Review Article

Milk Bioactive Peptides: Antioxidant, Antimicrobial and Anti-Diabetic Activities

Mahmoud El-Sayed[1], Sameh Awad[2, *]

[1]Department of Dairy Technology Research, Food Technology Research Institute, ARC, Giza, Egypt

[2]Laboratory of Dairy Microorganisms and Cheese Research (DMCR), Department of Dairy Science and Technology, Faculty of Agriculture, Alexandria University, Alexandria, Egypt

Email address:
mahmoud_im1981@yahoo.com (M. El-Sayed), Sameh111eg@yahoo.com (S. Awad)
[*]Corresponding author

Abstract: The main milk proteins are caseins and whey proteins, some other minor proteins and peptides, are also found in milk. Proteins are vital ingredients for human because they provide all the essential amino acids needed for body and human health. Milk proteins are very important sources of bioactive peptides. The bioactive peptides are inactive within the sequence of the parent protein and can be released by proteolytic enzymes, during gastrointestinal digestion or during milk processing, for example the adding coagulation enzymes and starter culture. Once bioactive peptides are present in the body, these peptides may act as regulatory compounds with hormone-like activity. Furthermore, Bioactive peptides from milk proteins have many biological activities such as antimicrobial, antihypertensive, antithrombotic, antioxidant, mineral binding, and anti-diabetic. Bioactive peptides have potential health and have pharmaceutical applications. Antimicrobial peptides are recognized as an important component of innate immunity, particularly at mucosal surfaces such as the lungs and small intestine that are constantly exposed to a range of potential pathogens. The ability of protein hydrolysates to inhibit deleterious changes caused by lipid oxidation appears to be related to the nature and composition of the different peptide fractions. Milk protein hydrolysate possesses free-radical-scavenging and anti-inflammatory activities have many beneficial effects on the increase of the glucose-induced insulin secretion and reduction in postprandial glycemia. This article is tried through exposure in some detail to review characteristics of some milk protein peptides and its positive effects on human health.

Keywords: Milk Proteins, Bioactive Peptides, Anti-diabetic, Antioxidant, Antimicrobial, Anti-hypertensive

1. Introduction

The two major milk proteins are caseins and whey proteins. Caseins represent about 80% of the total protein in cow milk; exist mainly in macromolecular complexes as casein micelles consisting of more than 1,000 casein submicelles. Caseins are known to be precursors of several different bioactive peptides. Casein is phosphoproteins and consists of about 30 different components including genetic variants. Casein consists mainly of αs- (α$_{s1}$-, α$_{s2}$-), β-, and κ-casein [1]. The whey proteins, which presented about 20% of the total milk proteins, whey proteins are excellent source of both nutritious and functional proteins. The main whey protein constituents, α-lactalbumin and β-lactoglobulin, account for 70–80% of the total whey proteins in bovine milk. Other minor components include bovine serum albumin (BSA), immunoglobulins (Igs) (mainly the G type), lactoferrin (LF), lactoperoxidase (LP), protease-peptones (PP), and many enzymes [2]. Milk proteins have been identified as a very important source of most bioactive peptides, these peptides are in an inactive state within the milk protein molecule and can be released during enzymatic digestion *in vitro* and *in vivo* [3].

2. Milk Protein Derived Bioactive Peptides

Milk proteins are known as the very important sources of bioactive peptides [4]. The health benefits of these peptides are classified as cytomodulatory, mineral binding, antimicrobial, immunomodulatory, blood-pressure lowering (Angiotensin-converting enzyme ACE- inhibitory), antithrombotic, antioxidant and opioid like, in addition to cholesterol-lowering and mineral absorption/bioavailability enhancers [5-8]. Bioactive peptides have been classified as specific fragments of protein that have a positive impact on body functions or conditions and may ultimately influence health [9, 10]. The release of bioactive peptides from milk proteins in the gastrointestinal tract results from the action of digestive enzymes such as pepsin and pancreatic enzymes (trypsin, chymotrypsin, carboxy- and aminopeptidases). The efficiency of physiological activity of biopeptides depends on their ability to maintain integral state during transport to the various functional systems of the body [11, 12]. Additionally, bioactive milk peptides can be absorbed intact from the intestinal lumen into the blood circulation, these may thus serve as novel functional food ingredients or pharmaceutical agents [13].

2.1. Production of Bioactive Peptides

Bioactive peptides can be released from the parent protein by enzymatic hydrolysis during gastrointestinal digestion, fermentation or maturation during food processing or proteolysis by food-grade enzymes derived from different origins (animal, plants or microorganisms) [1-3, 14, 15].

2.1.1. Enzymatic Hydrolysis
The hydrolysis of proteins by enzymes is a vital bioprocess to improve the physicochemical, functional and nutritional properties of original proteins or to prepare extensively hydrolyzed proteins for hypoallergenic infant diets and nutritional therapy [16-18]. The most used way to produce bioactive peptides is through enzymatic hydrolysis of whole protein molecules. Many of bioactive peptides have been produced using gastrointestinal enzymes, usually pepsin and trypsin, for example, Angiotensin-converting enzyme inhibitory (ACEI) peptides and calcium-binding phosphopeptides (CPPs), are most commonly produced by trypsin from milk proteins [19-22]. The most notable enzymes are pepsin, trypsin and chymotrypsin that have been shown to release bioactive peptides such as antihypertensive peptides, calcium-binding phosphopeptides (CPPs), antibacterial, immunomodulatory and opioid peptides from different casein (α-, β- and κ-casein) and whey proteins, e.g., α-lactalbumin (α-la), β-lactoglobulin (β-lg) and glycomacropeptide (GMP) [23, 24]. Bioactive peptides have been isolated from many milk and dairy products including cheese, kefir and yoghurt. These peptides are inactive within protein molecules and can be released in three ways by enzymatic hydrolysis by digestive enzymes predominantly alcalase, pepsin, trypsin, pancreatin, thermolysin and chymotrypsin, fermentation of milk with proteolytic starter cultures or proteolysis by enzymes derived from microorganisms or plants [25-27]. It was reported that the enzymatic hydrolysis of whey proteins had yielded bioactive peptides those similarity opioid receptor ligands *invitro* and *invivo* [28]. Opioid agonists have been found in whey proteins such as α-LA, ß-LG, and BSA, whereas opioid antagonists have been isolated from lactoferrrin. Peptides from both α-LA or ß-LG contain sequences in their primary structure similar to typical opioid peptide sequences, whereas serorphin (BSA f (399–404)) can be classed as an atypical opioid peptide with a dissimilar amino sequence. The complete hydrolysis is desirable for the production of bioactive peptides; meanwhile partial hydrolysis increases the number of peptides. The intricacy which induces a variety of bio-functional and techno-functional peptides can be reduced by using selective enzymes in incorporation with downstream processes [29].

2.1.2. Microbial Fermentation
The proteolytic system of lactic acid bacteria (LAB) is very complicated. It is possessed of an extracellular located serine proteinase, a transport system specific for di-, tri-, and oligopeptides, and a multitude of intracellular peptidases. Proteinases of lactic acid bacteria may hydrolyze more than 40% of the peptide bonds of α_{s1}- and β-caseins, producing oligopeptides of 4 to 40 amino acid residues [30]. Most of industrially utilized dairy starter cultures have proteolytic systems. Bioactive peptides can be generated by the starter and non-starter bacteria used in the production of fermented dairy products. Todays, the proteolytic system of most lactic acid bacteria (LAB) strains, e.g. *Lactococcus lactis, Lactobacillus helveticus* and *Lb. delbrueckii* ssp *bulgaricus*, is already well characterized. This system consists of a cell wall-bound proteinase and several distinct intracellular peptidases, including endopeptidases, aminopeptidases, tripeptidases and dipeptidases [31]. *Lactobacillus helveticus* is widely used as a dairy starter in the manufacture of traditional fermented milk products, such as Emmental cheese and highly proteolytic *Lb. helveticus* strains capable of releasing ACE-inhibitory peptides. The best-known ACE-inhibitory peptides, Val-Pro-Pro (VPP) and Ile-Pro-Pro (IPP), have been identified in milk fermented with *Lb. helveticus* strains [32, 33]. *Lb. rhamnosus* GG was studied for capable of suppressing immune function by generating peptides from the hydrolysis of casein [34]. It was reported that the digests of casein by peptidases produced by *Lb. rhamnosus* inhibited protein kinase C translocation and down regulated IL-2 expression. Taken together, these results indicate suppression of T cell activation by casein digests. *Lactobacillus paracasei* was investigated for capable of suppressing immune function by generating peptides from the hydrolysis of β-lactoglobulin (β-lg) [35]. It was found that the *Lb. paracasei* was capable of inducing oral tolerance to β-lg by producing peptidases that can hydrolysis of β-lg. The proteolytic activities of several dairy LAB cultures and probiotic strains (*Lactobacillus acidophilus*, *Bifidobacterium*

lactis and *Lactobacillus casei*) were studied as determinant of growth and *in vitro* ACE-inhibitory activity in milk fermented with these single cultures [36]. All the cultures released ACE-inhibitory peptides during growth with a *Bifidobacterium longum* strain and the probiotic *Lb. acidophilus* strain.

2.2. General Classification of Milk Bioactive Peptides

In most studies the primary classes of bioactive milk peptides, based on their specific physiological functions [37-40]:
1. Antimicrobial Peptides.
2. ACE Inhibitors.
3. Antithrombotic Peptides.
4. Opioid Milk Peptides.
5. Immunomodulatory peptides.
6. Mineral binding peptides.
7. Antidiabetic peptides.

To exert physiological effects *in vivo*, bioactive peptides must be released during intestinal digestion and then reach their target sites at the luminal side of the intestinal tract or after reabsorption, in the peripheral organs.

2.2.1. Antimicrobial Peptides

The antibacterial properties of milk have been known for a long time. In fact, the incidence of diseases like diarrhea or respiratory infections is significantly lower in breastfed infants than in formula-fed infants; a variety of protective factors in human milk are thought to be responsible for this effect. During the first few days postpartum, the specific activity of immunoglobulins is the dominant factor for immunity [41, 42]. The first discovery about the antimicrobial properties of milk was made by Jones and Simms [43]. They identified substance capable of inhibiting the growth of streptococci called lactenin. However, the first antimicrobial activities attributable to peptides isolated from bovine milk peptide were discovered in the late 1960s. These poorly defined peptide fractions were derived from chymosin digestion of casein and referred to as casecidins [44]. Later, another group identified a group of basic, high molecular weight polypeptides released from heated and rennin-treated casein [45]. These polypeptides, denoted as casecidins, were probably derived from κ-casein and α$_{s1}$-casein, and displayed bactericidal properties against several resistant strains of *Staphylococcus aureus*, as well as against lactobacilli. The antimicrobial and immunological functions of some of the bovine milk proteins; and their proteolytic products have been proposed to assist the survival of the neonate, which lacks a well-developed immune system. The best characterized of the antimicrobial proteins in bovine milk include lactoferrin, lactoperoxidase and lysozyme. In some species minor proteins, including a folate binding protein, are also regarded [10, 46].

Many peptides arising from the hydrolysis of milk protein have already been demonstrated to have important non-nutritional roles including immunomodulatory, mineral stabilizing, antibacterial, antiviral and antifungal activities. In addition, it has been recognized for a longtime that breast-feeding of infants provides protection from a range of enteric and respiratory infections. Antibacterial peptides are recognized as an important component of innate immunity, particularly at mucosal surfaces such as the lungs and small intestine that are constantly exposed to a range of potential pathogens [46-49]. Antimicrobial peptides are usually composed of a hydrophobic surface and a hydrophilic surface. This distinct amphipathic characteristic is believed to play a role in the antimicrobial mechanism of action allowing the peptide to interact with the bacterial membrane [50]. Antimicrobial peptides usually have less than 50 amino acid residues, of which nearly 50% are hydrophobic and have a molecular weight below 10 kDa. These peptides can be generated in vitro by enzymatic hydrolysis. Many of these small molecules (< 10 kDa; 3–50 amino acid residues) have proven to be potent antimicrobial substances with promising applications in medicine or food preservation. Therefore, intensive research work has been carried out to detect, purify and characterize as many of these peptides as possible for application in industrial production [51, 52]. Most Antimicrobial peptides, with their amphipathic nature, directly act on the membrane of the pathogen. The cationic properties of Antimicrobial peptides are implicated in their selective interaction with the negatively charged surfaces of microbial membranes, resulting in the accumulation of Antimicrobial peptides on the membrane surface. Then, their hydrophobic portions are responsible for the interaction with hydrophobic components of the membrane. From this complex interaction with the membrane, major rearrangements of its structure occur, which may result from the formation of peptide-lipid specific interactions, the peptide translocation across the membrane and interaction with intracellular targets or the most common mechanism, a membranolytic effect [53, 54]. The antimicrobial activity of ovine whey proteins and of their peptic hydrolysates was measured against different pathogenic microbial strains. The peptic hydrolysates inhibited the growth of *Escherichia coli* HB101*, Escherichia coli* Cip812*, Bacillus subtilis* Cip5265, and *Staphylococcus aureus*, but no peptide identification was carried out [55]. The digestion of caprine whey proteins was investigated *in vitro* by two-steps degradation assay, using human gastric juice at pH 2.5 and human duodenal juice at pH 7.5 [56]. The protein degradation and antibacterial activity obtained were compared with those obtained after treatment with commercial enzymes, by using pepsin and a mixture of trypsin and chymotrypsin. The two methods resulted in different caprine protein and peptide profiles. Active growing cells of *E. coli* were inhibited by the digestion products from caprine whey obtained after treatment with human gastric juice and human duodenal juice. Cells of *Bacillus cereus* were inhibited only by whey proteins obtained after reaction with human gastric juice, while the products after further degradation with human duodenal juice demonstrated no significant effect.

2.2.1.1. Whey Proteins Derived Antimicrobial Peptides

Lactoferricin is a potent bactericidal peptide specifically generated by enzymatic degradation of lactoferrin, also exhibit antimicrobial activity against both Gram-positive and Gram-negative microorganisms. Lactoferricin B obtained from bovine lactoferrin and lactoferricin H obtained from human lactoferrin. The fragments were characterized and named human (H) and bovine (B) lactoferricin. The structure activity relation of lactoferricin fragment has been studied during last year's. Some studies have been shown that antimicrobial, antifungal, antitumor, and antiviral properties of lactoferricin can be associated to tryptophan/Arginine-rich proportion of the peptide. Also the anti-inflammatory and immunomodulating properties are associated to a positively charged region of the molecule [57, 58]. It has been demonstrated that Lactoferricin starts electrostatic interaction with the negatively charged membranes of bacteria in initial binding, lipopolysaccharide and teichoic acid as binding site in Gram-negative and Gram-positive bacteria have been identified. It has been demonstrated the peptide approach the cytoplasm and suppress the bacterial protein synthesis that exact mechanism is not clear [59]. Lactoferrampin is an antimicrobial cationic domain in the N1-domain of lactoferrin. It includes amino acids 268-284 of bovine lactoferrin. It has been demonstrated that lactoferrampin display higher candidacidal activity than the lactoferrin. Furthermore, lactoferrampin has antimicrobial activity against of *Bacillus subtilis, Escherichia coli*, and *Pseudomonas aeruginosa*, but not against of the fermenting bacteria, *Actinomyces naeslundii, Porphyromonas gingivalis, Streptococcus mutans* and *Streptococcus sanguis*. Lactoferrampin plays a critical role in membrane- mediated activities of lactoferrin [60]. ß-Lactoglobulin exhibit around half of the whole protein in bovine whey, while human milk contains no ß- lactoglobulin. Proteolytic digestion of bovine ß-lactoglobulin by trypsin bears four peptide segments with bactericidal activity. These peptides corresponded to ß-lactoglobulin f (15–20), f (25–40), f (78–83) and f (92–100). These peptides only effect on Gram-positive bacteria and inhibit them. The amino acid sequence of peptide fragment f (92–100) is altered by replacing of Asp with Arg and the substitute of a Lys residue at the C-terminal end to enhance the bactericidal activity to Gram-negative bacteria [61]. The charge, hydrophobicity and cationic=anionic character are important factors for bactericidal activity. The β-Lactoglobulin as source of bioactive peptides 261 negative charge of these peptides explains why they were only weakly effective against Gram-negative bacteria whose membranes contain lipopolysaccharide, a negatively charged molecule [62]. The antimicrobial potential of whey protein isolate hydrolyzed by gastrointestinal enzymes was revealed that the pepsin hydrolysates exhibited significant activity [63]. Fractionation of 60-min hydrolysate by reversed-phase high performance liquid chromatography yielded five fractions that were antibacterial, with minimum inhibitory concentrations comprised between 20 and 35 mg mL^{-1}. Five peptide fragments derived from β-Lb, and one fragment from

α-La (f117-121) were identified as antibacterial. Another antibacterial fragment (f14-18) very close to a peptide sequence previously identified was also derived from β-Lg [63]. Some studies have been shown that α-lactalbumin provided bactericidal peptides after digestion with trypsin and chymotrypsin, but not with pepsin. It has bactericidal activity against of Gram-positive bacteria [61]. The antibacterial peptides derived from α-La were negatively charged at the pH of the antibacterial assay. This character may explain why they were weakly active against Gram-negative bacteria whose outer membranes contain negatively charged lipopolysaccharide as a major component [61].

2.2.1.2. Casein Derived Antimicrobial Peptides

Isracidin, is that N-terminal segment of αs$_1$-CN (2770 Da, f (1–23) that inhibit the *in vitro* growth of *Lactobacilli* and other Gram positive bacteria. This peptide also protects sheep and cows against mastitis. Isracidin has a strong protective effect against *Staphylococcus aureus, Streptococcus pyogenes* and *Listeria monocytogenes* when administered at low dose as 10 μg per mouse prior to bacterial challenge [64]. Casein derived immunopeptides including immunopeptides from α$_{SI}$-casein (residues 194-199) and αs-casein (residues 63-68 and 191-193) have been shown to stimulate phagocytosis of sheep red blood cells by murine peritoneal macrophages, and to exert a protective effect against *Klebsiella pneumoniae* [65]. Conversely, *in vivo*, isracidin has proven competitive with antibiotics in therapeutic use and provided a strong protective effect in mice against *Staphylococcus aureus, Streptococcus pyogenes* and *Listeria monocytogenes* [66]. The isracidin is active against emerging pathogens of major concern to food safety, including *Escherichia coli* O157: H7, *Enterobacter sakazakii* and *Staph. aureus* [67]. The production of three peptides, generated by *Lactobacillus acidophilus* DPC6026 fermentation of α$_{s1}$-casein (Caseicin A, B and C), have common features with other reported antibacterial peptides, given by a high degree of homology with isracidin for istance [67]. Caseicin A and B were able to inhibit *Escherichia coli* O157: H7 and *Enterobacter sakazakii*, while Caseicin C displayed only minor activity against *Listeria innocua*. Casocidin-1 is α$_{S2}$-casein (150–188), has a mass of 4870 Da, and a regular alternation of hydrophobic and hydrophilic residues. It also contains a high proportion of basic amino acyl residues (10 of 39) and as a consequence has a pI of 8.9. With such characteristics, Casocidin-1 resembles the amphipathic defensins and was proposed to cause disruption of bacterial membranes, which is responsible for its antimicrobial activity against *Staphylococcus carnosus* and *E. coli*. In addition, another α$_{S2}$-casein peptide, α$_{S2}$-casein (183–207), displayed antibacterial activity against similar bacterial species to α$_{S2}$-casein (164–179) and had an over representation of basic amino acyl residues in its sequence [68]. Chymosin digest of bovine casein released five different antibacterial peptides originated from the C-terminal of bovine αs2-casein. The identified peptides, f (181-207), f (175-207), and f (164-207),

were active against a wide variety of Gram-positive and also Gram-negative bacteria, with MIC ranging from 21 to 168 mg mL^{-1}, 10.7e171.2 mg mL^{-1}, and 4.8e76.2 mg mL^{-1}, respectively [69]. In addition, these peptides inhibited sensitive Gram-positive bacteria as effectively as nisin and lactoferricin B. Therefore, they had a high potential to be used as food-grade preservatives [69]. Four antibacterial peptides have been identified from a pepsin hydrolysate of ovine αs2-casein. The peptides correspond to sequences αs2-casein (f165–170), (f203– 208), (f165–181), and (f184– 208), taking into account that the last two fragments were homologous to those previously identified in the bovine protein. In this study, the ovine αs2-casein peptides (f165–181) showed the highest antibacterial activity against all bacteria tested while the fragment (f203– 208) revealed itself a good example of a multifunctional peptide because it exhibited not only antimicrobial activity, but also, potent antihypertensive and antioxidant activity [70]. The αs$_{2-}$casein peptide f (183-207) generated pores in the outer membrane of Gram-negative bacteria and in the cell wall of Gram-positive. In the Gram-negative bacteria, the f (183-207) originated the cytoplasm condensation, and in the Gram-positive bacteria, the cytoplasmic content leaked to the extracellular medium [71]. Substitution of the also positively charged Lys residues at positions 15 and 17 of the α$_{s2}$-casein f (183–207) peptide also caused a significant reduction of the effectiveness against *C. sakazakii*, which points toward the importance of the positive charge of the peptide for its biological activity [72]. Fractions of human ß-casein have also a protective effect against *Klebsiella pneumoniae* in mice. The immunomodulatory peptide derived from bovine ß-casein f (193-209), was shown to enhance the antimicrobial activity of mouse macrophages [37]. Kappacin is an antimicrobial peptide that obtained from κ-casein. This peptide was non-glycosylated portion of human κ-casein f (63–117) and it was obtained after acidification of human milk and incubation with pepsin [73]. Kappacin corresponds to the non-glycosylated, phosphorylated form of caseinomacropeptide (CMP) which exhibited growth inhibitory activity against Gram-positive (*Streptococcus mutans*) and Gram negative (*Porphyromonas gingivals*) bacteria. It has been demonstrated that active component of this peptide that exhibit growth inhibitory activity against Gram-positive (*Streptococcus mutans*) and Gram-negative *Porphyromonas gingivals*) bacteria is phosphorylated and non-glycosylated. Also, it has been demonstrated that non-phosphorylated and glycosylated forms don't exhibit any activity against *Streptococcus mutans* [74]. The mechanism that by this kappacin limit gastrointestinal tract infection in the growing neonate, may be release of kappacin in stomach that by which increase sensitivity of bacteria to gastric acid by collapsing important trans membrane cation gradients. Molecular modeling suggests that the reason why the glycosylated forms of kappacin don't have antimicrobial activity because of sugar moieties would block pore formation [75]. κ-casecidin is a pentapeptide with antimicrobial activity that was isolated from trypsin digest of bovine κ-casein. It can suppress growth of some pathogenic bacteria such as *Staph. aureus, E. coli* and *Salmonella typhimurium*. Also, this peptide displays cytotoxic activity against some mammalian cells such as human leukemic cell lines. Cytotoxic effect of this peptide may be inducing apoptosis [39]. Mean over a C-terminal chymosin-digest of bovine κ-casein [κ-CN f (106-169)], called caseinomacropeptide (CMP), and exerts *in vitro* antibacterial activity against major oral pathogens (e.g. *Streptococcus mutans, Porphyromonas gingivalis* and *Actinomyces naeslundii*) and *E. coli* [48, 74]. Caseinomacropeptide (CMP) interacts with toxins, viruses and bacteria, thus it can promote health. Glycosylated CMP suppresses the binding of cholera toxins to their oligosaccharide receptors on cell walls and defends cells from infection induced by influenza virus. CMP also suppresses the adhesion of cariogenic bacteria such as *Streptococcus mutans, S. sanguis* and *S. sobrinus* to the oral cavity and regulates the composition of the dental plaque micro biota. This could help to influence acid formation in the dental plaque, in turn reducing hydroxyapatite dikssolution from tooth enamel and promoting remineralisation. For this, it has applied for oral care products to prevent dental caries [76].

2.2.2. ACE-inhibitory Peptides

Angiotensin-converting enzyme (ACE; EC 3.4.15.1) plays a dual role in the regulation of hypertension: it catalyzes the production of the vasoconstrictor angiotensin II and it inactivates the vasodilator bradykinin. By inhibiting these processes, ACE inhibitors have antihypertensive effects. Peptides derived from milk proteins can have ACE-inhibiting properties and may thus be used as antihypertensive components [77]. Through fermentation, peptides that have an ACE-inhibiting and thus a blood pressure–lowering effect can be derived from milk proteins [78]. A fermented milk product with the biologically active peptides valyl-prolyl-proline (Val-Pro-Pro) and isoleucyl-prolyl-proline (Ile-Pro-Pro) was shown to lower blood pressure in spontaneously hypertensive rats [32]. Many ACE inhibitory peptides have been isolated and identified in enzymatic hydrolysates of bovine casein. The isolation of ACE inhibitory peptides from whey protein is usually limited by the rigid structure of native β-lactoglobulin because it is resistant to digestive enzymes such as pepsin and pancreatin [79]. Several ACE inhibitory and antihypertensive peptides prepared by enzymatic digestion of cheese whey proteins as byproducts from the manufacture of cheese [80].

2.2.3. Antioxidative Peptides

The importance of oxidation in the body and in foodstuffs has been widely recognized. Oxidation is a vital process in aerobic organisms, particularly in vertebrates and humans although it leads to the formation of free radicals [52]. When an excess of free radicals is formed, they can overwhelm protective enzymes like superoxide dismutase, catalase and peroxidase, and cause destructive and lethal cellular effects (e.g. apoptosis) by oxidizing membrane lipids, cellular proteins, DNA and enzymes, thus shutting down cellular

respiration. It is well known that lipid peroxidation occurring in food products causes deteriorations in food quality, for example rancid flavour, unacceptable taste and shortening of shelf life. In addition, it has been recognized that oxidative stress plays a significant role in a number of age specific diseases [81-83]. Radical scavenging is the main mechanism by which antioxidants act in foods. The radical scavenging assays primarily operate by direct measurement of hydrogen donation or electron transfer from the potential antioxidant to a free radical in simple "lipid free" systems. The ability to scavenge specific radicals may be targeted as, for example, hydroxyl radical, superoxide radical or nitric oxide radical. Peptides generated from the digestion of various proteins are reported to have antioxidative activities. Studies with peptides containing histidine have demonstrated that these peptides can act as metal-ion chelators, active-oxygen quencher, and hydroxyl radical scavenger. The ability of protein hydrolysates to inhibit deleterious changes caused by lipid oxidation appears to be related to the nature and composition of the different peptide fractions produced, depending on the protease specificity [83]. Antioxidant peptides contain of 5–16 amino acid residues. Antioxidative peptides from foods are considered safe and healthy compounds with low molecular weight, low cost, high activity and easy absorption. They have some advantages in comparison to enzymatic antioxidants; that is, with simpler structure they have more stability in different situation and no hazardous immunoreactions [84]. The peptides generated from the digestion of milk proteins are reported to have antioxidant activity. These peptides are composed of 5–11 amino acids including hydrophobic amino acids, proline, histidine, tyrosine or tryptophan [85]. Treatment with milk bioactive peptides improved activities of antioxidant enzymes (catalase, superoxide dismutase, reduced glutathione, glutathione-S-transferase, and glutathione peroxidase) in healthy and diabetic rats [86].

A. Casein Derived Antioxidative Peptides

Caseins have been shown to provide antioxidant activity against TBARS in both Fe/ascorbate induced peroxidation of arachidonic derived liposomes and model linoleic acid systems [87]. Caseins have polar domains that contain phosphorylated serine residues, and their characteristic sequences, -SerP-SerP-SerP-Glu-Glu, are effective cation chelators that form complexes with calcium, iron and zinc. Thus, phosphorylated caseins and/or their peptides in the aqueous phase could be a source of natural chelators to control lipid oxidation in food emulsions by binding and partitioning transition metals away from the emulsion droplet [88]. Suetsuna *et al.* [89] found that a casein hydrolysate exerted (Tyr–Phe–Tyr–Pro– Glu–Leu) scavenging activity towards the superoxide anion, hydroxyl radical, and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical. It is suggested that the casein-derived Glu-Leu sequence is important for this radical scavenging action, thus the primary structure of the protein plays a role in determining the antioxidant activity.

Tryptic β-casein digest and tryptic and subtilisin digests of whole casein retained their inhibitory properties. The highest inhibition of linoleic acid oxidation was observed in the fraction containing β-casein f (169–176) with a trace amount of β-casein f (33–48). The effect was lower than that of the unfractionated digest but higher than the fraction containing the undigested casein [90, 91]. Casein hydrolysates and low molecular weight casein hydrolysates had better peroxyl radical scavenging activities than enriched CPP at equal phosphorous content. Antioxidant properties might, therefore not be uniquely attributed to chelating metals by phosphoseryl residues but also to scavenging of free radicals [88]. Casein hydrolysates were reported to have higher concentration of histidine, lysine, proline and tyrosine than caseinophosphopeptides CPP, and all these amino acids have been previously found to act as free radical scavengers [92].

Casein hydrolysates affected both cellular catalase activity and GSH content in Jurkat cells [14]. In addition, they found that casein hydrolysates contained a certain degree of electron donating capacity as determined by the ferric reducing antioxidant power (FRAP) assay (17–32 mmol L^{-1}). The bioactive peptides in commercial Cheddar cheese showed the highest inhibition of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) [93]. The results indicate that the higher the concentration of peptide, the higher the inhibition of DPPH. The antioxidant activity increased in all the casein fractions (β-, κ- and αs-caseins) after their hydrolysis by pepsin, trypsin and chymotrypsin, the effect was particularly remarkable in the κ-casein fraction, which increased its antioxidant activity almost threefold [94]. Further assays in a linoleic acid oxidation system showed that κ -casein hydrolysate inhibited lipid peroxidation. The CPP reduced glutathione (GSH) concentration and increased GSH-reductase activity in Caco-2 cells [95]. Yak casein hydrolysate possesses free-radical-scavenging and anti-inflammatory activities, and thus it can possibly be used in the prevention of oxidative stress and inflammation related disorders [96]. The antioxidant activities, as determined using the 2, 2_-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), of the 24-h and 48-h hydrolysates of bovine casein after fermentation with *Bifidobacterium longum* KACC91563 were higher than that of the 4-h hydrolysate [97]. Three fractions (≥ 10 kDa, ≥ 3 but < 10 kDa, and < 3 kDa) were separated from the 24-h hydrolysate by ultrafiltration. Among these fractions, the < 3 kDa fraction exhibited the highest antioxidant activity (936.7 μ*M*) compared with the other fractions (42.1 and 34.2 μ*M* for > 10 kDa and 3–10 kDa fractions, respectively) [97].

B. Whey Proteins Derived Antioxidative Peptides

The AO activity of the WPC was attributed to the Cys content [98]. And the ability to elevate cellular GSH Whey protein hydrolysates can help decrease oxidative stress by their radical scavenging activity and by their ability to increase the production of antioxidant enzymes *in vivo*. Similarly, caseins and casein-derived peptides have been associated with radical-scavenging properties *in vitro* and

with their ability to increase cellular catalase activity and GSH levels in human lymphocyte (Jurkat) cells [99, 100]. Hydrolysates of WPI have been shown to possess AO activity. Five hour digestion with Alcalase produced a hydrolysate with strong reducing power (Ferric reducing antioxidant power FRAP). When fractionated on the basis of molecular mass, the low molecular weight fraction (0.1–2.8 kDa) was most potent [101]. A Corolase PP digest of β-Lg produced the most potent peptide (f19–29; Trp–Tyr–Ser–Leu–Ala–Met–Ala– Ala–Ser–Asp–Ile). Synthetic β -Lg f19–29 had a higher radical scavenging ability than BHA (2.62 μmol Trolox/μmol peptide vs. 2.43 μmol Trolox/μmol BHA). The Antioxidant (AO) activity was attributed to the presence of tryptophan (Trp), Tyr and Met residues in the peptide. The radical scavenging ability of another β -Lg peptide (f42–46; Tyr–Val– Glu–Glu–Leu) was compared to an equimolar mixture (Tyr + Val + 2 (Glu) + Leu) of amino acids and the peptide was more potent (0.8 μmol Trolox/μmol peptide vs. 0.4 μmol Trolox/μmol amino acid mixture). This suggests that in some instances the peptide bond or structural conformation of the peptide can enhance AO activity [102]. Timón *et al.* [103] identified three peptides (derived from β-casein and αs₁-casein) with radical scavenging activity from Burgos type cheese. Peptides derived from β-casein (f193-209 (YQQPVLGPVRGPFPIIV) and (f191-209 (LLYQQPVLGPVRGPFPIIV)) have already being described as antioxidant peptides; however, peptide fromαs₁-casein (f180-199 (SDIPNPIGSENSEKTTMPLW)) could become a potential new antioxidant peptide.

2.2.4. Anti-diabetic Peptides

The worldwide incidence of type2 diabetes is increasing. It was estimated in 2000 that there were 171 million diabetics, while incidences for 2030 are estimated to reach 366 million people [104]. Diabetes is prevalent in about 3% to 5% of the population in industrial countries. The major forms of diabetes, type 1 and type 2 diabetes, contribute at about 10 percent and 90 percent, respectively [105]. The World Health Organization has estimated that there are 3.2 million deaths per year because of this disease [106]. The number of patients (20– 79 years) diagnosed with diabetes reached 425 million in 2017 and 90% of which were classified as T2DM cases [107]. Type 2 diabetes is a heterogeneous clinical syndrome characterized by elevated blood glucose levels due to defective insulin secretion and/or insulin action [108]. When insulin secretion cannot compensate for insulin resistance, type 2 diabetes develops [109]. Insulin deficiency lead to various metabolic aberrations in animal such as increased blood lipid, total cholesterol, and free fatty acids arrive at the liver in large amount and triglyceride accumulates in the liver which becomes fatty [110]. Whey and casein ingestion stimulate increased insulin secretion. Ingestion of whey protein leads to more rapid secretion of insulin than micellar casein, however, hydrolysis of casein speeds up the absorption of AAs and secretion of insulin relative to the micellar form of casein [111]. The intake of hydrolysed milk proteins generally results in higher *in vivo*

insulinotropic effects compared with unhydrolysed proteins [112]. Also whey-derived bioactive compound is the tripeptide Ile-Pro-Ala, released from β-lactoglobulin hydrolysis, which may act as inhibitor of dipeptidyl peptidase-4, reducing glucose levels and stimulate insulin [113]. In the same trend, other reports mention the possible role of whey bioactive peptides in reducing type 2 diabetes and obesity [114]. The levels of blood glucose of β-CM-7 treatment rats group decreased compared with model group (*P* < 0.01) accompanied with their alleviated symptoms of diabetes [115].

2.2.5. Dipeptidyl Peptidase-IV Inhibitory Peptides

Glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) are gut-derived peptides (so called incretin hormones) that potentiate insulin secretion from the islet β-cells in a glucose-dependent manner [116]. The aminopeptidase, dipeptidyl peptidase-4 (DPP-IV (EC 3.4.14.5)) is the principal enzyme responsible for the rapid degradation of these peptides *in vivo* [113]. DPP-IV inhibitors can improved the insulin blood level, decreased the blood glucose level and limit hypoglycaemia and caused increase in body weight [117]. The degradation of GLP-1 and GIP by DPP-IV results in a loss in the bioactive properties of these hormones. DPP-IV drug inhibitors are utilized to prevent incretin degradation *in vivo*, thereby increasing their half-life [118]. The stimulatory effect of whey protein on GLP-1 may have many beneficial effects not only on the increase of the glucose-induced insulin secretion and reduction in postprandial glycemia. Enhanced GLP-1 levels also increase the synthesis of proinsulin and insulin stores in β-cells; promote differentiation of precursor cells into β-cells; lead to proliferation of β-cell lines resulting in increased β-cell mass; and reduce the rate of β-cell apoptosis [119, 120]. Uchida *et al.* [121] found that a DPP-IV inhibitory tryptic digest of β-lactoglobulin induced a decrease in blood glucose level in mice following an oral glucose tolerance test when administered orally at 300 mg/kg body weight. The DDP-IV inhibitory peptide Leu-Pro-Glu-Arg-Ile-Pro-Pro-Leu from Gouda type cheese induced a significant reduction of blood glucose in rats following a glucose challenge [122]. The DPP-IV inhibitory activity of sodium caseinate, skim milk powder and milk protein concentrate hydrolysates increased over the course of *in vitro* pepsin-pancreatin digestion, whey protein isolate (WPI) hydrolysate showed highest inhibitory activity following peptic digestion [123]. Hydrolysates produced from sodium caseinate using 11 different proteases displayed higher inhibitory activity than most WPI hydrolysates. However, among all enzymatic treatments investigated, peptic digestion of WPI resulted in the greatest DPP-IV inhibitory activity (IC₅₀ of 0.075 mg mL⁻¹). It was found that three amino acids (Met, Leu and Trp) and eight dipeptides (Phe-Leu, Trp-Val, His-Leu, Glu-Lys, Ala-Leu, Val-Ala, Ser-Leu and Gly-Leu) released from whey proteins hydrolysate inhibited DPP-IV. Trp and Trp-Val were multifunctional inhibitors of xanthine oxidase (XO) and DPP-IV [124]. Protein hydrolysates and dipeptides which can

be released by the action of food-grade gastrointestinal enzyme preparations on milk proteins were shown to inhibit DPPIV [100]. An LF-derived hydrolysate (LFH1) and Trp-Val may act as multifunctional agents in the management of type 2 diabetes. Milk proteins, particularly, milk protein-derived peptides and amino acids have also been linked with the regulation of postprandial glycaemia and insulin secretion in normoglycaemic and type 2 diabetic subjects. Silveira *et al.* [125] studied the peptides with DPP-IV inhibitory activity which released from β-lactoglobulin by hydrolysis with trypsin. Some of the identified sequences showed moderate or high inhibitory activity. Jakubowicz and Froy [114] studied the effect of dietary whey protein on obesity and Type 2 diabetes and they found that whey protein, via bioactive peptides and amino acids generated during gastrointestinal digestion, enhances the release of several hormones, such as gastric inhibitory peptide (GIP), glucagon-like peptide 1 (GLP-1) and insulin, that lead to reduced food intake and increased satiety. Insulin secretion is associated with the glucose lowering effect and with the control of food intake. The mechanism by which whey protein leads to the increased insulin secretion is currently not known and should be investigated. One possible mechanism is the production of bioactive peptides that serve as endogenous inhibitors of Dipeptidyl peptidase-4 (DPP-4) in the proximal gut, preventing the degradation of the insulinotropic incretins GLP-1 and GIP. Another mechanism may involve branched-chain amino acids (BCAAs), specifically leucine, which activate the mammalian target of rapamycin (MTOR) signaling pathway and protein synthesis leading to elevated hormone expression and secretion and increased thermogenesis. The treatment of animals with alloxn alone caused a significant increasing ($p < 0.05$) in plasma glucose level by about three-fold when compared to control group [40]. Meanwhile, treatment with oral intake milk protein concentrate hydrolysate MPCH of diabetic rats caused significant ($p < 0.05$) decreasing of plasma glucose level compared to diabetic group, but the glucose level was not reached the values of control group. These results showed that the MPCH had a best significant effect in reduction blood glucose level of diabetic rats while there was no effect on normal healthy rats.

3. Conclusion

Bioactive peptides have been isolated from many milk and dairy products including cheese, kefir and yoghurt. These peptides are inactive within protein molecules and can be released in three ways by enzymatic hydrolysis. Bioactive peptides have been defined as specific protein fragments that have a positive impact on body functions or conditions and may ultimately influence the health benefits. The activity of peptides is based on their inherent amino acid composition and sequence. The size of active sequences may vary from 2 – 20 amino acid residues, and many peptides are known to reveal multifunctional properties. The best-characterized sequences include antihypertensive, antithrombotic,

antimicrobial, antioxidative, immunomodulatory, and opioid peptides. Milk protein hydrolysate is characterized by a high biological value such as improvement the kidney and liver functions of diabetic rats. Milk proteins could be used as a natural source of peptides with antioxidant activities. So, these peptides may be play an important role in human health specially in reducing the harmful of T2 diabetic, free radical and pathogenic bacteria. It also encourages the use of milk proteins and its hydrolysates as antioxidant agents for human consumption and as an ingredient in nutraceutical and pharmaceuticals as well as in different health-oriented food products to enhance its functionalities and shelf life.

References

[1] Swaisgood, H. E., 2003. Chemistry of the caseins. In Fox, P. F. and McSeeney, P. L. H. (Eds.), Advanced Dairy Chemistry, Vol. 1, Proteins, Part A (pp. 139–201). Kluwer Academic/Plenum Press. New York. USA.

[2] Fox, P. F., 2003. Milk proteins: General and historical aspects. Fox, P. F. and McSeeney, P. L. H. (Eds.), Advanced Dairy Chemistry, Vol. 1, Proteins (pp. 1–48). Kluwer Academic/Plenum Press. New York. USA.

[3] Dziuba, M., Dziuba, B., Iwaniak, A., 2009. Milk proteins as precursors of bioactive peptides. Acta Sci. Pol., Technol. Aliment, 8 (1): 71-90.

[4] Korhonen, H., 2009a. Milk-derived bioactive peptides: From science to applications. Journal of Functional Foods, 1: 177 –187.

[5] Korhonen, H., Pihlanto, A., 2003. Food-derived bioactive peptides opportunities for designing future foods. Current Pharmaceutical Design, 9: 1297–1308.

[6] Hartmann, R., Meisel, H., 2007. Food-derived peptides with biological activity: from research to food applications. Curr. Opin. Biotechnol, 18: 163–169.

[7] Hajirostamloo, B., 2010. Bioactive component in milk and dairy product. World academy of science, Engineering and Technology, 72: 162-166.

[8] Plaisanciéa, P., Claustrec, J., Estiennea, M., Henryd, G., Boutroud, R., Paqueta, A., Léonild, J., 2013. A novel bioactive peptide from yoghurts modulates expression of the gel-forming MUC2 mucin as well as population of goblet cells and Paneth cells along the small intestin. Journal of Nutritional Biochemistry, 24: 213–221.

[9] Kitts, D. D., Weiler, K., 2003. Bioactive proteins and peptides from Food sources. Applications of bioprocesses used in isolation and recovery. Current Pharmaceutical Design, 9: 1309–1323.

[10] Atanasova, J., Ivanova, I., 2010. Antibacterial peptides from goat and sheep milk proteins. Biotechnol. & Biotechnol; 1799: 1803.

[11] Vermeirssen, V., Van Camp, J., Verstraete, W., 2004. Bioavailability of angiotensin I-converting enzyme inhibitory peptides. British Journal of Nutrition, 92: 357–366.

[12] Picariello, G., Ferranti, P., Fierroa O., Mamonea, G., Cairaa, S., Di Luccia, A., Monica, S., Addeon, F., 2010. Peptides surviving the simulated gastrointestinal digestion of milk proteins: Biological and toxicological implications. Journal of Chromatography; B 878: 295-308.

[13] Phelan, M., Kerins, D., 2011. The potential role of milk-derived peptides in cardiovascular disease. Food Funct., 2: 153-167.

[14] Phelan, M., Aherne, A., Fitzgerald, R. J., O'Brien, N. M., 2009. Casein- derived bioactive peptides: biological effects, industrial uses, safety aspects and regulatory status. International Dairy Journal, 19: 643-654.

[15] Hernández-Ledesma. B., Contreras, M. M., Recio, I., 2011. Antihypertensive peptides: production, bioavailability and incorporation into foods. Advances in Colloid and Interface Science, 165: 23–35.

[16] Wei, Q. and Zhimin, H. 2006. Enzymatic hydrolysis of protein: mechanism and kinetic model. Front. Chem. China, 3: 308−314.

[17] Vanhoute, M., Froidevaux, R., Pierlot, C., Krier, F., Aubry, J. M., Guillochon, D., 2008. Advancement of foam separation of bioactive peptides using an aeration column with a bubbling-draining method. Separation and Purification Technology, 63: 460–465.

[18] El-Sayed, M. I., 2013. Functional Properties of Goat and Buffalo Milk Proteins. pp 206- 235. Lampert Academic Publishing. Germany.

[19] 19. FitzGerald, R. J., Murray, B. A., Walsh, D. J., 2004. Hypotensive peptides from milk proteins. Journal of Nutrition, 134: 980S–988S.

[20] Gobbetti, M., Minervini, F., Rizzello, C. G. 2004. Angiotensin I-converting enzyme-inhibitory and antimicrobial bioactive peptides. International Journal of Dairy Technology, 57: 172–188.

[21] Korhonen, H., Pihlanto, A., 2006. Bioactive peptides: Production and functionality. International Dairy Journal, 16: 945–960.

[22] Meisel, H., FitzGerald, R. J., 2003. Biofunctional peptides from milk proteins: Mineral binding and cytomodulatory effects. Current Pharmaceutical Design, 9: 1289–1295.

[23] Yamamoto, N., Ejiri, M., Mizuno, S., 2003. Biogenic peptides and their potential use. Current Pharmaceutical Design, 9: 1345–1355.

[24] Gobbetti, M., Minervini, F., Rizzello, C. G., 2007. Bioactive Peptides in Dairy Products. In Y. H. Hui (Edt.), Handbook of food products manufacturing, pp. 489–517. Hoboken, New Jersey: John Wiley and Sons, Inc.

[25] Kilara, A., Panyam, D., 2003. Peptides from milk proteins andtheir properties. Critical Reviews in Food Science and Nutrition, 43: 607–633.

[26] Korhonen, H., 2008. Bioactive Components in Bovine Milk. In Park. Y. W (edt). Bioactive Components in Milk and Dairy Products. pp, 15: 42. Wiley-Blackwell.

[27] Korhonen, H. J., 2009b. Bioactive milk proteins and peptides: from science to functional applications. The Australian Journal of Dairy Technology, 64: 16–25.

[28] Morris, P. E., FitzGerald, R. J., 2008. Whey Proteins and Peptides in Human Health. In Whey Processing, Functionality and Health Benefits. Charles I. Onwulata _Peter J. Huth, Edt. Blackwell Publishing and the Institute of Food Technologists. pp 285-344. 2121 State Avenue, Ames, Iowa 50014-8300, USA.

[29] Holder, A., Thienel, K., Klaiber, I., Pfannstiel, J., Weiss, J., Hinrichs J., 2014. Quantification of bio- and techno-functional peptides in tryptic bovine micellar casein and b-casein hydrolysates. Food Chemistry, 158: 118–124.

[30] Kunji, E. R. S., Mierau, I., Hagting, A., Poolman, B., Konings, N., 1996. The proteolytic system of lactic acid bacteria. Antonie Leeuwenhoek 70: 187–221.

[31] Christensen, J. E., Dudley, E. G., Pederson, J. A., Steele, J. L., 1999. Peptidases and amino acid catabolism in lactic acid bacteria. Antonie van Leeuwenhoek; 76: 217–246.

[32] Nakamura, Y., Yamamoto, M., Sakai, K., Okubo, A., Yamazaki, S., Takano, T., 1995. Purification and characterization of angiotensin I converting enzyme inhibitors from sour milk. Journal of Dairy Science, 78, 777–783.

[33] Sipola, M., Finkenberg, P., Korpela, R., Vapaatalo, H., Nurminen, M. L., 2002. Effect of long-term intake of milk products on blood pressure in hypertensive rats. Journal of Dairy Research, 69, 103–111.

[34] Pessi, T., Isolauri, E., Sutas, Y., Kankaanranta, H., Moilanen, E., Hurme, M., 2001. Suppression of T-cell activation by Lactobacillus rhamnosus GG-degraded bovine casein. International Immunopharmacology, 1: 211–218.

[35] Prioult, G., Pecquet, S., Fliss, I., 2004. Stimulation of interleukin-10 production by acidic β-lactoglobulin- derived peptides hydrolyzed with Lactobacillus paracasei NCC2461 peptidases. Clinical and Diagnostic Laboratory Immunology, 11, 266–271.

[36] Donkor, O., Henriksson, A., Vasiljevic, T., Shah, N. P., 2007. Proteolytic activity of dairy lactic acid bacteria and probiotics as determinant of growth and in vitro angiotensin-converting enzyme inhibitory activity in fermented milk. Lait, 86: 21–38.

[37] Silva, S. V., Malcata, F. X., 2005. Milk caseins as a source of bioactive peptides. International Dairy Journal, 15 (1): 1-15.

[38] Iwaniak, A., Minkiewicz, P., 2007. Proteins as the source of physiologically and functionally active peptides. Acta Sci. Pol., Technol. Aliment. 6 (3), 5-15.

[39] Tidona, F., Criscione, A., Guastella, A. M., Zuccaro, A., Bordonaro, S. and Marletta, D. 2009. Bioactive peptides in dairy products. Ital. J. Anim. Sci., 8: 315-340.

[40] El-Sayed, M. I, Awad S, Wahba A, El Attar A, Yousef MI and Zedan M. 2016. In Vivo Anti-diabetic and Biological Activities of Milk Protein and Milk Protein Hydrolyaste. (2016). Advances in Dairy Research. Vol 4: 1-6.

[41] Van Hooijdonk, A. C. M., Kussendrager, K. D., Steijns, J. M., 2000. In vivo antimicrobial and antiviral activity of components in bovine milk and colostrums involved in nonspecific defence. British Journal of Nutrition, 84: 127–134.

[42] López-Expósito, I., Recio, I., 2008. Protective effect of milk peptides: Antibacterial and antitumor properties. In Bösze. Z (edt). Bioactive Components of Milk. Springer ScienceBusiness Media, New York, NY 10013, USA.

[43] Jones, F. S., Simms, H. S., 1930. The bacterial growth inhibitor (lactenin) of milk. Journal of Experimental Medicine, 51: 327–339.

[44] Hill, R. D., Lahov, E., Givol, D., 1974. A rennin-sensitive bond in alpha and beta casein. Journal of Dairy Research, 41: 147–153.

[45] Lahov, E., Edelsten, D., Sode-Mogensen, M. T., Sofer, E., 1971. Properties of basic glycopeptides released from cow milk protein by heat. Milchwissenschaft, 26, 489–495.

[46] Floris, R., Recio, I., Berkhout, B., Visser, S., 2003. Antibacterial and antiviral effects of milk proteins and derivatives thereof. Current Pharmaceutical Design, 9, 1257–1275.

[47] Clare, D. A., Catignani, G. L., Swaisgood, H. E., 2003. Biodefense properties of milk: The role of antimicrobial proteins and peptides. Current Pharmaceutical Design, 9: 1239–1255.

[48] Dashper. S. G., Liu. S. W., Reynolds. E. C., 2007. Antimicrobial Peptides and Their potential as oral therapeutic Agents. International Journal of Peptide Research and Therapeutics, 13 (4): 505–516.

[49] Haque, E., Chand, R., 2008. Antihypertensive and antimicrobial bioactive peptides from milk proteins. Eur Food Res Technol 227: 7–15.

[50] Brogden, K. A., 2005. Antimicrobial peptides: pore formers or metabolic inhibitors in bacteria?. Nat. Rev. Microbiol, 3: 238–250.

[51] Benkerroum, N., 2010. Antimicrobial peptides generated from milk proteins: a survey and prospects for application in the food industry. International Journal of Dairy Technology, 63: 320-338.

[52] Najafian, L., Babji, A. S., 2012. A review of fish-derived antioxidant and antimicrobial peptides: Their production, assessment, and applications. Peptides, 33: 178–185.

[53] Seo, M., Won, D., Kim, H. S., Mishig-Ochir, J. H., Lee,. B. J., 2012. Antimicrobial Peptides for Therapeutic Applications: A Review. Molecules, 17: 12276-12286.

[54] Teixeira, V., Feio, M. J., Bastos, M., 2012. Role of lipids in the interaction of antimicrobial peptides with membranes. Prog. Lipid Res., 51, 149–177.

[55] El-Zahar, K., Sitohy, M., Choiset, Y., Métro, F., Haertle´, T., Chobert, J. M., 2004. Antimicrobial activity of ovine whey protein and their peptic hydrolysates. Milchwissenschaft; 59: 653–656.

[56] Almaas, H., Holm, H., Langrud, T., Flengsrud, R., Vegarud, G. E., 2006. In vitro studies of the digestion of caprine whey proteins by human gastric and duodenal juice and the effects on selected microorganisms. British Journal of Nutrition; 96: 562–569.

[57] Vogel, H. J., Schibli, D. J., Weiguo, J., Lohmeier-Vogel, E. M., Epand, R. F., Epand, R. M., 2002. Towards a structure-function analysis of bovine lactoferricin and related tryptophan and arginine containing peptides. Biochem. Cell Biol., 80, 49-63.

[58] Farnaud, S., Evans, R. W., 2003. Lactoferrin- a multifunctional protein with antimicrobial properties. Mol Immunol 40: 395-405.

[59] Ulvatne, H., Samuelsen, O., Haukland, H. H., Kramer. M., Vorland, L. H., 2004. *Lactoferricin B inhibits bacterial macromolecular synthesis in Escherichia coli and Bacillus subtilis. FEMS Microbiol. Lett. 237: 377-384.*

[60] Van der Kraan, M. I. A., Groenink, J., Nazmi, K., Veerman, E. C. I., Bolscher, J. G. M., Nieuw Amerongen, A. V. 2004. Lactoferrampin: A novel antimicrobial peptide in the N1-domain of bovine lactoferrin. Peptides, 25, 177–183.

[61] Pellegrini, A., Thomas, U, Bramaz, N., Hunziker, P., Von Fellenberg, R., 1999. Isolation and identification of three bactericidal domains in the bovine α–lactalbumin molecule. Biochim. Biophys. Acta; 1426: 439–448.

[62] Pellegrini, A., Dettling, C., Thomas, U., Hunziker, P., 2001. Isolation and characterization of four bactericidal domains in the bovine β-lactoglobulin. Biochim. Biophys. Acta, 1526: 131–140.

[63] Théolier, J., Hammami, R., Labelle, P., Fliss, I., Jean, J., 2013. Isolation and identification of antimicrobial peptides derived by peptic cleavage of whey protein isolate. Journal of Functional Foods, 5: 706–714.

[64] Lahov, E. and Regelson., W., 1996. Antibacterial and immunostimulating casein-derived substances from milk: casecidin, isracidin peptides. Food Chem. Toxicol, 34: 131–145.

[65] Kayser, H., Meisel, H., 1996. Stimulation of human peripheral blood lymphocytes by bioactive peptides derived from bovine milk proteins. FEBS Letters 383: 18-20.

[66] Kolb, A. F., 2001. The prospects of modifying the antimicrobial properties of milk. Biotechnology advances, 19: 299–316.

[67] Hayes, M., Ross, R. P., Fitzgerald, G. F., Hill, C., Stanton C., 2005. Casein-derived antimicrobial peptides generated by lactobacillus acidophilus DPC6026. Appl. Environ. Microbiol. 72: 2260-2264.

[68] Recio, I., Visser, S., 1999. Identification of two distinct antibacterial domains within the sequence of bovine αs2-casein. Biochim. Biophys. Acta 1428: 314-326.

[69] McCann, K. B., Shiell, B. J., Michalski, W. P., Lee, A., Wan, J., Roginski, H., 2005. Isolation and characterisation of antibacterial peptides derived from the f (164–207) region of bovine αS2-casein. International Dairy Journal, 15: 133–143.

[70] López-Expósito, I., Gómez-Ruiz, J. A., Amigo, L., Recio, I., 2006. Identification of antibacterial peptides from ovine as2-casein. Int. Dairy J. 16: 1072- 1080.

[71] López-Expósito, I., 2007. Novel peptides with antibacterial activity derived from food proteins. Study of the mode of action and synergistic effect. Dissertation Tesis. Faculty of Science. Universidad Auto´ noma de Madrid.

[72] Alvarez-Ordóñez, A., Begley, M., Clifford, T., Deasy, T., Considine, K., Hill. C. 2013. Structure-Activity Relationship of Variants of the Milk-Derived Antimicrobial Peptide α$_{s2}$-Casein f (183–207). *Appl. Environ. Microbiol;* 79 (17): 5179-5185.

[73] Liepke, C., Zucht, H. D., Forsmann, W. G. and Standker, L., 2001. *Purification* of novel *peptide antibiotics* from *human milk. J. Chromatogr., 752: 369-377.*

[74] Malkoski, M., Dashper, S. G. O'Brien-Simpson, N. M., Talbo, G. H., Macris, M., Cross, K. J., Reynolds, E. C., 2001. Kappacin, a novel antibacterial peptide from bovine milk. Antimicrob. Agents. Chem., 45: 2309–2315.

[75] López-Expósito, I., Recio, I., 2006. Antibacterial activity of peptides and folding variants from milk proteins. International Dairy Journal, 16: 1294–1305.

[76] Thoma- Worringer, C., Sorensen, J., Lopez-Fandino, R., 2006. Health effects and technological features of caseinomacropeptide, Int Dairy J, 16: 1324-1333

[77] Seppo, L., Jauhiainen, T., Poussa, T. and Korpela, R. 2003. A fermented milk high in bioactive peptides has a blood pressure-lowering effect in hypertensive subjects. Am J Clin Nutr., 77: 326.

[78] Yamamoto N, Takano T., 1999. Antihypertensive peptides derived from milk proteins. Nahrung43: 159–64.

[79] Saito, T. 2008. Antihypertensive Peptides Derived from Bovine Casein and Whey Proteins. Z. Bösze (ed.), Bioactive Components of Milk. Springer. New York.

[80] Abubakar, A., Saito, T., Kitazawa, H., Kawai, Y., Itoh, T. 1998. Structural analysis of new antihypertensive peptides derived from cheese whey protein by proteinase K digestion. Journal of Dairy Science, 81: 3131–3138.

[81] Halliwell, B., 2001. Role of free radicals in the neurodegenerativediseases: Therapeutic implications for antioxidant treatment. DrugsAging, 18, 479–484.

[82] Liu, Q., Raina, A., Smith, M., Sayre, L., Perry, G., 2003. Hydroxynonenal, toxic carbonyls, and Alzheimer disease. Molecular Aspects of Medicine, 24: 305–313.

[83] Pihlanto, A., 2006. Antioxidative peptides derived from milk proteins. International Dairy Journal, 16: 1306–1314.

[84] Sarmadi, B. H., Ismail, A., 2010. Antioxidative peptides from food proteins: A review. Peptides, 31: 1949–1956.

[85] 85. Kamau, S. M., Lu, R. R., Chen, W., Liu, X. M., Tian, F. W., Shen, Y., Gao. T., 2010. Functional significance of bioactive peptides derived from milk proteins. Food Reviews International, 26: 386–401.

[86] Awad S., El-Sayed MI., Wahba A., El Attar A., Yousef MI and Zedan M. 2016. Antioxidant activity of milk protein hydrolyaste in alloxan-induced diabetic rats. (2016). Journal of Dairy Science, 99: 8499–8510.

[87] Wong, P. Y. Y. and Kitts, D. D., 2003. Chemistry of buttermilk solid antioxidant activity. Journal of Dairy Science, 86, 1541–1547.

[88] Diaz, M., Dunn, C. M., McClements, D. J., Decker, E. A., 2003. Use of caseinophosphopeptides as natural antioxidants in oil-inwater emulsions. Journal of Agricultural and Food Chemistry, 51: 2365–2370.

[89] Suetsuna, K., Ukeda, H., Ochi, H., 2000. Isolation and characterization of free radical scavenging activities peptides derived from casein. Journal of Nutritional Biochemistry, 11: 128–131.

[90] Rival, S. G., Boeriu, C. G., Wichers, H. J., 2001a. Caseins and casein hydrolysates. 2. Antioxidative properties and relevance to lipoxygenase inhibition. Journal of Agricultural and Food Chemistry, 49: 295–302.

[91] Rival, S. G., Fornaroli, S., Boeriu, C. G., Wichers, H. J., 2001b. Caseins and casein hydrolysates. 1. Lipoxygenase inhibitory properties. Journal of Agricultural and Food Chemistry, 49, 287–294.

[92] Diaz, M., Decker, E. A., 2004. Antioxidant mechanisms of caseinophosphopeptides and casein hydrolysates and their application in ground beef. Journal of Agricultural and Food Chemistry, 52: 8208–8213.

[93] Pritchard, S. R., Phillips, M., Kailasapathy, K., 2010. Identification of bioactive peptides in commercial Cheddar cheese. Food Research International, 43: 1545–1548.

[94] Gómez-Ruiz, J. A., López-Expósito, I., Pihlanto, A., Ramos, M., Recio, I., 2008. Antioxidant activity of ovine casein hydrolysates: identification of active peptides by HPLC–MS/MS. Eur. Food. Res. Technol, 227: 1061–1067.

[95] Laparra, J. M., Alegrı´a, A., Barbera´, R., Farre´, R., 2008. Antioxidant effect of casein phosphopeptides compared with fruit beverages supplemented with skimmed milk against H2O2-induced oxidative stress in Caco-2 cells. Food Research International, 41: 773–779.

[96] Mao, X., Cheng, X., Wang, X., Wu, S., 2011. Free-radical-scavenging and anti-inflammatory effect of yak milk casein before and after enzymatic hydrolysis. Food Chemistry, 126: 484–490.

[97] Chang, O. K., Seol, K. H., Jeong, S. G., Oh, M. H., Park, B. Y., Perrin, C., Ham, J. S., 2013. Casein hydrolysis by Bifidobacterium longum KACC91563 and antioxidant activities of peptides derived therefrom. J. Dairy Sci; 96: 1–12.

[98] Gad, A., Khadrawy, Y., El-Nekeety, A. A., Mohamed, S. R., Hassan, N. S., Abdel-Wahhab, M. A., 2011. Antioxidant activity and hepatoprotective effects of whey protein and spirulina in rats. Nutrition; 27 (5): 582–589.

[99] Phelan, M., Aherne-Bruce, S. A., O'Sullivan, D., FitzGerald, R. J., O'Brien, N. M., 2009b. Potential bioactive effects of casein hydrolysates on human cultured cells. International Dairy Journal; 19, 279–285.

[100] Nongonierma, A. B., FitzGerald. R. J., 2013. Dipeptidyl peptidase IV inhibitory and antioxidative properties of milk protein-derived dipeptides and hydrolysates. Peptides; 39: 157–163.

[101] Peng, X., Xiong, Y. and Kong, B., 2009. Antioxidant activity of peptide fractions from whey protein hydrolysates as measured by electron spin resonance. Food Chem., 113 (1): 196 –201.

[102] Hernández-Ledesma, B., Dávalos, A., Bartolomé, B., Amigo, L., 2005. Preparation of antioxidant enzymatic hydrolysates from α-lactalbumin and β-lactoglobulin. Identification of active peptides by HPLC-MS/ MS. Journal of Agricultural and Food Chemistry, 53: 588–593.

[103] Timón, M. L., Parra, V., Otte, J., Broncano, J. M., Petrón, M. J. 2014. Identification of radical scavenging peptides (< 3 kDa) from Burgos-type cheese. LWT - Food Science and Technology, 57: 359-365.

[104] WHO, 2006. Definition and diagnosis of diabetes mellitus and intermediate hyperglycemia: report of a WHO/IDF consultation.

[105] 105. Schrezenmeir, J. and Jagla, A. 2000. Milk and diabetes. Journal of the American College of Nutrition, Vol. 19, No. 2: 176S–190S.

[106] WHO., 2011. Global status report on noncommunicable diseases 2010. World Health Organization, WHO Press.

[107] IDF. (2017). IDF diabetes atlas, 8th ed. Brussels: International Diabetes Federation.

[108] 108. Bailey, C. J., Flatt, P. R., 1995. Development of antidiabetic drugs. In: Ioannides, C., Flatt, P. R. (Eds.), Drugs, Diet and Disease: Mechanistic Approaches toDiabetes. Ellis Horwood, London, pp. 279–326.

[109] Tamrakara, A. K., Jaiswala, N., Yadavb, P. P., Mauryab, R. and Srivastavaa, A. K. 2011. Pongamol from Pongamiapinnata stimulates glucose uptake by increasing surface GLUT4 level in skeletal muscle. Molecular and Cellular Endocrinology, 339: 98–104.

[110] Baynes, J. W., 1991. Role of oxidative stress in development of complications in diabetes. Diabetes, 40: 405.

[111] Nilsson, M., Holst, JJ., Bjorck IM., 2007. Metabolic effects of amino acid mixtures and whey protein in healthy subjects: studies using glucose-equivalent drinks. Am. J. Clin. Nutr., 85: 996–1004.

[112] Power, O., Hallihan, A., Jakeman, P., 2009. Human insulinotropic response to oral ingestion of native and hydrolysed whey protein. Amino Acids, 37, 333e339.

[113] Tulipano, G., Sibilia, V., Caroli, A. M., Cocchi, D., 2011. Whey proteins as source of dipeptidyl dipeptidase IV (dipeptidyl peptidase- 4) inhibitors. Peptides, 32 (4), 835–838.

[114] Jakubowicz, D., Froy, O., 2013. Biochemical and metabolic mechanisms by which dietary whey protein may combat obesity and Type 2 diabetes. Journal of Nutritional Biochemistry, 24: 1–5.

[115] Han, D. N., Dong-Hui Zhang, D. H., Wang, L. P., Zhang. Y. S., 2013. Protective effect of _-casomorphin-7 on cardiomyopathy of streptozotocin-induced diabetic rats via inhibition of hyperglycemia and oxidative stress. Peptides, 44: 120–126.

[116] Drucker, DJ., 2006. Enhancing the action of incretin hormones: a new whey forward. Endocrinology, 147: 3171–2.

[117] Yan, J., Zhao, J., Yang, R & Zhao, W., 2019. Bioactive peptides with antidiabetic properties: a review. International Journal of Food Science and Technology, 1: 11. doi: 10.1111/ijfs.14090

[118] Bjelke, J. R., Christensen, J., Nielsen, P. F., Branner, S., Kanstrup, A. B., Wagtmann, N., 2006. Dipeptidyl peptidases 8 and 9: Specificity and molecular characterization compared with dipeptidyl peptidase IV. Biochemistry Journal; 396: 391–399.

[119] Nauck, M. A., Vilsboll, T., Gallwitz, B., Garber, A. and Madsbad, S., 2009. Incretin-based therapies: viewpoints on the way to consensus. Diabetes Care; 32 Suppl. 2: S223-231.

[120] Portha, B., Tourrel-Cuzin, C., Movassat, J., 2011. Activation of the GLP-1 receptor signaling pathway: a relevant strategy to repair a deficient beta-cell mass. Exp. Diabetes Res., 376509.

[121] Uchida, M., Ohshiba, Y., Mogami, O., 2011. Novel dipeptidyl peptidase- 4-inhibiting peptide derived from β-lactoglobulin. Journal Pharmacological Science, 117: 37–63.

[122] Uenishi, H., Kabuki, T., Seto, Y., Serizawa, A., Nakajima, H., 2012. Isolation and identification of casein-derived dipeptidyl-peptidase 4 (DPP-4)-inhibitory peptide LPQNIPPL from gouda-type cheese and its effect on plasma glucose in rats. International Dairy Journal, 22: 24–30.

[123] Lacroix, I. M. E. and Li-Chan, E. C. Y., 2012. Dipeptidyl peptidase-IV inhibitory activity of dairy protein hydrolysates. International Dairy Journal, 25 (2): 97–102.

[124] Nongonierma, A. B., Mooney, C., Shields, D. C., FitzGerald. R. J., 2013. Inhibition of dipeptidyl peptidase IV and xanthine oxidase by amino acids and dipeptides. Food Chemistry, 141: 644–653.

[125] Silveira, S. T., Martínez-Maqueda, D., Recio, I., Hernandez-Ledesma, B., 2013. Dipeptidyl peptidase-IV inhibitory peptides generated by tryptic hydrolysis of a whey protein concentrate rich in b-lactoglobulin. Food Chemistry, 141: 1072–1077.