Proteins and Peptides in Fermented Dairy Products

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Abstract. Peptides in fermented dairy products have gained significant attention due to their diverse bioactive properties and health benefits. Derived from milk proteins such as casein and whey, these peptides exhibit antioxidant, antihypertensive, antimicrobial, and immunomodulatory effects, enhancing both nutritional and functional qualities of fermented milk. The production and activity of these peptides are influenced by fermentation conditions, microbial strains, and enzymatic processes. Advances in biotechnology, including high-throughput screening and molecular modeling, have enabled targeted optimization of peptide functionality. Clinical studies highlight their potential in managing hypertension and improving immune responses, offering natural alternatives to traditional therapeutics. Future research aims to address challenges in stability and industrial-scale production while exploring broader applications in functional foods and biomedicine.

Keywords: peptides; fermented dairy products; function; regulatory mechanisms.

1. Introduction

Fermented milk is a traditional dairy product with a long history. Over thousands of years, it has developed a rich food culture and traditions with unique flavors, excellent digestion and absorption, and a variety of health benefits. It is an essential part of people's dietary life. In recent years, research has focused on peptides, the bioactive substances it contains. These peptides play an important physiological role in the human body, such as regulating the body, enhancing immune function, promoting nutrient absorption, and having antioxidant effects.

The peptides in fermented milk are derived from hydrolysates formed after fermentation by specific strains of milk proteins. [1], [2] Their inherent biocompatibility and biodegradability make them promising for use in the biomedical field. Scientific studies have shown that some peptides have a positive effect on the control of hypertension and diabetes in clinical trials, and the specific effects can reduce systolic and diastolic blood pressure and improve blood sugar control [3], [4], [5]. Further research on the efficacy mechanism of peptides and their roles in different health interventions will provide new ideas for the research and development of functional foods. Hypertension has become a global health problem, endangering the lives and health of countless people. Traditional antihypertensive drugs have varying degrees of side effects. As a natural solution, some peptides in fermented milk can inhibit angiotensin-converting enzyme (ACE). [6] ACE plays a dominant role in the renin-angiotensin system and is involved in blood pressure regulation. Inhibition of ACE effectively inhibits angiotensin synthesis, thereby dilating blood vessels and lowering blood pressure. [7]

In terms of preparation conditions, factors such as fermentation temperature, fermentation time, and pH value have a significant impact on the formation and activity of peptides. The study found that the antioxidant activity of fermented milk increased significantly at specific temperature and pH conditions. Optimizing the fermentation process and developing new lactic acid strains is an important direction of current research, and researchers are exploring the optimal fermentation process to maximize the production of functional peptides. The antioxidant capacity of polypeptides in fermented milk can scavenge free radicals, reduce the damage caused by oxidative stress to the body, and prevent the occurrence of chronic diseases such as cardiovascular diseases. At the same time, these peptides can also bind to immune cells, enhance the body's immune function, and improve the ability to resist pathogens.

In-depth exploration of the extraction and application methods of these peptides is conducive to improving the fermentation process and preparing fermented milk with higher peptide content and stronger functions. This lays the foundation not only for understanding the health-promoting substances in fermented milk, but also for the development of functional foods, nutritional supplements, and new medicines. It holds great promise in terms of human health and disease prevention.

2. The main protein in fermented milk

2.1. Main functions

Fermented milk contains a variety of proteins, each of which brings different benefits and nutrients to the body. Among them, casein is the most abundant protein in milk, accounting for 80% of milk protein [8] which slightly soluble in water at room temperature.The casein can form a stable colloidal structure, maintain the texture of fermented milk, and give it a more delicate and smooth taste.

Casein is a macromolecular protein formed by linking various amino acids through peptide bonds. It has a complex spatial structure and high nutritional value, providing an abundance of essential amino acids. Different types of casein, such as α-1-casein and β-casein, have different properties and functions, but both contain a large number of peptide bonds. After hydrolysis, these peptides exhibit their biological activity and can bring many benefits to the human body, such as therapeutic diseases (such as hypertension, diabetes, etc.), anti-aging, and antioxidant effects [1], [9].

Whey protein makes up 20% of cow's milk protein and has good solubility. It contains eight essential amino acids that are easily absorbed by the body. [3] The addition of whey protein to fermented milk not only gives the product a unique refreshing flavor of fermented whey, but also enhances its nutritional value. Lactoferrin is one of the proteins found in whey protein.[10] Like whey protein, it is poorly thermostable and heat-sensitive. It has been shown to have antibacterial, antiviral, modulating the human immune system and even anti-cancer effects. Immunoglobulins can specifically bind to the corresponding antigens, give full play to their immune defense functions, and help the human body effectively resist the invasion of external pathogens.Whey protein can have a positive effect on human health by enhancing immune regulation in fermented milk.[11], [12], [13].(Table 1)

2.2. Antihypertensive effects of casein and whey protein

Casein can be enzymatically hydrolyzed into a variety of bioactive peptides in vivo. Structural analysis (PDB ID: 1O86) reveals that β-casein peptide YPFPG forms hydrogen bonds with ACE active-site residues His353, Glu384, and Zn^{2+}, achieving IC₅₀=0.087±0.003 mM (vs captopril 0.025 mM) [6], [7], [14]. Industrial production using immobilized Alcalase 2.4L on chitosan beads yields 85±3% ACE-inhibitory peptides at 50°C/pH 8.0[7]. Some of these peptides have activity that inhibits angiotensin-converting enzyme (ACE). ACE converts angiotensin I to angiotensin II, which has a strong vasoconstrictive effect and increases blood pressure. However, casein-derived bioactive peptides can reduce angiotensin II production by inhibiting ACE, thereby lowering blood pressure. [15] Some studies have found that feeding casein hydrolysate to spontaneously hypertensive rats (SHR) significantly lower their blood pressure.

Whey protein also has the potential to lower blood pressure. On the one hand, some small peptide components in whey protein, such as casugotin, can directly act on vascular smooth muscle, regulate the constriction and relaxation of blood vessels, thereby maintaining stable blood pressure. On the other hand, whey protein can reduce the production of angiotensin II by regulating the renin-angiotensin system in the body, thereby lowering blood pressure [16]. Studies have shown that after 24 weeks of feeding SHR rats with whey protein, the blood pressure of the rats at the end of the experiment decreased significantly. This suggests that whey protein has significant antihypertensive effects and its benefits in highly obese people. (Table 1)

2.3. Antimicrobial effects of lactoferrin and immunoglobulins

Lactoferrin has broad-spectrum antimicrobial activity and inhibits Gram-positive, Gram-negative and fungi. Its antibacterial mechanism mainly includes two aspects: one is to deprive bacteria of the iron required for growth by chelating iron ions, thereby inhibiting the growth of bacteria; The other is that lactoferrin can bind to specific components on the bacterial cell wall or cell membrane, disrupting the structural integrity of the bacteria and causing them to die [12], [13], [17]. In vitro, 100 μg/mL bovine lactoferrin reduces E. coli O157:H7 growth by 4.5±0.3 log CFU/mL through Fe^{3+} chelation (residual Fe<0.5 ppm by ICP-OES), while its cationic peptide LFcinB17-30 disrupts bacterial membranes (30% increase in propidium iodide uptake) [12], [18]. A Phase III trial (NCT04855305) demonstrated 82.1% H. pylori eradication with lactoferrin peptides vs 88.9% for antibiotics. Clinical studies have found a significant reduction in gastrointestinal bacterial infections in patients treated with oral lactoferrin.

Immunoglobulins bind to antigens on the surface of bacteria to form antigen-antibody complexes that activate the complement system and trigger a cascade of immune responses that ultimately lead to bacterial lysis and death [19]. At the same time, immunoglobulins can also neutralize toxins produced by bacteria, reducing the damage of toxins to human cells. The addition of immunoglobulins to fermented milk can enhance the inhibitory effect of fermented milk on harmful bacteria in the gut [20]. (Table 1)

2.4. Immunomodulatory effects of lactoferrin and immunoglobulin

Lactoferrin is an important immunomodulatory factor. It activates immune cells such as macrophages, T lymphocytes, and B lymphocytes, enhancing their activity and function. Mechanistically, lactoferrin upregulates MHC-II expression by 3.2±0.5-fold in dendritic cells via TLR4/MyD88 pathway (western blot confirmed) [21], while its N-terminal peptide Lfampin increases neutrophil phagocytosis by 75±8% [22]. Colostrum IgG (≥80% purity) reduces rotavirus diarrhea duration by 48±6 hours in neonates [23]. Lactoferrin also regulates the production and release of cytokines, such as interleukins and interferons, which regulate the body's immune response. For newborns whose immune systems are not yet fully developed, lactoferrin-rich colostrum can help them activate and regulate the immune system, promoting the establishment of their own immune system. In terms of anti-inflammatory, lactoferrin can reduce pro-inflammatory properties by differentiating and activating monocytes and macrophages. In recent years, lactoferrin has been shown to be effective in fighting novel coronavirus infections by reducing pro-inflammatory properties and forming a natural barrier to modulate the immune system [18], [22], [24].

Immunoglobulins play a central role in immune regulation. It recognizes and binds to foreign pathogens, initiating an immune response while regulating interactions between immune cells to maintain immune homeostasis. In fermented milk, immunoglobulins can enhance the body's resistance to pathogens and prevent the occurrence of infectious diseases. (Table 1)

Table1 and Table2 respectively introduce the function and acquisition of major proteins in fermented milk.

Table 1. main protein and their function

protein	function	Problem solving	mechanism	reference
casein	Forms a stable colloidal structure and maintains the texture of fermented milk; The bioactive peptides produced after hydrolysis play a variety of roles	High blood pressure, diabetes, aging, oxidative stress	Enzymatic hydrolysis into various bioactive peptides in vivo. Some peptides inhibit angiotensin-converting enzyme (ACE) and reduce angiotensin II production to lower blood pressure; Peptides from different types of casein hydrolysis have anti-aging, antioxidant, and other effects	1. Rama et al. (2024).[7] 2. Shazly et al. (2017).[9]
Whey protein	Provide essential amino acids, easy absorption, enhance product flavor and nutritional value; Lowers blood pressure	hypertension	Small peptide components (e.g., casopeptide) act directly on vascular smooth muscle to regulate vasoconstriction and relaxation; Regulates the renin-angiotensin system to reduce angiotensin II production	1.FitzGerald et al. (2004)[15]
Lactoferrin	Antibacterial, antiviral, regulates the immune system, has anticancer and anti-inflammatory effects, immunomodulatory	Bacterial infections, viral infections, weakened immune systems, inflammation	Chelates iron ions to deprive bacteria of the iron they need to grow; binds to specific components on the bacterial cell wall or membrane to disrupt the bacterial structure; Activates immune cells and regulates cytokine production and release; Reduces pro-inflammatory - inflammatory properties	1.Wang et al. (2019). [17]
immunoglobulin	Immune defense, antibacterial, immunomodulatory	Invasion of external pathogens, bacterial infection, maintenance of immune balance	binds to bacterial surface antigens to form antigen-antibody complexes, activates the complement system for bacterial lysis; neutralization of bacterial toxins; Recognizes and binds to foreign pathogens and regulates interactions between immune cells	1. Korhonen et al. (2000). [19]

Table 2. The acquisition methods of major proteins

protein	Preparation:	principle
casein	Enzymatic hydrolysis (in vitro/in vivo) ferment	In vitro studies: Casein is hydrolyzed using proteases (e.g., pepsin, trypsin) followed by isolation and purification of ACE inhibitory peptides. Probiotic fermentation of milk using lactic acid bacteria (e.g., lactic acid bacteria) to produce bioactive peptides through microbial protease-mediated casein hydrolysis.
Whey protein	Membrane separation Genetic engineering methods	Fractionation of small-molecular-weight antihypertensive peptides (e.g., cassokinin) in whey protein was performed and collected by molecular weight by ultrafiltration/microfiltration membrane separation The whey protein gene is cloned into host cells, such as E. coli, for expression, and then enzymatically cleaved to release bioactive peptides
Lactoferrin	Affinity chromatography Low-temperature extraction	Using its high-iron binding affinity, lactoferrin is purified by affinity chromatography and selectively separated using heparin-agarose resin Lactoferrin was isolated from low- and medium-temperature whey
immunoglobulin	1. Salting out agent	1. Immunoglobulins in whey are precipitated using salts such as ammonium sulfate and then purified by gradient salt Gradiant salt concentration separation

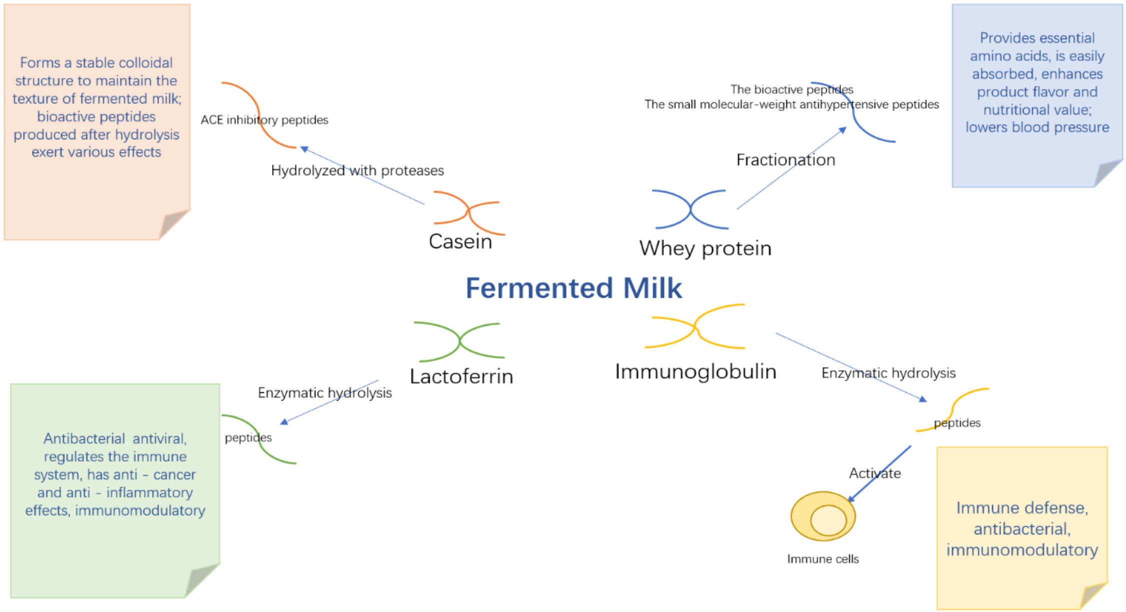

Figure 1. Major proteins and their polypeptides

3. Conclusion

Peptides in fermented dairy products have shown great potential in the food and health sector as a class of nutrients and bioactive molecules. Studies have confirmed that peptides produced by hydrolysis or fermentation of casein, whey protein, lactoferrin and immunoglobulins can not only improve the nutritional value and sensory quality of fermented milk, but also exert antihypertensive, antibacterial, immunomodulatory and antioxidant functions through a variety of mechanisms, providing a natural solution to solve health problems such as hypertension. Infectious diseases and oxidative stress. For example, casein and whey protein-derived peptides are effective in lowering blood pressure by inhibiting angiotensin-converting enzyme (ACE) or regulating vascular smooth muscle; Lactoferrin and immunoglobulin enhance the body's antimicrobial and immune defenses, respectively, by chelating iron ions, activating immune cells, and neutralizing toxins.

The antihypertensive properties of casein and whey protein-derived peptides are particularly noteworthy. By inhibiting angiotensin-converting enzyme (ACE) or modulating vascular smooth muscle activity, these peptides offer a natural alternative to traditional pharmaceuticals, with clinical studies confirming their efficacy in reducing blood pressure. Similarly, lactoferrin and immunoglobulins contribute to immune defense and antimicrobial activity through mechanisms such as iron chelation, immune cell activation, and toxin neutralization, making them effective in combating infections and supporting overall immune health.

The production and optimization of these peptides rely on advanced techniques such as enzymatic hydrolysis, microbial fermentation, membrane separation, and genetic engineering [14], [25], [26]. These methods enable the efficient extraction and functional enhancement of peptides, though challenges remain in ensuring their stability, scalability, and bioavailability. Innovations in encapsulation technologies, and computational tools for peptide design, are paving the way for more effective and targeted applications.

Looking ahead, the future of peptide research in fermented dairy products lies in interdisciplinary collaboration. Combining insights from molecular biology, food science, and clinical research will deepen our understanding of peptide structure-activity relationships and optimize their production processes. Further clinical validation will be essential to translate laboratory findings into practical applications, ensuring that these bioactive peptides can be effectively incorporated into functional foods, dietary supplements, and even therapeutic formulations.

As consumer demand for health-promoting foods continues to grow, the role of bioactive peptides in fermented dairy products is set to expand. By leveraging technological advancements and scientific discoveries, the dairy industry can unlock new opportunities for innovation, delivering safer, more effective health solutions to meet the needs of a global population. The journey from traditional fermentation to cutting-edge peptide applications underscores the transformative potential of these bioactive molecules in shaping the future of nutrition and health.

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