

Review

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Posted Date: 10 June 2024

doi: 10.20944/preprints202406.0540.v1

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Review

Whey as an Optimal Substrate for the Development of Functional Products Enriched with Bioactive Peptides

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Abstract: Milk serves as a crucial source of natural bioactive compounds essential for human nutrition and health. The gastrointestinal digestion of milk proteins releases various biologically active peptides that positively impact human health and enhance physiological performance. With the growing production of cheese, Greek yogurt, and other dairy products, the volume of unutilized acid whey is significantly increasing. Acid whey, containing almost all whey proteins and approximately one-quarter of casein, presents a valuable raw material for generating peptides with potential health benefits. These peptides exhibit properties such as antioxidant, antimicrobial, anti-inflammatory, anticarcinogenic, antihypertensive, antithrombotic, opioid, mineral-binding, and growth-stimulating activities, contributing to improved human immunity and the treatment of chronic diseases. Enzymatic hydrolysis of milk proteins has been developed as a method to produce these peptides, facilitating their use in food fortification. To incorporate whey into functional beverages, it undergoes fermentation by lactic acid bacteria with proteolytic activity. To specifically enrich these beverages with peptides exhibiting targeted bioactivities, it is essential to select lactic acid bacteria strains possessing the requisite protease profiles. Additionally, the sensory properties of the final product can be enhanced using starter cultures with diverse metabolic capabilities, even in the absence of added flavoring agents. Given the imperative to develop waste-free technologies and enhance the profitability of the dairy industry, there is a growing need to efficiently utilize and transform acid whey into a broad spectrum of valuable products and food additives. This approach is justified not only by the nutritional benefits of the bioactive components in whey but also by the commercial potential of safe and health-enhancing products.

Keywords: functional products; peptides; whey; fermentation; lactic acid bacteria; proteolytic activity

1. Introduction

The rapid population growth on Earth, coupled with the impossibility of expanding arable land and further intensifying food production, necessitates the development of innovative methods to enhance the nutritional and biological value of existing food products. Additionally, there is a need for more efficient use of raw materials and the creation of waste-free production systems. One promising direction is the utilization of whey, which is increasingly recognized not as a mere by-product of dairy production, but as a valuable raw material for further biotechnological processing.

Advanced methods such as chemical hydrolysis, fermentation, enzymatic treatment, and environmentally friendly technologies like ultrasonic and thermal treatments, have been successfully employed to extract peptides from whey protein [1]. These peptides exhibit excellent functional properties that positively impact cardiovascular, digestive, endocrine, immune, and nervous systems. Consequently, research on their application for health enhancement, through both the optimization of food product compositions and the development of pharmaceuticals, is highly relevant [2-4]. The development of peptide drugs is currently one of the most prominent topics in

pharmaceutical research [5]. Commercial production of functional probiotic products containing bioactive peptides (BAPs) offers significant contributions not only due to their health benefits but also by reducing the risk of spoilage in dairy products. The inclusion of natural antioxidants in yogurt and other dairy products can prevent the rancidity of milk fat, thus extending shelf life and maintaining quality [6].

The use of whey in dietary and therapeutic nutrition is highly appropriate due to its high nutritional and biological value, relative cost-effectiveness, and availability. Moreover, utilizing whey waste from the dairy industry addresses environmental concerns, making this approach one of the most promising directions in the development of functional products [7].

2. Milk and Whey

2.1. Milk

The value of milk as a primary food product for newborn humans and animals is due to its content of complete protein, conjugated linoleic acid, omega-3 fatty acids, vitamin D, selenium, and calcium, and most importantly, biologically active peptides that play a key role in the physiological activity of the human body [8,9].

Previous claims about the adverse effects of dairy fats on human health have now been largely discredited [10]. Moreover, an inverse correlation has been shown between dairy consumption and cardiovascular diseases [11,12], as well as a direct correlation between increased dairy consumption and improved metabolic health [13]. Milk is a rich source of antioxidants, including vitamins A and E, and there is evidence that milk consumption may be associated with a reduced risk of chronic diseases [14]. Milk and dairy products, both naturally containing probiotics and with added probiotics, are beneficial for health due to their functional food properties by improving the microbial balance of the gut, influencing immune response, and other important biological functions.

Milk contains two main classes of proteins: caseins and whey proteins. Caseins are divided into four main types (α s1-, α s2-, β -, and κ -casein), while whey proteins include β -lactoglobulin, α -lactalbumin, whey albumin, lactoferrin (LF), and other bioactivators (Figure 1). The different biological activities of these proteins are significant for the prevention and treatment of numerous dietary, physiological, and metabolic diseases. For example, LF—one of the most important bioactivators in milk—performs biological functions such as regulating iron absorption and modulating immune responses and possesses antimicrobial, antiviral, antioxidant, anticancer, and anti-inflammatory activities [3]. LF is considered one of the milk components modulating the gut flora of infants. It increases the number of beneficial bacteria, and protects the host organism from infection and inflammation, partially restoring gut homeostasis and the integrity of both the intestinal wall and lung tissue. By acting locally on the intestine and microbiota, LF is also seen as a promising candidate for the systemic treatment of influenza [15]. It has been shown that LF has anticarcinogenic activity against many human and animal tumors, modulating cell-level proliferation, differentiation, maturation, activation, migration, and immune cell function [16], as well as antioxidant and antigenotoxic activity concerning gastric and hepatic cells [17].

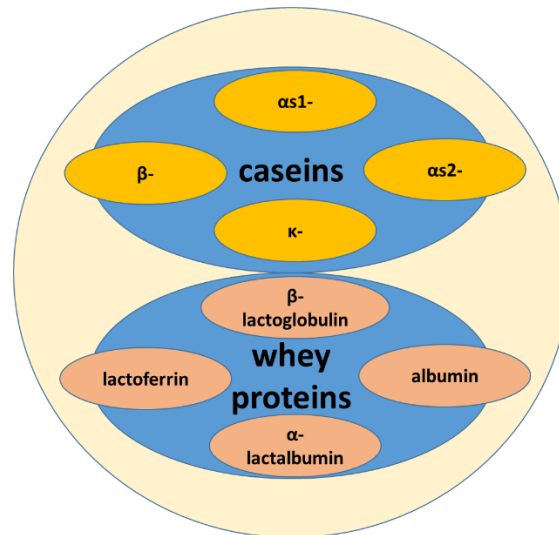


Figure 1. Milk and whey proteins.

Caseins and whey proteins are almost completely assimilated and are extremely important in the prevention and treatment of numerous dietary, physiological, and metabolic diseases. Their amino acid composition is equivalent to meat proteins but does not contain harmful purine bases, unlike meat proteins. Osteopontin is found in the highest concentrations in milk—a multifunctional protein involved in immune system activation and regulation; biomineralization; tissue remodeling processes, including the growth and development of the intestine and brain; interactions with bacteria; and much more [18].

Milk is a very important source of natural bioactive components for human nutrition and health [19,20]. Various BAPs released during gastrointestinal digestion directly affect numerous biological pathways, causing behavioral, gastrointestinal, hormonal, immunological, neurological, and dietary responses [21,22]. Notably, some beneficial properties are attributed not to the original proteins of both human and animal milk but exclusively to the bioactive peptides released from them [23].

2.2. Whey

Every year, more than 160 million tons of whey are generated globally [24]. Approximately half of the dry components of milk, containing a variety of compounds, are transferred to whey (including up to 100% whey proteins; up to 88% minerals; up to 25% casein; up to 12.5% fat; up to 99% lactose, along with vitamins, enzymes, and organic acids) [25]. The increase in cheese production, Greek yogurt, and other dairy products has led to an increase in unused acidic whey, further exacerbating its detrimental effects on the environment [26]. Contrary to sweet whey (produced during hard cheese making with enzymes), acidic whey, due to its properties (elevated lactic acid and calcium phosphate levels, increased oxygen demand), necessitates higher processing expenses [27,28] and substantial efforts for its proper utilization [29].

From a nutritional point of view, whey proteins outperform others, particularly caseins, due to their abundant essential branched-chain amino acids crucial for metabolism [30]. The biological functionality of whey proteins is closely linked to their structural characteristics, allowing them to function as intact molecules, partially hydrolyzed forms, or small bioactive peptides. Extensive research has explored the biological effects of whey proteins in animal and human models, highlighting benefits such as improved nutrient uptake and decreased susceptibility to chronic diseases [31].

Whey protein stands as a vital by-product of the dairy sector, yet it poses a significant environmental threat. Therefore, the enzymatic breakdown of whey protein concentrate to produce peptides presents an innovative strategy that can enhance the value of whey by transforming a low-value waste by-product into high-quality commodities, specifically antioxidant and cytoprotective

peptides. These peptides, when incorporated as functional constituents [32], have the potential to enhance consumer health by fortifying the immune, cardiovascular, nervous, and gastrointestinal systems [33].

3. Peptides

Peptides with high biological activity — evolutionarily conserved molecules produced by living organisms of various origins — are regarded as a new generation of bioactive regulators designed to protect and stimulate the host's metabolism. They regulate gene expression and protein synthesis in plants, microorganisms, insects, birds, rodents, primates, and humans. Low molecular weight peptides, usually <10 kDa, with hydrophobic properties and aliphatic and aromatic chains, play an important role in molecular interactions to ensure necessary activity [34].

Short peptides consisting of 2–7 amino acid residues can penetrate the nuclei and nucleoli of cells and interact with the nucleosome and histone proteins, as well as with both single-stranded and double-stranded DNA. DNA-peptide interactions, including sequence recognition in gene promoters, are important for template-driven synthetic reactions, replication, transcription, and repair. Peptides can regulate DNA methylation status, which is an epigenetic mechanism of gene activation or repression, both in normal conditions and in cases of pathology and aging [35].

BAPs derived from milk are potential ingredients for health-beneficial functional food products. The wide range of physiological functions of BAPs includes antimicrobial, antihypertensive, antithrombotic, anticancer, antioxidant, opioid, anti-appetite, immunomodulatory, mineral-binding, and growth-stimulating activities [8,36,37]. They can enter the body in intact form, that is, as structures exhibiting their properties directly in the gastrointestinal tract (GIT), as well as in the form of specific protein and peptide precursors [38]. Often, these compounds are first subjected to the action of digestive enzymes of the macroorganism or the residing microflora and then, entering the bloodstream as peptides or even individual essential amino acids, become structural elements of the body's bioactive polypeptides. They are targeted at treating chronic diseases related to nutrition: obesity, cardiovascular diseases, and diabetes. Peptides derived from cow, goat, sheep, buffalo, and camel milk have multifunctional properties (Figure 2), including antimicrobial, immunomodulatory, antioxidant, and antithrombotic, as well as inhibitory and antagonistic activities against various toxic agents. By regulating immunological, gastrointestinal, hormonal, and neurological responses, they play a vital role in the prevention of cancer, osteoporosis, hypertension, diabetes, and other diseases [39,1].

The biological activity of peptides and their contribution to maintaining organic homeostasis, immunomodulation, and protection of the body from oxidative and other adverse processes, is well described in the literature. Reports on the activity of milk peptides in glycemic control, antihypertensive activity, and their ability to inhibit uric acid formation are summarized, as well as the need to address gaps related to their interaction with cellular targets and their use in human therapy [34].

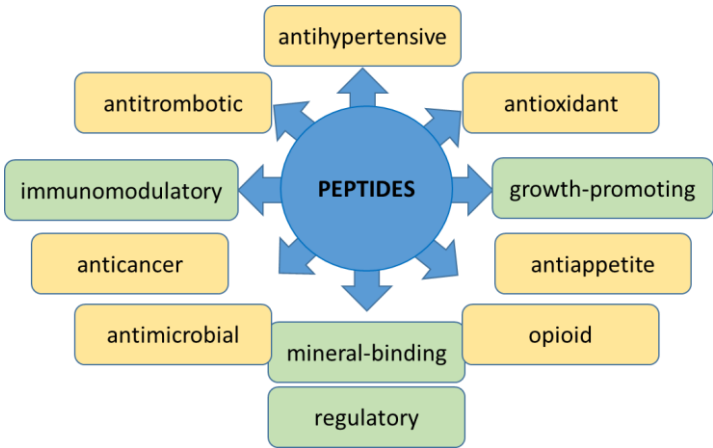


Figure 2. Physiological effects of peptides.

In recent decades, the number of approved peptide drugs has steadily increased, and by 2021, the average growth rate of the global peptide therapy market was 7.7% [40]. As potential therapeutic agents, they have numerous advantages: low production cost, low toxicity, and the possibility of storage at room temperature [41]. They are used against HIV infection [5]. With the development of sequencing technologies and synthesis methods, more therapeutic peptides with two or more functional characteristics are being discovered, which is crucial for creating new drugs [42].

The antibacterial peptide-based therapeutic approach has significant potential in addressing infections caused by antibiotic-resistant bacteria, which present a growing menace to human health. In contrast to the bactericidal mechanism of conventional antibiotics with a singular target, antimicrobial peptides can eliminate pathogens by affecting multiple targets, thereby notably diminishing the emergence of antibiotic-resistant bacteria and positioning them, owing to their wide spectrum of activity, as one of the prime substitutes to intricate antibiotics [12,43]. They function through various mechanisms, augmenting natural immune responses and decreasing susceptibility to chronic diseases [44].

A favorable attribute of antimicrobial peptides is their compatibility with living organisms and degradability, thermal stability, and elevated specificity, which is highly appealing for food conservation [45]. Moreover, unlike antibiotics, peptides disintegrate entirely within the organism without manifesting adverse repercussions. One such peptide, nisin, derived from *Streptococcus lactis* [46], is presently utilized commercially (as E234). The consumer demand for food items devoid of synthetic preservatives has propelled the search for natural antimicrobial substances with a broad antimicrobial spectrum and enhanced characteristics.

Over the past decade, viral infections have become a substantial health concern. The concept of antimicrobial peptides encompasses antiviral peptides, which exhibit notable potential in safeguarding the human body against various viral ailments [47]. Due to the necessity of devising experimental and computational techniques for antiviral peptide recognition, strategies for feature depiction, categorization algorithms, and assessment criteria for performance are being formulated [48]. Recently, the employment of sophisticated machine learning approaches for the design of peptide-centered therapeutic agents has become increasingly advantageous due to their noteworthy outcomes [49-52]. A web server has been established for the prediction of anti-coronavirus peptides [53].

Antimicrobial peptides constitute a pivotal component of the inherent immunity of organisms. Recent investigations have shown that minor cationic peptides can regulate cell viability in diverse human cell cultures, inhibit cancer cells, and serve as therapeutic agents against cancer [54]. They exhibit immune cell functions, encompassing lymphocyte proliferation, antibody production, and cytokine control [55], stimulate macrophages' phagocytic capability, and inhibit specific cytokine secretion [56,57]. Anticancer peptides have emerged as promising therapeutic agents for managing and preventing cancer. Their utilization may evolve into a feasible substitute for traditional treatments for this illness, as they are notably safer and more selective than conventional agents [58,59], and, owing to their heightened specificity and reduced toxicity, do not jeopardize the functionality of vital organs [60]. Nevertheless, with the rapid escalation in the number of peptide sequences, predominantly unveiled through time-intensive experimental screening, there exists a requirement for the formulation of dependable and precise predictive models. Currently, systems that outperform existing methodologies, achieving nearly 100% precision, have been devised and will prove beneficial in the development of anticancer medications [61-64].

BAPs can also lower blood pressure by inhibiting angiotensin-converting enzyme (ACE), which plays a central role in blood pressure regulation. Angiotensin is one of two polypeptide hormones and a powerful vasoconstrictor that acts in the body to control blood pressure through the contraction of smooth muscle in blood vessels [65]. In addition, ACE inactivates bradykinin, an endothelium-dependent vasodilator that increases blood pressure [66], which forms the basis of one of the treatment strategies for hypertension. Ong and Shah [67] showed that the addition of *Lactobacillus acidophilus* to cheese starter increased the production of a number of BAPs that lower blood pressure

by inhibiting ACE. The findings strongly suggest that the antihypertensive and antioxidant peptides found in whey protein fermentate may be useful in the development of pharmacologically active ingredients for health in the coming years [68].

The highest ACE inhibitory activity, as well as antioxidant activity, was demonstrated by goat milk treated with alkaline protease, which should be considered in the development of functional dairy products for the treatment of hypertension [69,70]. Goat milk is widely recognized as an excellent source of dairy protein. Casein and whey protein BAPs derived from it also exhibited antibacterial properties. Goat milk protein is a promising source for the development of high-quality protein products with high safety standards that have potential applications in the pharmaceutical and food industries [71].

Among the products of whey protein hydrolysis (WPH), short-chain peptides predominate, demonstrating low allergenicity and high essential amino acid content. WPH can be used in the production of various dairy products as a replacement for skim milk in the preparation of normalized mixtures, in the production of prophylactic nutrition products for people with cow's milk protein allergies, and as a primary ingredient in beverages for regular consumption [72]. Products derived from whey are also used in the manufacture of pharmaceuticals, cosmetics, and skincare products (Figure 3). They are used by athletes to stimulate muscle protein synthesis, provide energy, and enhance endurance. Moreover, bioactive components derived from whey have numerous biomedical, pharmaceutical, and therapeutic applications [73]. There are already more than 60 peptide drugs on the market, and several hundred new therapeutic peptides are in preclinical and clinical development [74].

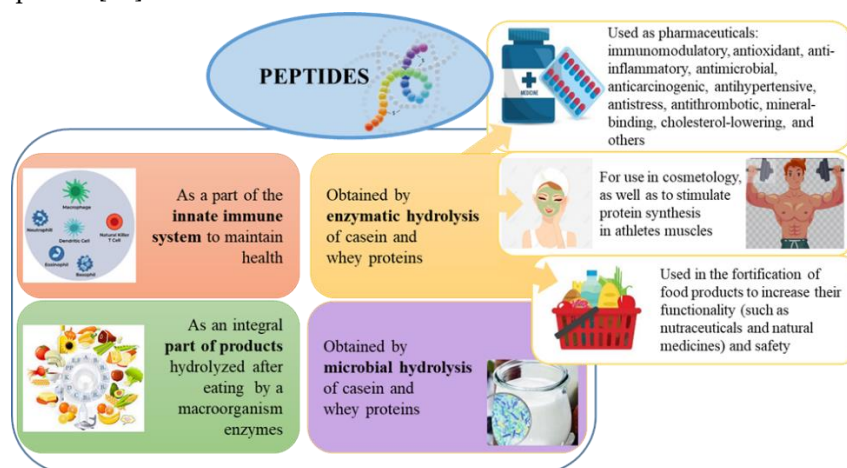


Figure 3. Sources and significance of milk and whey peptides.

Among the products of proteolytic cleavage of milk whey proteins, opioid peptides have been found to play a protective role, for example, by reducing environmental stress during breastfeeding [75]. Additionally, peptides with immunomodulatory and hypocholesterolemic effects, as well as peptides that influence intestinal motility, have been identified [37]. There is evidence that peptides derived from milk whey proteins are involved in the realization of biological functions such as calcium ion absorption, antioxidant activity, appetite regulation, and anticancer activity [76]. It has also been shown that some peptides derived from lactoferrin and casein exhibit antithrombotic activity [36,77]. According to Peipei Dou [78], WPH significantly suppresses the development of high blood pressure and tissue damage caused by hypertension by inhibiting ACE activity and reducing renin concentration, thereby lowering systolic blood pressure in spontaneously hypertensive rats. Increased *Akkermansia*, *Bacteroides*, and *Lactobacillus* bacteria also contributed to lowering blood pressure, reducing heart weight, and decreasing cardiomyocyte damage (hypertrophy and degeneration). The proteomic results showed that the expression of 19 proteins in the heart, mainly related to the Wnt and apelin signaling pathways, was altered after WPH intake. In particular, oxidative stress in serum decreased, as evidenced by a reduced malondialdehyde content, increased total antioxidant capacity, and superoxide dismutase activity. The data obtained indicated that WPH

exhibits promising antihypertensive capabilities in vivo and could become a potential alternative to antihypertensive dietary supplements.

BAPs derived from whey protein and added to probiotic fermentation products and confectionery items (chocolate, cookies, or cream) influence human health both directly and indirectly. They increase the viable count of probiotic bacteria, enhance the stability of probiotic products, act in the human body as antioxidants and ACE inhibitors, stimulate the proliferation of intestinal epithelial cells, and more. According to Pasin and Comerford [79], the benefits of dairy products in diabetes for insulin secretion and glycemic control are attributed to the high content of essential amino acids and BAPs that stimulate insulin secretion, as well as specific combinations of macronutrients and micronutrients and the unique strains of probiotics and BAPs they contain.

Due to their numerous proposed and proven health benefits, along with recent advancements in their discovery and identification [80], bioactive peptides from milk are already being used in formulations of new functional food products as nutraceuticals and natural medicines [81,82]. Fermented dairy products, such as yogurt, cheese, and sour milk, are excellent sources of milk peptides and are gaining popularity worldwide. Furthermore, both fermented and non-fermented dairy products have been shown to be associated with a lower risk of hypertension, coagulopathies, stroke, and cancer [83].

The antioxidant and ACE-inhibitory activity in fermented dairy products can be enhanced through co-fermentation with probiotic lactic acid bacteria (LAB) cultures, due to their relatively high proteolytic activity [84]. Probiotic strains (depending on their specificity) can contribute to various health benefits for the consumer, attributed to BAPs, during the fermentation of milk whey [85]. For instance, it has been shown that the probiotic *L. plantarum* subsp. *plantarum* PTCC 1896 or its components are promising for future use in treating type II diabetes by inhibiting DPP4 [86]. A proteomic profile study of kefir (an alcoholic fermented milk drink) identified 257 peptides, mainly released from β -casein [87]. Among these, 16 peptides demonstrated antimicrobial, immunomodulatory, ACE-inhibitory, opioid, antithrombotic, mineral-binding, and antioxidant activities. Yogurt peptides, when modified by treatment at pH 6.0 or above 50°C, exhibited higher antioxidant activity compared to the control [88].

4. Whey-Based Drinks

Native whey is rarely used as a beverage due to its unattractive taste [89]. However, developing various types of beverages based on whey is one of the most promising directions for utilizing whey for food purposes. This is not only due to the properties and composition of whey and its suitability for dietary and therapeutic nutrition but also due to its relative affordability and availability, and undeniably, its potential to address environmental pollution issues. In most cases, the range of whey beverages with improved taste is expanded by adding fruit or vegetable syrups, as well as other plant components enriched with bioactive substances, thus combining their positive properties. Without the addition of flavoring ingredients, the taste of the product can be altered using starter cultures, such as in studies by Skryplonek et al. [90,91] using *Lactobacillus acidophilus* LA-5 and *Bifidobacterium animalis* ssp. *lactis* Bb-12. Since acid whey virtually lacks casein, replacing part of the milk with acid whey reduces the gel strength and viscosity of the product [91]. To improve the texture, Skryplonek et al. added more milk solids to the product, either dry skimmed milk or replacing part of the milk with condensed milk. In another study, the addition of a stabilizer resulted in products with high viscosity and water-holding capacity for all tested samples [92]. Research by Oleinikova and coworkers showed that the best sensory performance and structure were achieved with a 3:2 ratio of milk to whey [93].

The biological value of whey is significantly enhanced by its use in cultivating LAB. The chemical composition of whey provides an ideal nutrient matrix for the growth and viability of LAB [94- 96]. The proper selection of LAB can be used to specifically influence the functionality of the resulting beverages. To create beneficial and appealing drinks from acid whey, it is possible to modify and monitor the impact of flavoring ingredients and starter cultures with varying metabolic orientations. Additionally, research has shown that homogenization after fermentation can improve

the physical stability of the product and provide a smoother taste. Lievore et al. [89] prepared a liquid beverage in which acid whey replaced water, resulting in a more homogeneous product. When developing fermented dairy products with whey, it is important to consider the addition of stabilizers, homogenization, and the type of beverage to compensate for texture changes. A notable area of interest is the growth of probiotics in the presence of whey derivatives, such as lactulose, a derivative of lactose that is a highly sought-after prebiotic in functional feeding [97]. Another approach is the simultaneous addition of prebiotics to whey along with probiotic starter cultures. For example, the addition of wheat bran, which imparts synbiotic properties to the beverage, also enhances the survival of probiotic microorganisms under conditions of acid and bile stresses [98].

Over the past two decades, a variety of whey-based beverages and similar products containing isolated whey components (mainly whey proteins) have emerged on the market. These beverages are often refreshing, using starter and/or probiotic cultures capable of metabolizing lactose, thereby defining the unique taste and texture of the fermented drink [99]. Both mono- and mixed cultures of LAB are used, and to improve the organoleptic properties, the beverages are enriched with fruit and berry additives, enhancing their consumer appeal. For developing products for children and adolescents, a formulation was created to replace milk with whey, also including iron [100]. Numerous randomized, double-blind, and placebo-controlled clinical trials have demonstrated the promising therapeutic applications of whey-derived products for preventing type 2 diabetes, obesity, cardiovascular diseases, phenylketonuria, eliminating excessive free radicals formed as a result of oxidative stress, inhibiting tumor development, providing anti-proliferative effects, and treating metastatic carcinoma [73]. The use of acid whey in beverage formulations will not only expand the range of dairy products with high nutritional and biological value but also contribute to increased milk processing efficiency through the implementation of resource-saving technologies.

Thus, due to their significant technological and functional potential, LAB are key microorganisms for the food industry. In the fermented dairy products they produce traditionally used worldwide, LAB not only help preserve the raw materials but are also valued as bio-protective and health-promoting agents. This is due to the presence of probiotic microorganisms among them, as well as their nutritional and organoleptic properties, and they are responsible for the flavor development of the final products [60,101]. The choice of specific starter cultures depends on the particular phenotypes that benefit the product, ensuring the shelf life, safety, texture, and taste of the final product. Studies have shown that whey promotes the production of bacteriocins by LAB [102, 103]. It has also been shown that the introduction of acetic acid bacteria into the starter culture contributes to anti-*Candida* antagonism in whey-based fermented beverages [104]. Rationally selected LAB can also be used to develop functional beverages with reduced beta-lactoglobulin whey protein content, which is the main cause of milk allergy [95].

5. Proteolytic Activity of Lactic Acid Bacteria

As a nitrogen source for LAB growth, a sufficient amount of free amino acids is required, and therefore, for development in high-protein environments, particularly milk, they have evolved the ability to hydrolyze proteins, producing not only free amino acids but also a wide range of biologically active peptides [105], perceived as potential therapeutic tools and important components of personalized nutrition suitable for the prevention of many civilization- and nutrition-related diseases.

To increase the production of bioactive peptides from milk proteins, which are promising for the functionalization of fermented dairy products, various strategies have been developed, including the observance of optimal hydrolysis conditions for whey protein concentrate [106,86] and the use of LAB proteolytic systems [107]. The use of both specific and nonspecific proteases is the most common method for obtaining bioactive peptides, requiring less time and allowing control over the hydrolysis process to obtain peptides with specific molecular weights and amino acid compositions. Their production increases with processing due to the action of proteolytic enzymes (endo- and exopeptidases), both endogenous and from microbial fermentation flora. This hydrolysis involves the action of endopeptidases, which cleave proteins into major fragments. Then, they are further

cleaved by exopeptidases into smaller peptides and free amino acids. Hydrolysis leads to the accumulation of peptides and free amino acids in food. Some of the peptides obtained may be bioactive and thus have a beneficial effect on consumer health [108].

Among the products of proteolysis, short-chain peptides predominate, demonstrating low allergenicity and high levels of essential amino acids. The resulting hydrolysate can be used in the technology of preparing various dairy products to replace skim milk in normalized blends, to produce products for the preventive nutrition of people with cow's milk protein allergy, and as the main ingredient in beverages for regular consumption [72].

Microbial fermentation with the release of bioactive peptides encoded in milk proteins has now been recognized as a natural, safe, and economically efficient strategy for obtaining these highly effective health regulators. The proteolytic system of LAB mainly consists of cell wall-bound proteinases (initially cleaving casein into oligopeptides), peptide transporters (transporting oligopeptides into the cytoplasm), and various intracellular peptidases, including endopeptidases, aminopeptidases, tripeptidases, and dipeptidases, converting peptides into small molecules and generating free amino acids [109,110]. The features of the LAB proteolytic system are associated with their taxonomic affiliation. Its activity is most pronounced in *Lactobacillus helveticus* [111], followed by *Lact. delbrueckii* subsp. *bulgaricus*, *Lact. delbrueckii* subsp. *lactis/diacetylactis*, and *Lact. delbrueckii* subsp. *lactis/cremoris* [94]. Using *Lact. helveticus* as an example, it has been shown that products fermented by different strains of the same genus and species differed from each other in terms of texture, protein content, total and non-protein nitrogen, as well as proteolysis index [112].

6. Perspectives

In view of the necessity to establish technology that produces minimal waste and enhances the profitability of the dairy sector, the need for improving the effectiveness of whey utilization and conversion into a broad array of valuable products and food supplements is steadily increasing. This is supported not only by its health-promoting food elements but also by the marketability of the products created, positioned as Generally Acknowledged as Safe (GRAS) [113,114,4]. The ideal collection of vital amino acids found in whey proteins, which elevate their biological worth in comparison to proteins from other sources, enables them, as they undergo fermentative processes both in the digestive system and during microbial fermentation, to act as a reservoir of BAPs. To enhance the process of producing BAPs, hybrid approaches, presently utilized in biopharmaceuticals, have been suggested, founded on computational simulation combined with heuristics and mechanistic simulation. Criteria for industrial procedures concerning the liberation and resilience of peptides based on multiple process variables have been consolidated, and certain techniques for enriching peptides derived from whey, potentially suitable for industrial use, have been examined [115].

The worldwide market for BAPs is progressively expanding due to rising consumer consciousness of the favorable correlation between functional food items and health, alongside the potential to use them as replacements for conventionally marketed medications, particularly those subject to stringent legislation or those that have become ineffective. Different nations have established diverse regulatory frameworks to shield consumers from hazards and misleading assertions regarding BAPs. Scientific verification of their safety and effectiveness is a crucial factor that can substantially influence the introduction of BAPs into the market. Assertions regarding the health advantages of BAPs must be substantiated by substantial human research findings. Regulatory standards for the utilization of BAPs obtained from various food protein sources, including milk, whey, fish, and soybeans, are present in numerous countries' markets [116].

A scientometric evaluation of studies published between 2011 and 2021 by Brazilian researchers indicated a rising interest in fundamental investigations involving BAPs from various protein origins, encompassing food items and agro-industrial remnants. The most extensively studied characteristics of these biomolecules are their antioxidant properties and their linked antimicrobial capabilities. Furthermore, there are accounts of other impacts, such as anxiolytic, anti-adipogenic, anticoagulant,

anticonvulsant, anti-sclerotic, and cyto-regulatory functions, which ascertain the potential for utilizing these biomolecules in the innovation of novel products [117].

Considering the continuous growth in the number of peptide sequences, predominantly identified through laborious experimental screening, coupled with several challenges, there is a necessity to devise a dependable and precise model for their forecasting. Systems have now been devised that outperform existing techniques, achieving almost complete accuracy, which will be particularly advantageous in the design of anticancer medications [61-64].

One method to enhance the effectiveness of BAPs release investigations and diminish the quantity of labor-intensive experiments is the utilization of computational modeling of enzymatic protein hydrolysis. Through the application of bioinformatics modeling methodologies, a digital representation of the peptide complex of various kinds of whey with anticipated biological activity, safety, and sensory characteristics has been formulated. The investigation was carried out using proteomic database instruments founded on a formerly established algorithm for amalgamated bioinformatics modeling. With respect to the positioning of the protein proportion in the protein profile, hydrolysis by the protease complex chymotrypsin C-subtilisin was identified as the most effective technique for liberating peptides with both antioxidant and ACE-inhibitory functions. It was additionally observed that bioactive peptides procured as a consequence of *in silico* hydrolysis modeling following gastrointestinal digestion can be deemed safe in relation to allergic responses and toxicological impacts [118].

An effort to develop an extensive repository of bioactive peptides delineated in the literature, graphically correlated to the sequences of precursor proteins, which could be employed for seeking particular roles of bioactive peptides derived from milk proteins from any mammalian origin, was executed by Nielsen et al. [119].

7. Conclusion

Recently, there has been growing recognition of the key role of food and beverages in the prevention and treatment of diseases. The production and consumption of functional food products have become significant because they provide health benefits beyond basic nutritional functions. Currently, beverages are undoubtedly the most active category of functional food products due to their convenience and the ability to meet consumer demands for content, size, shape, and appearance of packaging, as well as the ease of distribution and storage of chilled and long-shelf-life products. Moreover, they serve as excellent vehicles for delivering nutrients and bioactive compounds, including vitamins, minerals, antioxidants, omega-3 fatty acids, plant extracts, as well as fiber, prebiotics, and probiotics.

In recent years, beverages based on whey have gained prominence among health foods due to their nutritional and health-promoting qualities [120]. Formulations of such beverages are developed according to physiological characteristics of the body, their own medical-biological requirements are established, new highly sensitive methods for safety and functional activity assessment suitable for use in production conditions are developed.

The growing interest in BAPs prompts the scientific community and the food industry to develop new food additives and functional products based on them. Their use is facilitated by the simplicity of the procedure for their production and separation [121]. Currently, such products are recognized as the only rational option for survival in the modern world [122]. They provide nutritional benefits beyond a normal balanced diet and rightfully can be called "products of the future" [123], especially as the nutritional status is described as one of the leading risk factors for severe diseases, including the currently relevant acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [124].

Pure BAPs of food origin are already appearing abundantly on the market as nutraceuticals, and understanding their mechanism of action allows regulating their use for therapeutic purposes similar to medicinal drugs [125].

For the production of fermented beverages based on whey enriched with biologically active components, the selection of a strain of LAB with high proteolytic and peptidolytic activity towards

milk proteins is a crucial parameter. A deep understanding of the functionality and regulation of the proteolytic system of LAB opens up great opportunities to influence the final result of obtaining food products with the desired potential properties contributing to health reinforcement.

Foundations have already been laid for developing new commercial dairy products with antihypertensive effects [56,126], which opens up excellent opportunities for obtaining functional dairy products that improve heart health by reducing blood pressure and heart rate.

Many studies report the isolation of new bioactive peptides from milk proteins using new technologies as pretreatment. Ultrasonic treatment, which changes the conformation of proteins, enhances the biological functions of enzymes and the reaction rate of enzymatic hydrolysis, ensuring easy control, ease of operation, gentle operating conditions, the possibility of achieving industrial scaling and production, as well as effective influence of auxiliary enzymatic hydrolysis, has been recognized as innovative and most efficient [127].

Expanding the range of specialized functional beverages based on whey can also be achieved by using various biologically active plant raw materials in their production, enriching their chemical composition, improving the taste characteristics and consistency of products, and increasing consumer appeal.

Author Contributions: Writing—original draft preparation, SM.; writing—review and editing, YO, AR, SM, ZY, EK.

Funding: This research is funded by the Science Committee of the Ministry of Science and Higher Education of the Republic of Kazakhstan (Grant No. AP19674760).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: No new data were created.

Conflicts of Interest: The authors declare no conflicts of interest.

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