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Review

Applied Advances in Whey Bioactive Peptides: Enzymatic Generation, Mechanisms of Action, and Health-Related Applications

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Abstract

Whey is a major by-product of the dairy industry and represents a valuable source of proteins that can be enzymatically converted into bioactive peptides with diverse health-related functions. In recent years, increasing attention has been given to whey-derived peptides due to their antioxidant, antihypertensive, antimicrobial, anti-inflammatory, antithrombotic, immunomodulatory, and anticancer activities, highlighting their potential use as functional ingredients and nutraceutical compounds. The generation and biological functionality of these peptides are strongly influenced by the protein source, processing conditions, enzymatic or microbial hydrolysis strategies, and peptide structure. Unlike the existing literature, this review provides an analysis of individual peptide sequences, meticulously linking their specific chemical structures to their diverse biological activities, such as antioxidants, antihypertensive, and immunomodulatory effects. By moving beyond general protein hydrolysis, this work offers a unique comparative framework that evaluates how these distinct peptide fractions perform under industrial conditions. Furthermore, it bridges the gap between laboratory discovery and commercial implementation, focusing on critical parameters for large-scale production, stability in functional food matrices, and the regulatory pathways required for market-ready nutraceuticals. This integrated approach provides a strategic roadmap for translating molecular bioactivity into high-value industrial applications. This review provides an applied overview of recent advances in the production of whey bioactive peptides, emphasizing enzymatic generation methods, structure–activity relationships, and underlying mechanisms of action associated with their biological effects. In addition, current and emerging applications of whey-derived peptides in functional foods, nutraceuticals, and health-oriented formulations are critically discussed. Finally, key challenges related to peptide stability, bioavailability, industrial scalability, and regulatory aspects are addressed to identify future perspectives for the effective translation of whey bioactive peptides from research to practical applications.

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1. Introduction

Cow's milk contains approximately 3% protein, of which nearly 80% corresponds to caseins, while the remaining 20% is represented by whey proteins. Whey proteins are mainly composed of β-lactoglobulin (β-Lg), α-lactalbumin (α-La), immunoglobulins (Ig), bovine serum albumin (BSA), proteose–peptone fractions, and glycomacropeptide (GMP), whereas lactoferrin (Lf) and lactoperoxidase are present in smaller amounts. Collectively, these proteins have attracted considerable scientific interest because they can be hydrolyzed to generate peptides with diverse biological activities [1].

The biological functionality of peptides derived from whey proteins is largely influenced by their amino acid sequence, length, and composition. Even peptides with identical sequences and molecular sizes may exhibit different biological activities due to variations in their chemical modifications, hydrophobicity, or conformational structure [2]. These characteristics determine peptide–target interactions and play a critical role in defining their physiological effects.

In recent years, bioactive peptides derived from whey proteins have gained increasing attention due to their potential applications in the development of functional foods, nutraceuticals, and health-oriented formulations. Enzymatic hydrolysis, gastrointestinal digestion, and microbial fermentation are among the main processes responsible for releasing these peptides from parent proteins, enabling the expression of antioxidant, antihypertensive, antimicrobial, immunomodulatory, and other health-related activities. As a result, whey-derived peptides are currently considered promising natural alternatives for food enrichment and disease risk reduction strategies.

Despite the extensive research conducted on whey-derived bioactive peptides, several challenges remain, particularly those related to production optimization, peptide stability during gastrointestinal digestion, limited bioavailability, and the translation of in vitro findings into consistent in vivo effects. Factors such as processing conditions, hydrolysis methods, and peptide stability significantly influence the biological activity and applicability of these compounds. Therefore, a comprehensive and applied understanding of whey-derived peptides, including their generation mechanisms, structure–activity relationships, and potential health benefits, is essential.

This review compiles and analyzes current knowledge on bioactive peptides obtained from whey proteins, with particular emphasis on their production strategies, biological activities, and practical applications in food and health-related fields.

A comprehensive search of the peer-reviewed literature published between 2015 and 2025 was performed using major scientific databases such as PubMed, Scopus, ScienceDirect, and Google Scholar. Keywords relating to whey proteins, bioactive peptides, enzymatic hydrolysis, functional foods, nutraceuticals, and health applications were combined to identify eligible records. Studies were included if they provided original research or clinical data on therapeutic outcomes and formulation strategies, as well as some reviews which provided relevant data for this compilation. Non-English texts and commentaries were excluded. The selection process favored high-impact publications from the last ten years, ensuring a focus on contemporary evidence while acknowledging foundational research.

2. Major Whey Proteins and Associated Bioactive Peptides

2.1. β-Lactoglobulin

For example, β-lactoglobulin (β-Lg) (Figure 1A) is the most important whey protein, constituting around 50% of its protein composition. It has been reported that this whey protein is not hydrolyzed by gastric pepsin in the gastrointestinal tract; therefore, it reaches the upper intestine intact [1]. This protein has 162 amino acids, of which 84 are essential amino acids [2]. In addition, in its structure, there are two disulfide bonds and a sulfhydryl group that, after denaturation by enzymatic action or heat treatment, is exposed to interact with other proteins and form protein agglomerates [3,4]. By enzymatic action, it generates peptide fractions with overall antioxidant activity [5], although it has also been reported to have antihypertensive, antimicrobial, and immunomodulatory activities [6]. Additionally, β-Lg binds hydrophobic ligands such as fatty acids, steroid vitamins, vitamin D, and cholesterol [7]. The ability of β-Lg to form ligands is of dietary interest to improve the bioaccessibility of compounds such as highly soluble fatty acids, thus improving their absorption during gastrointestinal digestion [8].

2.2. α-Lactalbumin

In whey, α-lactalbumin (α-La) is the second most abundant protein, representing 20% of its protein composition (Figure 1B). α-lactalbumin has 123 amino acids, of which 67 are essential amino acids, with a high content of tryptophan, lysine, and cysteine. In its structure, there are four disulfide bonds, but it lacks free sulfhydryl groups [9]. It has been reported that α-La produces antimicrobial and antistress peptides due to high concentrations of tryptophan (Trp, W) [10]. On the other hand, there are reports that in an acid medium, human α-La generates peptides with antimicrobial and bactericidal activity and can produce HAMLET (human alpha-lactalbumin made lethal to tumor cells), an oleic acid complex that positively affects human health by inhibiting cancer tumor growth and stimulating malignant cell apoptosis in the organism [11]. The bovine equivalent of this complex is called bovine α-lactalbumin made lethal to tumor cells (BAMLET), and its cytotoxic effect on tumor cancer cells has been proven, since it increases the permeability of the lysosomal membrane.

2.3. Bovine Serum Albumin

Bovine serum albumin (BSA) represents 0.7–1.3% of all whey proteins (Figure 1C). Given its low concentration in whey, studies on BSA bioactivity are still limited. However, albumin has proven to have an activity like that of opioids [12]. This protein is confirmed by 582 amino acids and has a molecular weight of 66.26 kDa. In its structure, it has 17 intermolecular disulfide bridges and a thiol group at residue 34 [13]. Due to its structure, it can bind to lipids and free fatty acids [14]. This protein can be used as a source of essential amino acids, mainly tryptophan (Trp, W), valine (Val, V), and phenylalanine (Phe, F). The main biological interest is due to its high capacity to inhibit tumor growth, due to the modulation of the activity of various growth-regulating factors [15]. In addition, like other milk whey proteins, it could reversibly form ligands, allowing its use as a vehicle for fatty acids, with potential benefits to human health, or use these ligands in lipid synthesis [15,16].

2.4. Proteose–Peptone

Proteose–peptone (PP) is found at low concentrations in whey; still, its enzymatic hydrolysis generates peptides with bacteriostatic activity against Gram-positive and Gram-negative bacteria and antifungal activity probed in *Candida* species [17,18]. These effects are largely due to the protein's capability to permeate lipid layers of the cell membrane [19]. The

peptides generated from proteose–peptone are constituted by short chains of hydrophobic and positively charged amino acids. This allows the peptides to disturb the lipid bilayer of microorganisms, leading to an alteration like the one produced by proteins and triggering cell death due to ion and metabolic substance loss.

2.5. Glycomacropeptide

Glycomacropeptide (GMP) is a hydrophilic protein derived from κ-casein that is generated because of enzymatic coagulation of milk caseins during cheese making [20]. Its chain is made up of 64 amino acids and has a molecular weight of 6.8 kDa [21]. It is considered one of the whey proteins of greatest biological interest because it contains aromatic amino acids such as phenylalanine, tyrosine, and tryptophan [22]. In addition, this protein tends to be soluble in an acid medium; it is resistant to chemical agents, and it presents low biological degradation, so it remains stable during its gastrointestinal digestion, allowing its absorption by the blood circulation [23]. Glycomacropeptide represents 10–15% of whey proteins. Its controlled enzymatic hydrolysis renders peptides with antimicrobial activity. Such peptides fight tooth decay and inhibit certain oral pathogen bacteria such as *Streptococcus mutans* and *Porphyromonas gingivalis* [19]. Additionally, these peptide fractions promote the growth and development of other beneficial bacteria in the oral cavity [24]. Peptide fractions from glycomacropeptide hydrolysis have been proven to have an antithrombotic effect, inhibiting platelet aggregation [25]. A potent antihypertensive peptide constituted by κ-casein f(108–110) of GMP, corresponding to IPP, a tripeptide, Isoleucine–Proline–Proline, was purified from a Japanese dairy drink [26].

2.6. Lactoferrin

Lactoferrin (Lf) is an iron-chelating monomeric glycoprotein with a molecular weight of 80 kDa [27] (Figure 1D). Lactoferrin exhibits biological activities without hydrolyzation. It has proven to have antimicrobial and antioxidant capabilities and anti-inflammatory, anticancer, and immunoregulatory properties [28].

This whey protein has a potential antimicrobial effect due to its ability to chelate iron, limiting the availability of the mineral for pathogenic microorganisms; however, its efficiency may be limited, since some studies have reported that iron-saturated lactoferrin has reduced antimicrobial activity [29]. On the other hand, it has been shown that the lactoferrin protein can interact at the membrane level with the lipopolysaccharide (LPS) of Gram-positive bacteria, causing an antimicrobial effect [30]. An electrostatic union can be generated between the net positive charge of lactoferrin and the negative charge of the LPS of the bacteria, causing openings that distort the structure of the outer membrane of the bacteria, facilitating the entry of the peptide to bind to the charged cytoplasmic membrane negatively and exert an antimicrobial effect [31]. When hydrolyzed, Lf generates peptides with bacteriostatic and bactericide effect upon Gram-positive and negative bacteria and fungicide activity against *Candida* sp. [32]. It is commonly known as the antimicrobial protein of milk; furthermore, it can inhibit virus replication [33]. The proteolysis of Lf by pepsin produces fragments rich in N-terminal arginine (R) (lactoferricin) with antimicrobial, antiviral, and antiparasitic activity [34]. The mechanism through which peptides are generated from whey proteins is mostly enzymatic action, yet microorganism activity in fermentation also contributes to a lesser degree.

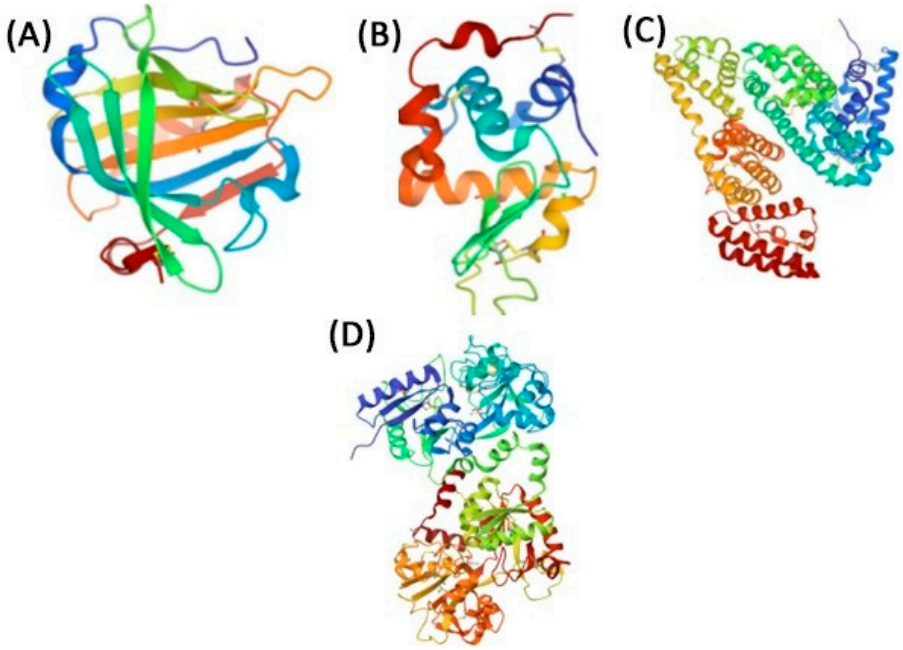

Figure 1. 3D-structure of the main whey proteins. (**A**) β-lactoglobulin (PDB ID: 3BLG) [35]; (**B**) α-lactalbumin (PDB ID: 1F6S) [36]; (**C**) bovine serum albumin (PDB ID: 4JK4) [37]; (**D**) lactoferrin (PDB ID: 1QJM) [38].

3. The Methods for Generating Bioactive Peptides

Peptides can be generated by two mechanisms: soluble protein consumption (mostly lactoglobulins) and the release of enzymes (as proteases and peptidases) during yeast fermentation. In the first case, there are advances using bioinformatics-selected proteases, which are evaluated for their ability to mitigate β-LG allergenicity. It has been indicated that pepsin and stem bromelain are the most effective at cleaving linear epitopes and reducing structural hydrophobicity. These findings support the use of targeted enzymatic hydrolysis to produce safer, dairy-based functional materials [39]. Furthermore, the use of metagenomics as a revolutionary tool to identify next-generation enzymes is transforming the discovery of novel proteases, enabling more precise and efficient peptide production [40]. Other strategies involve a multi-step filtration process and enzymatic hydrolysis, with trypsin as the most effective enzyme, achieving the highest degree of hydrolysis (17.80%) and antioxidant activity (0.93 mg/mL). This strategy was validated using the Ordinal Priority Approach (OPA methods) for industrial use [41].

On the other hand, the fermentation process to obtain bioactive peptides has emerged to express specific sequences. When combined with cost-effective, food-grade purification such as hexametaphosphate (HMP) precipitation, this approach offers a scalable and sustainable alternative to traditional chromatography, ensuring high-purity protein fractions with preserved functional properties [42]. Also, the economic feasibility of precision fermentation by optimizing β-lactoglobulin production was proposed using *Aspergillus oryzae*. By refining pH, temperature, and carbon input via a Design of Experiments (DoE) approach, the design achieved a 70% carbon yield and an 80% increase in protein titers. These results significantly lower production costs toward the 10 USD/kg target and support FDA GRAS regulatory submissions, establishing a scalable high-value food protein production [43]. Also, the environmental aspect is relevant in the production of recombinant peptides or protein fractions by using precision fermentation (PF), which demonstrates that using high-yield sugar crops or surplus sugar streams can significantly reduce the environmental footprint. Findings show that PF could feasibly replace substantial volumes of animal pro-

tein while drastically lowering land requirements compared to the 19–68 m^2/kg footprint of dairy-based β-LG. While challenges remain regarding green ammonia and renewable energy integration, PF offers a strategic pathway to mitigate climate change and enhance global food security by 2050 [44].

The distribution of peptide fractions and their composition are highly dependent on the extraction method and the protein source. In this case, peptide profiles of whey can vary according to lactic acid bacteria and others present in milk [45]. Several species of microorganisms are used in fermentation as a starter culture, mainly lactic acid bacteria, which are probiotic and are proven to have proteolytic capabilities. Bacteria hydrolyze whey proteins, increasing the number of peptides available in the medium [46]. These peptides not only have a certain biological activity but also improve the sensory characteristics (smell, taste, and texture) of the fermented dairy product [47]. When generating peptides from fermentation, the selection of the strain must be considered; the microorganism must be able to start proteolysis and release and lyse oligopeptides to obtain peptides with biological activity available in the medium [48]. Studies have proven the generation of peptides with antihypertensive activity when lactic acid bacteria are used, especially *Lactobacillus helveticus* [49]. Whey has also been fermented with *Kluyvermyces marxinaus* var *marxianus* and subsequently hydrolyzed with pepsin and trypsin from which an angiotensin-converting enzyme (ACE) inhibitor peptide has been isolated. Additionally, *K. marxianus* needs proteases and peptidases to hydrolyze soluble proteins and generate oligopeptides [50,51].

Controlled enzymatic hydrolysis is a good quality method regularly used in foods. In this method, the action of certain enzymes generates proteolysis; the process can be stopped by controlling the temperature to inactivate enzymes when the hydrolysis level is reached [52]. The incubation of medium at 100 °C for 10 min is adequate to inactivate most of the enzymes; however, there are reports of proteases, such as bromelain, that maintains 20% of the original activity after incubation under these conditions [53]. Pancreatic enzymes, especially trypsin and chymotrypsin, are used as proteases generating biologically active peptides from whey [54]. However, proteolytic enzymes such as pepsin, thermolysin, and alcalase have also been used to generate diverse short peptides such as VHLKP [47,55,56]. Similarly, pepsin generates peptides with antimicrobial activity, mainly against *Escherichia coli* K12 [57]. Despite the methods used, peptides can provide positive effects on health to the immune, gastrointestinal, nervous, and cardiovascular systems, among others; also, the benefits that peptides may produce are related to the size, sequencing, and source of the peptide.

The generation of specific peptide sequences during whey protein hydrolysis is strongly influenced by the type and specificity of the proteolytic enzymes used. Proteases can be broadly classified into endopeptidases and exopeptidases. Endopeptidases cleave peptide bonds within the protein chain, producing peptide fragments of different sizes, whereas exopeptidases act sequentially at the N- or C-terminal ends, releasing individual amino acids or short peptides and further modifying the peptide profile [58,59].

Among the most used endopeptidases, trypsin selectively cleaves peptide bonds at the carboxyl side of lysine and arginine residues, generating peptides with basic C-terminal amino acids. Pepsin preferentially hydrolyzes bonds involving hydrophobic and aromatic residues such as phenylalanine, leucine, and tyrosine under acidic conditions, mimicking gastric digestion. Alcalase, a microbial serine endoprotease, exhibits broad specificity and is widely used for industrial hydrolysis due to its high efficiency and ability to produce a wide range of low-molecular-weight peptides. Other enzymes, such as chymotrypsin, preferentially cleave at aromatic residues, while flavourzyme contains both

endo- and exopeptidase activities, enabling extensive hydrolysis and the generation of shorter peptides and free amino acids [60–62].

Therefore, the final peptide composition depends on enzyme specificity, substrate structure, and hydrolysis conditions, and results from sequential proteolysis rather than peptide synthesis. The release of bioactive peptides occurs when encrypted sequences within the native protein structure become accessible and are selectively liberated during enzymatic treatment.

4. Biological Activities of Whey-Derived Peptides

Enzymatic hydrolysis is widely used to obtain peptides, but also, peptide fractions obtained have different biological activities (Figure 2), and that activity depends on the enzyme used, which leads to a specific biological activity [63]. The peptide sequences discussed in this manuscript are expressed using the standard single-letter amino acid code, in which each letter corresponds to a specific amino acid residue. This notation is commonly used to simplify the representation of short peptide sequences.

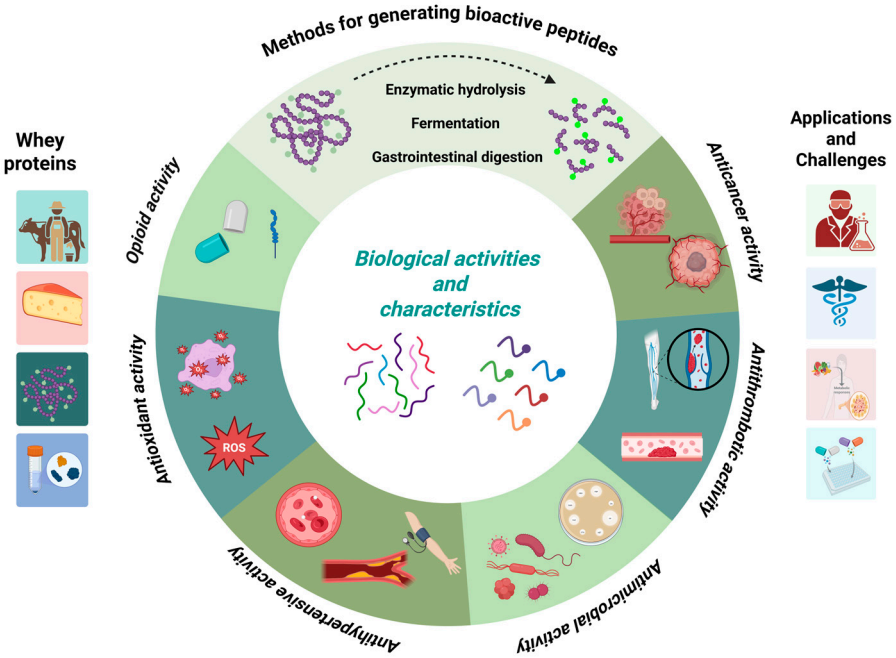

Figure 2. Overview of the generation of whey-derived bioactive peptides and their structure–function relationships.

Also, stability in the gastrointestinal tract, intestinal absorption, and systemic metabolism are determinants for bioactive activity and metabolism in the human body. Whey peptides can be studied through metabolomics analysis; some reports indicate that whey protein hydrolysates (WPH) supplementation effectively maintains metabolic homeostasis by increasing pre-exercise amino acids and carbohydrate levels, resulting in fewer metabolic disruptions compared to control groups. Furthermore, the study identified the peptide YGLF as a highly stable and bioavailable compound through peptidomics and molecular docking. In vivo tests confirmed that YGLF (100 mg/kg) significantly enhances glucose and glycogen levels while reducing fatigue markers like lactate and urea nitrogen, establishing a robust framework for identifying potent anti-fatigue peptides [64]. Another example is the action of the tripeptide Pro-Glu-Trp (PEW), which mitigates hyperuricemia (HUA) by focusing on previously unclear intestinal pathways. Beyond suppressing uric acid (UA) synthesis via XOD inhibition, PEW was found to enhance intestinal UA excretion by upregulating transporters like ABCG2 and GLUT9. These findings demonstrate

that PEW targets HUA through a multi-faceted approach involving metabolic regulation, microbiota modulation, and anti-inflammatory effects [65].

Some peptides increase their bioavailability using supplements like ferrous chloride and ferrous gluconate by significantly increasing hemoglobin levels and achieving superior iron bioavailability (331.9%). Beyond replenishing iron stores, WPP-Fe mitigated oxidative stress and systemic inflammation while favorably modulating the gut microbiota. Specifically, it increased the *Firmicutes* to *Bacteroidetes* ratio and enriched *Ileibacterium*, a genus positively correlated with improved hematological parameters. These findings suggest that WPP-Fe provides a synergistic approach to managing IDA through enhanced absorption and microbial regulation in mouse models [66].

Additionally, the effect of satiety-modulating potential of whey peptides can be measured by using the standardized INFOGEST in vitro digestion protocol combined with peptidomics and STC-1 enteroendocrine cell assays. The results highlighted that the <3 kDa intestinal peptide fraction exhibited the strongest stimulation of cholecystokinin (CCK), a key hormone in the gut–brain axis for appetite regulation. Peptidomic analysis identified bioactive sequences derived from β-lactoglobulin (β-La) and α-lactalbumin (α-La), specifically those enriched in hydrophobic and aromatic residues. Furthermore, in silico profiling predicted multi-functional bioactivities, including ACE-inhibitory, DPP-IV-inhibitory, and antioxidant effects, suggesting that WPC-derived low-molecular-weight peptides are promising candidates for nutritional strategies targeting obesity and cardiometabolic health, as we described in more detail in subsequent sections [67].

Overall, the biological activity of whey-derived peptides is strongly influenced by structural features such as molecular size, amino acid composition, hydrophobicity, and the presence of specific residues at the C-terminal region. In general, peptides with molecular weights below 3 kDa show higher bioactivity due to improved interaction with biological targets and greater absorption potential. Hydrophobic and aromatic residues, such as Trp, Tyr, and Phe, are frequently associated with antioxidant and ACE-inhibitory activities, while positively charged amino acids (Arg and Lys) contribute to antimicrobial and enzyme-inhibitory effects. However, the reported potency of these peptides varies considerably among studies due to differences in enzyme specificity, hydrolysis conditions, peptide purification level, and assay methodologies. Therefore, direct comparison of activity values should be interpreted with caution.

Importantly, most reported biological activities are based on in vitro assays, whereas in vivo and clinical evidence remain limited. Factors such as gastrointestinal stability, bioavailability, metabolism, and peptide transport represent critical challenges for the translation of these bioactivities into physiological effects.

4.1. Antihypertensive Activity

The conversion of angiotensin I to angiotensin II is a key factor in the regulation of blood pressure, since angiotensin II is an effective vasoconstrictor that, in turn, inactivates the vasodilator bradykinin [68]. Therefore, there is interest in finding bioactive peptides that can interact with the active site of the angiotensin-converting enzyme (ACE) and inhibit its activity. Caseins and whey proteins have antihypertensive activity [47]. When they undergo enzymatic hydrolysis, their enzymatic activity increases due to peptide generation, especially when their size is smaller than 3 kDa [54]. The peptides containing hydrophobic amino acids such as Trp (W), Tyr (Y), or Phe (F) at each of the three C-terminals are potent ACE-inhibitors [69]. Branched-chain aliphatic amino acids also promote ACE-inhibitory activity [70]. In addition, it has been reported that a positive charge of amino acids such as Arg (R) and Lys (K) at the C-terminal boosts the effect [71].

The most used enzyme to obtain hydrolysates is trypsin because it is used in both proteins α-La and β-Lg to obtain peptides from different fragments (f) of proteins with ACE-inhibitory action, antimicrobial activity, and antihypertensive action. Trypsin is one of the most used enzymes for the generation of whey-derived bioactive peptides because of its high specificity and reproducibility. This serine protease selectively cleaves peptide bonds at the carboxyl side of lysine and arginine residues, except when followed by proline, resulting in predictable peptide profiles. Such specificity facilitates the targeted release of short peptides with defined structural characteristics, including positively charged or hydrophobic residues that are frequently associated with biological activities such as ACE inhibition and antimicrobial effects. However, despite its advantages, the peptide profile obtained depends on hydrolysis conditions and substrate accessibility. Various peptides are obtained from β-Lg such as WYSLAMAASDI, VAGTWY f(81–83), ALPMHIR f(22–25), IIAEK f(32–40), IPAVFK, and LEKW [6,72–82]. These sequences are expressed using the standard single-letter amino acid code, in which each letter represents a specific amino acid residue, as commonly employed in peptide and protein chemistry. Also, dipeptides, like YP, and tripeptides, such as PEW, present the same activity, and PEW has important antidiabetic and antihypertensive responses (Table 1) [83–86]. It has been reported that the peptide ALPMHIR, derived from whey protein concentrate, exhibits marked inhibitory activity against ACE, reaching an IC_{50} value of 43 μM.

On the other hand, some studies have reported a significant decrease in systolic and diastolic pressure in hypertensive rats when administered whey from milk inoculated with *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. One of the peptides involved in this effect is the YPYY peptide [87,88].

Although numerous whey-derived peptides have demonstrated strong ACE-inhibitory activity in vitro, their antihypertensive efficacy in vivo is more variable [89]. Several animal studies have reported reductions in systolic and diastolic blood pressure following administration of whey protein hydrolysates [90]. However, the magnitude of the effect depends on factors such as peptide dose, gastrointestinal stability, and absorption efficiency. Therefore, further well-controlled human studies are required to confirm the physiological relevance of whey-derived antihypertensive peptides and to establish effective dose–response relationships.

4.2. Antioxidant Activity

It has been shown that oxidative stress is a condition caused mainly by the high production of reactive oxygen species (ROS); however, the increase in the concentration of these radicals leads to cell damage, modification in the protein structure, and mutation at the DNA level, which contributes to the development of other diseases [91]. Bioactive peptides with an antioxidant effect have been reported to exert their action through the intracellular conversion of cysteine (Cys, C) to glutathione, thus protecting cells from damage by ROS [92]. The mechanism of antioxidant activity in the peptides generated from hydrolyzed whey protein can be triggered by the inactivation of reactive oxygen species, hydrogen donors, the capturing of free radicals in the medium, and the chelation of metal ions, so that they inhibit lipid peroxidation.

The antioxidant potential of whey protein hydrolysates is also associated with low and high-molecular mass fractions [93]. On the other hand, peptide fractions with such biological activity contain 5–11 amino acids, including hydrophobic ones such as Pro, His, Tyr, and/or Trp. Peptides with Tyr (Y) and Trp (W) at the extremes show a higher antioxidant activity when compared against a phenolic acid such as gallic acid (Table 1) [94]. Also, some peptides such as LF and WYSL show the highest antioxidant activity, which is likely due to the capacity of indolic and phenolic groups such as hydrogen donors. Additionally,

this peptide contains the hydrophobic amino acid (Leu) in the extreme C-terminal region. These characteristics allow for an increase in its antioxidant activity [95,96]. Enzymes such as trypsin, pepsin, promatex, and flavourzyme are also able to hydrolyze β-Lg to obtain peptides such as LQKW f(58–61), which have a potent antioxidant effect because of Tyr (Y) and Trp (W), responsible for that activity [95,97–99]. Moreover, some peptides have been compared with synthetic antioxidants. For instance, the peptide VAGTWY obtained from whey is comparable with BHT (Butylated hydroxytoluene) [91,100,101].

Most antioxidant properties of whey-derived peptides have been demonstrated primarily using chemical assays or cell culture models, which may not fully represent physiological conditions in vivo [102,103]. In biological systems, antioxidant activity depends not only on the intrinsic radical-scavenging capacity of peptides but also on their gastrointestinal stability, bioavailability, and ability to interact with or modulate endogenous antioxidant defense systems, including glutathione metabolism and related enzymatic pathways [104]. Several animal studies have reported that whey protein hydrolysates can reduce oxidative stress markers, suggesting potential systemic antioxidant effects. However, attributing these effects to specific peptides remains challenging due to the complex composition of hydrolysate mixtures and the presence of multiple bioactive compounds [105]. Furthermore, comparisons among studies are often limited by differences in hydrolysis conditions, peptide fractionation strategies, and antioxidant assay methodologies. Consequently, additional well-designed in vivo experiments and human clinical studies are required to clarify the biological relevance of whey-derived antioxidant peptides and to determine their effective physiological concentrations.

4.3. Anticancer Activity

The antitumor activity of bioactive peptides is mainly due to mechanisms such as inhibition of angiogenesis and modulation of oncogene expression [106]. Peptides from the hydrolysis of milk whey proteins have been reported to have a high anticancer effect because they act as specific agents capable of inhibiting the cell viability of cancer cells, acting mainly on the degradation of the plasmatic membrane, generating pores, and inducing cell deformation and later apoptosis [107,108].

Lactoferrin is a promising protein with anticancer effects. It has been proven to remain stable in breast cancer cells and selectively inhibits their growth [109]. In experimental animals, the inhibition of tumor development and progression in the lungs, colon, and esophagus after Lf is administered has been evidenced [110]. The effects caused by this whey protein are associated with the suppression of tumor growth by the increase in glutathione levels and, consequently, the elimination of free radicals [111]. The presence of cysteine in peptides can contribute to the protective tumoral function, since it is a necessary amino acid for glutathione synthesis [112]. The modulatory effect upon immunity and cell viability constitutes another anticancer action mechanism of peptides [113]. Lactoferricin is a peptide obtained from Lf by hydrolysis using the acid. These peptides contain 25 amino acid residues and show cytotoxicity in different malignant cell lines in humans and experimental rats [114–116].

The anticancer potential of whey-derived peptides has been mainly evaluated in vitro using cancer cell lines, where effects such as reduced cell viability, induction of apoptosis, and inhibition of proliferation have been reported. Some animal studies, particularly with lactoferrin and its derived peptides, have shown inhibition of tumor growth and modulation of immune responses [117]. Nevertheless, the translation of these findings to clinical applications remains limited. Key challenges include peptide stability in the gastrointestinal tract, systemic bioavailability, target specificity, and the relatively high concentrations required to achieve cytotoxic effects in vitro. Therefore, more in vivo studies

and clinical trials are necessary to determine the therapeutic relevance and safety of whey-derived anticancer peptides

4.4. Antithrombotic Activity

Thrombosis, defined as the formation or presence of a blood clot within a blood vessel, is another risk factor in cardiovascular disease [118]. Bioactive peptides with antithrombotic activity can inhibit the binding of fibrinogen with thrombin, thus limiting platelet aggregation and therefore the formation of fibrin, inhibiting the formation of thrombus [119,120]. Glycomacropeptides (GMP) are the main source of peptides with an antithrombotic activity that has the capability of inhibiting platelet aggregation [121,122]. Some peptides derived from GMP and produced by tryptic hydrolysis inhibit platelet aggregation in systems in vitro [117]. Additionally, peptides derived from Lf are also involved in platelet adhesion [123]. There are reports on an antithrombotic peptide corresponding to j-CN f(113–116) isolated from yogurt [124]. Similarly, peptide PPK corresponding to j-CN f(109–111) has been reported to have the same structural homology as an antithrombotic peptide identified as MAIPPK. Peptide PPK is obtained from the water-soluble extract of fermented milk beverages [125,126].

Evidence supporting the antithrombotic activity of whey-derived peptides is primarily based on in vitro platelet aggregation assays. Although some peptides derived from glycomacropeptide and casein fragments have shown the ability to inhibit platelet aggregation, in vivo validation remains scarce. The physiological relevance of these effects may be limited by factors such as peptide degradation, low absorption, and the concentration required to achieve biological activity. Additionally, differences in experimental models and assay conditions make it difficult to directly compare results across studies.

4.5. Antimicrobial Activity

Peptides generated from whey proteins also exhibit antimicrobial activity and can permeabilize the membrane and generate cell lysis [127]. One of the main antibacterial mechanisms of bioactive peptides is due to electrostatic interactions between positively charged amino acid residues and negatively charged molecules on the microbial surface, specifically lipopolysaccharides (LPS), generating the decomposition and functionality of these compounds [128]. Protein α-La is hydrolyzed by trypsin and chymotrypsin, generating three hydrolysates with antagonistic effects against Gram-positive bacteria, especially *Bacillus subtilis*; β-Lg is also hydrolyzed by trypsin and has been reported to have the same effect against *Bacillus subtilis* [128]. When hydrolyzed, Lf can generate lactoferricin 27, a cationic peptide that produces cell death by increasing the permeability of the membrane [32]. The peptide lactoferricin B, generated by hydrolysis using pepsin, has an effect against Gram-positive and negative bacteria. Lactoferrampin f(268–284) has antibacterial activity against *Bacillus subtilis*, *Escherichia coli*, and *Pseudomonas aeruginosa*. Similarly, Kappacine f(138–158) of GMP has a phosphorylated serine residue that accumulates on the cell membrane, creating an anionic pore and promoting an antagonistic effect against Gram-positive (*Streptococcus mutans*) and negative (*Pseudomonas gingivalis* and *E. coli*) bacteria [129,130]

It is relevant to consider that the concentration of peptides, as well as the degree of their biological activity, will depend on the nature of the milk [131]. It has been proven that a greater antimicrobial effect is obtained from the hydrolysis of whey proteins in camel milk [132]. In addition, isolated peptides from camel milk whey reduce the growth of *E. coli* [133]. Due to the antimicrobial effect of whey hydrolysates, this product has been used as an additive to guarantee the innocuousness of food. However, several studies are

still needed to evaluate their allergenic effect and use them as GRAS (Generally Recognized as Safe) [134,135].

The antimicrobial properties of whey-derived peptides have been widely demonstrated in vitro against a broad range of microorganisms. However, their effectiveness under physiological or food system conditions may differ due to interactions with proteins, lipids, and salts that can reduce peptide activity. In vivo evidence is limited, and the concentration required for antimicrobial action is often higher than those achievable through normal dietary intake [134,135]. In addition, potential issues related to peptide stability, toxicity, and regulatory approval must be considered before their practical application as food preservatives or therapeutic agents.

4.6. Opioid Activity

Many drugs used to control pain diseases contain compounds with opioid capacity, such as morphine and codeine; however, excessive use of them generates side effects such as addiction [136]. That is why peptides with opioid activity emerge as an alternative due to their affinity. These peptides have an opioid receptor (μ, κ, δ) without exerting side effects and generating an analgesic effect [137]. Whey proteins can generate opioids, which allow for reaching cell receptors, and they can act as exogenous modulators of intestinal motility and epithelial permeability or through intestinal hormone release [138]. In general, opioid peptides exhibit a Tyr (Y) residue at the N-terminus and aromatic residues such as Phe (F) or Tyr (Y) at positions 3 or 4 of the amino acid sequence [139]. It is known that α-Lactorphin (YGLF), β-lactorphin (YLLF), and β-lactotensin (HIRL) are opioid peptides obtained from whey by enzymatic hydrolysis with pepsin and trypsin, and they can show antihypertensive activity [140].

Although several whey-derived peptides exhibit affinity for opioid receptors in vitro, their physiological relevance remains limited. Most food-derived opioid peptides are susceptible to enzymatic degradation during gastrointestinal digestion, which significantly reduces their stability and bioavailability. In addition, their absorption through the intestinal epithelium is often low, and only a small fraction of intact peptides may reach systemic circulation. Even when absorbed, the ability of these peptides to cross the blood–brain barrier is highly restricted due to their molecular size and hydrophilicity. Therefore, the central nervous system effects reported in experimental models may not be easily translated to humans. Current evidence suggests that the biological effects of milk-derived opioid peptides are more likely mediated through peripheral mechanisms, such as modulation of intestinal motility, hormone secretion, or interaction with local opioid receptors in the gastrointestinal tract. Consequently, further in vivo and clinical studies are required to clarify their bioavailability, transport mechanisms, and physiological significance [140].

Table 1. Peptides derived from whey protein with different biological activities.

Peptide Sequence	Hydrolyzing Enzyme	Biological Activity	Reference
	β-lactoglobulin		
WYSLAMAASDI	Trypsin, pepsin and thermolysin	Antioxidant	[141]
VAGTWY	Trypsin	Antihypertensive, antioxidant, antibacterial, antianxiety	[63]
IPAVFK	Pepsin and trypsin	ACE-inhibitory	[82]
ALPMHIR	Trypsin Thermolysin	Hypocholesterolemic, ACE-inhibitory ACE-inhibitory, hypocholesterolemic	[80]

Table 1. *Cont.*

Peptide Sequence	Hydrolyzing Enzyme	Biological Activity	Reference
VLVLDTDYK	Trypsin	Antibacterial Dipeptidyl peptidase IV (DPP-IV inhibiting)	[1]
GLDIQKVAGT	Pancreatin	ACE-inhibitory	[81]
IIAEKTKIPAVF	Pancreatin	Antibacterial	
GLDIQK	Trypsin	DPP-IV inhibiting	[70]
IPAVF	Trypsin	DPP-IV inhibiting	
IIAEK	Trypsin	Hypocholesterolemic	[78]
IIAEKTKIPAVF	Pancreatin	Antimicrobial	[79]
LKPTPEGDL	Pepsin	DPP-IV inhibiting, antidiabetic	[142]
WYSL	Alcalase	Antioxidant	[143]
LQKW	Thermolysin	Antioxidant	[144]
EQLTK	Trypsin	Antibacterial	[145]
YGLF	Trypsin	Antibacterial, ACE-inhibition	[146]
α-Lactalbumin			
VGINYWLAHK	Trypsin, pepsin, chimotrypsin	ACE inhibitor, opioid activity	[147]
CKDDQNPH	Chymotrypsin	Antibacterial	[148]
ALCSEK	Chymotrypsin	Antihypertensive	
S-Sf/S-S fractions	Chymotrypsin	Antibacterial	
INYW	Thermolysin	Antioxidant, antibacterial	[149]
LDQW	Thermolysin	Antioxidant	[150]
YLLF	Proteinase K Synthetic	ACE-inhibitory, antiosteoporosis	[99]

5. Applications for Whey-Derived Bioactive Peptides

In recent years, bioactive peptides have gained increasing relevance in the development of functional foods and nutraceutical products. Whey-derived peptides, in particular, have been incorporated into food formulations due to their biological activities and their compatibility with dairy and non-dairy matrices [151,152]. Their application aims not only to enhance nutritional value but also to provide additional health-related benefits to consumers.

Whey protein hydrolysates and peptide fractions have been mainly applied in dairy products such as yogurt, fermented milk, and flavored milk beverages. The incorporation of these peptides has been shown to improve functional properties, including angiotensin-converting enzyme (ACE) inhibitory activity and antioxidant capacity, without significantly compromising product quality when appropriate processing conditions are used [124,125]. In some cases, peptide addition has also contributed to improved physicochemical characteristics, such as texture and stability, as well as acceptable sensory attributes [126].

Beyond traditional dairy products, whey-derived peptides have been explored in beverages and other food systems as functional ingredients aimed at enhancing antioxidant activity and overall health value [127,128]. However, the successful application of bioactive peptides in food matrices depends on several technological factors, including peptide concentration, interaction with other food components, and stability during processing and

storage. Bitterness development and loss of activity remain important challenges that must be addressed through formulation strategies or advanced delivery systems.

In addition to food applications, whey bioactive peptides have shown potential for use in nutraceutical formulations and dietary supplements. In these systems, peptides can be delivered in more concentrated and controlled forms, allowing greater flexibility in achieving physiologically relevant doses. Nevertheless, considerations related to bioavailability, safety, and regulatory approval are critical for their commercialization.

Issues related to bioavailability, sensory, and stability aspects represent important challenges for the practical application of whey protein hydrolysates. One of the main limitations is the development of a pronounced bitter taste, which is commonly associated with the presence of low-molecular-weight peptides enriched in hydrophobic amino acids. This sensory characteristic often restricts the incorporation of hydrolysates into food formulations and may require additional processing steps such as debittering, fractionation, or encapsulation. Furthermore, due to the presence of exposed amino and carboxyl groups, peptides are chemically reactive and may undergo undesirable reactions during processing and storage. These include interactions with reducing sugars through Maillard reactions, oxidation, aggregation, or binding with other food components, which can affect both their stability and biological activity [55,112,116]. Therefore, controlling processing conditions and developing protective strategies are essential to preserve peptide functionality and ensure their effective application in functional foods and nutraceutical products.

Overall, whey-derived peptides represent versatile functional ingredients with promising applications in food and health-related products. Continued research focused on formulation strategies, delivery systems, and industrial scalability will be essential to fully exploit their potential and support their transition from research to commercial applications.

6. Challenges and Future Perspectives

Despite the growing body of evidence supporting the biological activity of whey-derived peptides, several challenges still limit their effective translation from laboratory-scale studies to real-world applications in functional foods, nutraceuticals, and therapeutic formulations. One of the main limitations is related to bioavailability and stability. Many bioactive peptides exhibit promising in vitro activity; however, their functionality may be compromised during gastrointestinal digestion, food processing, or storage. Enzymatic degradation, pH variations, and interactions with other food components can significantly affect peptide integrity and reduce their biological efficacy in vivo.

Another critical challenge concerns the dose–response relationship and physiological relevance of whey-derived peptides. In many studies, biological activities are demonstrated at peptide concentrations that may not be realistically achievable through normal dietary intake. This highlights the need for further in vivo studies and well-designed clinical trials to establish effective doses, safety profiles, and long-term health benefits. Additionally, interindividual variability, gut microbiota composition, and metabolic differences may influence peptide absorption and bioactivity, adding further complexity to their practical application.

From a technological perspective, scalability and standardization of peptide production remain significant obstacles. The biological activity of peptides is highly dependent on enzymatic specificity, hydrolysis conditions, and processing parameters, which can lead to variability between batches. Developing controlled, reproducible, and cost-effective production strategies is essential for industrial implementation. Advanced technologies such as membrane fractionation, targeted enzymatic hydrolysis, and in silico-assisted peptide prediction may contribute to improving process efficiency and product consistency. Some challenges for scalability and standardization of peptide production are the stability

and solubility challenges of key amino acids like glutamine, cysteine, and tyrosine. To overcome these constraints, some authors evaluate dipeptides as promising alternatives for CHO cell-based manufacturing to improve solubility and their impact on culture performance. To ensure process consistency and industrial scalability, the authors propose a rational design framework that integrates multi-omics, metabolic flux modeling, and artificial intelligence (AI) to tailor dipeptide strategies for specific bioprocessing needs [153]. Global whey production reaches 190 million tons annually, and the valorization of whey into bioactive peptides involved a multi-stage separation process depending on scalability and methodology; some processes include comprising skimming, microfiltration (MF), ultrafiltration (UF), and diafiltration (DF) to produce a purified protein fraction, which was subsequently hydrolyzed using enzymes such as trypsin, bromelain, and papain. While MF and UF effectively concentrated the protein to 23.24 mg/mL, DF efficiency was hindered by membrane fouling, reducing concentrations to 11.42 mg/mL. Also, this methodology identified that trypsin is the most effective enzyme for hydrolysis. These findings provide a scalable, integrated strategy for the dairy industry to mitigate environmental impact while producing high-value bioactive peptides [41]. An alternative approach to improve yields and reduce the environmental footprint is through two-step production routes, and the results indicate that the two-step fermentation process was the most efficient, achieving a competitive minimum selling price of €3.9–5.0/kg, which rivals both plant and animal-based proteins. Environmentally, this method offers a massive reduction in impact, up to 99.3% lower than traditional one-step fermentation. Furthermore, the study suggests that decentralized production is the most viable logistical strategy in this case for Europe, potentially replacing 7.2% of the continent's animal protein demand by valorizing 80% of available cheese whey permeate [154].

As mentioned above, whey proteins and their hydrolysates are key ingredients in functional foods, with their industrial use supported by rigorous safety standards such as US GRAS status and EFSA validations. While a regulatory gap still exists in the Codex Alimentarius regarding hydrolysates, ongoing efforts by AOAC International to standardize analytical methods are crucial for achieving global harmonization and further industrial scaling.

Some peptides with therapeutic applications, such as dipeptidyl peptidase IV (DPP-IV) inhibitory peptides derived from whey protein as natural supplements to synthetic treatments for Type 2 Diabetes (T2D), enhance insulin secretion and help manage hyperglycemia, offering a sustainable strategy against the rising global incidence of the disease. A bibliometric and VOS viewer analysis shows a steady increase in research since 2006, particularly in China, France, and the USA, and identifies a shift toward integrating food science and nutrition into chronic disease management. Ultimately, the research underscores the importance of functional foods in long-term health, though it highlights a critical need for clinical trials to fully validate the efficacy of these peptides in global health initiatives [155].

Regulatory aspects also represent an important bottleneck. Although whey proteins are widely recognized as safe, bioactive peptides intended for health-related claims must comply with strict regulatory frameworks. The classification of peptides as food ingredients, nutraceuticals, or therapeutic agents varies across regions, requiring comprehensive toxicological, allergenicity, and efficacy assessments. Clear regulatory guidelines and harmonized evaluation criteria will be crucial for facilitating market approval and consumer acceptance.

While global production reaches approximately 200 million tonnes annually, only half is currently valorized, highlighting a significant opportunity for circular-economy initiatives. There is global disparity in whey processing, noting that while industrialized nations valorize 70–90% of this byproduct, many regions still discharge untreated whey into sewer systems, causing significant environmental degradation due to high processing

costs and a lack of advanced technology. Conversely, the economic outlook for whey is exceptionally strong, with the global market projected to grow from USD 5.33 billion in 2021 to over USD 14 billion by 2030, driven largely by the demand for whey protein concentrates (WPC) and sports nutrition [156].

Future research should focus on integrating multidisciplinary approaches that combine proteomics, peptidomics, bioinformatics, and in vivo validation to better understand structure–activity relationships and mechanisms of action. Additionally, exploring novel delivery systems, such as encapsulation or peptide–matrix interactions, may enhance peptide stability and targeted release. Overall, addressing these challenges will be essential to fully exploit the potential of whey-derived bioactive peptides and to support their successful incorporation into applied bioscience and health-oriented innovations.

From a processing perspective, alternative production strategies such as immobilized enzyme systems have been proposed to improve hydrolysis efficiency, enzyme stability, and process reproducibility. Although these systems offer advantages such as enzyme reusability and better control of peptide profiles, their industrial application is still constrained by higher operational costs, mass transfer limitations, and challenges related to large-scale implementation.

In addition, the development of advanced delivery systems has emerged as a promising strategy to enhance the functional performance of bioactive peptides. Techniques such as microencapsulation, nanoemulsions, liposomes, and polymer-based carriers can protect peptides from gastrointestinal degradation, improve their stability during processing and storage, and potentially increase intestinal absorption. Despite these advantages, most studies remain at the laboratory scale, and important issues related to long-term stability, controlled release behavior, safety, regulatory approval, and cost-effectiveness must be addressed before their practical application in functional foods or nutraceutical products.

Overall, while these emerging technologies offer significant opportunities to improve the stability, bioavailability, and efficacy of whey-derived bioactive peptides, further research integrating computational prediction, experimental validation, and clinical evaluation is required to ensure their successful translation into real food and health applications.

7. Conclusions

The transformation of whey proteins into bioactive peptides represents a valuable strategy for the development of functional ingredients with potential health benefits. Through controlled enzymatic hydrolysis and fermentation processes, whey proteins can be converted into peptides exhibiting a wide range of biological activities, including antioxidant, antimicrobial, antihypertensive, antithrombotic, and immunomodulatory effects. These properties highlight the relevance of whey-derived peptides as natural compounds for application in food systems and health-oriented formulations.

Numerous whey-derived peptides have been identified, isolated, and characterized, demonstrating their capacity to interact with key biological targets and contribute to disease risk reduction. Their incorporation into food matrices has shown promising results in enhancing the nutritional and functional value of products, particularly in the context of functional foods and nutraceuticals. However, the effective translation of these bioactivities into practical applications remains dependent on factors such as peptide stability, bioavailability, and interactions within complex food systems.

Although significant advances have been achieved, further research is required to bridge the gap between experimental findings and real-world implementation. Greater emphasis should be placed on in vivo validation, dose–response relationships, and the assessment of long-term safety and efficacy. Addressing technological and regulatory challenges will be essential to support industrial scalability and consumer acceptance.

While bioactive peptides are generally considered safe when consumed through a standard diet, as their production mirrors natural human protein digestion, the misuse or excessive administration of these compounds can disrupt metabolic balance and lead to adverse effects. Current evidence indicates that food-derived peptides may exert their influence through two primary pathways: by directly entering the bloodstream or by modulating intestinal mucosal cells and the broader gut environment. Consequently, maintaining an appropriate dosage is essential to ensure therapeutic benefits without compromising physiological homeostasis.

Overall, whey-derived bioactive peptides constitute a promising and versatile class of compounds within applied biosciences. Continued interdisciplinary research and technological innovation will be key to unlocking their full potential and enabling their successful integration into functional foods, nutraceuticals, and future health-related applications.

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Abbreviations

The following abbreviations are used in this manuscript:

β-Lg	β-Lactoglobulin
α-La	α-Lactalbumin
Ig	Immunoglobulins
BSA	Bovine Serum Albumin
GMP	Glycomacropeptide
Lf	Lactoferrin
HAMLET	Human Alpha-lactalbumin Made Lethal to Tumor cells
BAMLET	Bovine Alpha-lactalbumin Made Lethal to Tumor cells
LPS	Lipopolysaccharide
ACE	Angiotensin-Converting Enzyme
ROS	Reactive Oxygen Species
BHT	Butylated Hydroxytoluene
GRAS	Generally Recognized as Safe

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