance peptide yield and bioactivity. Sequential treatment with specific proteases followed by fermentation optimizes peptide release and maintains bioactive properties [12].

Challenges and Future Perspectives

Limitations in Yield, Stability, and Bioavailability

Yield Limitations: The production of bioactive peptides through fermentation faces challenges related to low yields and inconsistent production. The complexity of fermentation processes and the influence of multiple variables make it difficult to achieve reproducible high yields. Scale-up from laboratory to industrial production presents additional challenges in maintaining optimal conditions [9].

Stability Issues: Bioactive peptides are susceptible to degradation by proteases, extreme pH, and temperature fluctuations. The stability of peptides during processing, storage, and gastrointestinal transit limits their practical applications. Developing strategies to enhance peptide stability without compromising bioactivity remains a significant challenge [9].

Bioavailability Concerns: The absorption and bioavailability of bioactive peptides in the human body are often limited by factors such as peptide size, charge, and resistance to digestive enzymes. Many peptides may be degraded before reaching their target sites, reducing their therapeutic efficacy [19].

Regulatory and Commercialization Aspects

The commercialization of fermentation-derived bioactive peptides faces regulatory challenges related to safety assessment, health claims validation, and standardization. Regulatory authorities require comprehensive toxicological studies and clinical trials to establish safety and efficacy. The complexity of peptide characterization and the need for consistent quality control add to the commercialization challenges [32].

Future Research Directions and Applications in Functional Foods

Advanced Characterization Techniques: Future research should focus on developing advanced analytical methods for peptide identification and quantification. Mass spectrometry-based approaches and bioinformatics tools will enhance our understanding of structure-activity relationships and facilitate the discovery of novel bioactive peptides [24].

Personalized Nutrition: The development of personalized fermented foods containing specific bioactive peptides tailored to individual health needs represents a promising future direction. Understanding genetic variations in peptide metabolism and response will enable the design of customized functional foods [25].

Synergistic Effects: Future research should explore the synergistic effects of multiple bioactive peptides and their interactions with other food components. Understanding these interactions will enable the development of more effective functional food formulations with enhanced health benefits [26].

Conclusion

Fermentation-derived bioactive peptides with antioxidant properties represent a promising avenue for developing functional foods with enhanced health benefits. While significant challenges remain in terms of yield optimization, stability, and bioavailability, continued research and technological innovations are addressing these limitations. The future of bioactive peptide production lies in the integration of advanced fermentation technologies, improved characterization methods, and personalized nutrition approaches to maximize their therapeutic potential and commercial viability.

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