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ARTICLE

Bioactive Peptides in Milk and Dairy Products: A Review

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Abstract

Functionally and physiologically active peptides are produced from several food proteins during gastrointestinal digestion and fermentation of food materials with lactic acid bacteria. Once bioactive peptides (BPs) are liberated, they exhibit a wide variety of physiological functions in the human body such as gastrointestinal, cardiovascular, immune, endocrine, and nervous systems. These functionalities of the peptides in human health and physiology include antihypertensive, antimicrobial, antioxidative, antithrombotic, opioid, anti-appetizing, immunomodulatory and mineral-binding activities.

Most of the bioactivities of milk proteins are latent, being absent or incomplete in the original native protein, but full activities are manifested upon proteolytic digestion to release and activate encrypted bioactive peptides from the original protein. Bioactive peptides have been identified within the amino acid sequences of native milk proteins. Due to their physiological and physico-chemical versatility, milk peptides are regarded as greatly important components for health promoting foods or pharmaceutical applications.

Milk and colostrum of bovine and other dairy species are considered as the most important source of natural bioactive components. Over the past a few decades, major advances and developments have been achieved on the science, technology and commercial applications of bioactive components which are present naturally in the milk. Although the majority of published works are associated with the search of bioactive peptides in bovine milk samples, some of them are involved in the investigation of ovine or caprine milk. The advent of functional foods has been facilitated by increasing scientific knowledge about the metabolic and genomic effects of diet and specific dietary components on human health.

Keywards: bioactive peptide, milk, proteins, functional foods, human health

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Introduction

Milk of all mammalian species contains a heterogeneous mixture of lacteal secretion which contains numerous components which exhibit a wide variety of chemical and functional activities. These diversified composition and functionality of milk can be found in even protein components alone. Proteins constitute a myriad of serum and glandular-derived compounds which are different in molecular size, concentration and functionality (Regester *et al*., 1997). The traditional and contemporary view of the role of milk has been markedly expanded beyond the horizon of nutritional subsistence of infants (Gobbetti *et al*., 2007; Park, 2009a). Milk has been more than a source of nutrients to any neonate of mammalian species, as well as for growth of children and nourishment of adult humans (Park, 2009b). It contains a wide range of proteins that provide protection against enteropathogens or are essential for the manufacture and characteristic nature of certain dairy products (Korhonen and Pihlanto-Leppälä, 2004).

Recent studies indicate that milk furnishes a broad range of biologically active compounds that guard neonates and adults against pathogens and illnesses, such as immunoglobulins, antibacterial peptides, antimicrobial proteins, oligosaccharides, lipids, besides many other components at low concentrations, so-called “minor” components, but with considerable potential benefits, illustrated in Fig. 1 (Park, 2009b). Thus, beyond nutritional values

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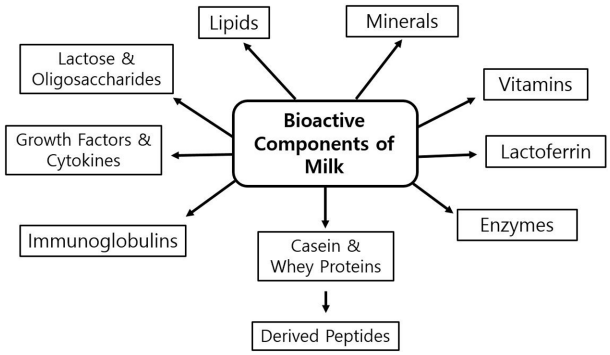

Fig. 1. Major bioactive functional compounds derived from milk (Park, 2009b).

of milk, milk-born biologically active compounds such as caseins, whey proteins and other minor constituents exhibit important physiological and biochemical functions that have crucial impacts on human metabolism and health (Gobbetti *et al*., 2007; Korhonen and Pihlanto-Leppala, 2004; Park, 2009a; Schanbacher *et al*., 1998). Bioactivity of milk components have been categorized as four major areas: (1) gastrointestinal development, activity, and function; (2) immunological development and function; (3) infant development; and (4) microbial activity, including antibiotic and probiotic action (Gobbetti *et al*., 2007).

Recently, fractionation and marketing of bioactive milk ingredients have emerged as a new lucrative sector for dairy industries and specialized bio-industries. Many of these components are being exploited for both dairy and non-dairy food formulations and even pharmaceuticals (Korhonen and Pihlanto, 2007a; Krissansen, 2007; Playne *et al.,* 2003; Shah, 2000). The dairy industry has played a leading role in the development of functional foods and also has already commercialized these products. Those products have enhanced bioactive functions in human health including the immune system, reduce elevated blood pressure, combat gastrointestinal infections, help control body weight and prevent osteoporosis (FitzGerald *et al*., 2004; Hartmann and Meisel, 2007; Korhonen and Marnila, 2006).

The objectives of this paper are to: (1) review the current knowledge on bioactive peptides derived from milk of major species concerning various forms of naturally occurring bioactive compounds, their physiological, biochemical and nutritional functionalities in human health, and (2) elucidate some recent studies on potential applications and development of functional foods using bioactive peptides and components in bovine milk as well as in other species milk.

Functionalities of Bioactive Peptides in Milk and Dairy Products

Numerous studies have been reported on functional properties of bioactive components in milk and dairy products especially in human and bovine milk, although more people drink the milk of goats than that of any other single species worldwide (Haenlein and Caccese, 1984; Park, 1990, 2006). On the other hand, the study of these bioactive components in milks has been difficult, because of the low concentrations of certain very potent agents in milks, their biochemical complexities, the need to develop specific methods to quantify certain factors due to their particular forms in milks, the compartmentalization of some of the agents, and the dynamic effects of lactation length and other maternal factors on concentrations or functions of the components of the systems (Goldman and Goldblum, 1995; Park, 2009a).

Among those bioactive constituents in milk and dairy products, bioactive peptides (BPs) are the most studied components in this regard. BPs have been defined as specific protein fragments that have a positive influence on physiological and metabolic functions or condition of the body and may have ultimate beneficial effects on human health (Kitts and Weiler, 2003). BPs can be delivered to the consumers in conventional foods, dietary supplements, functional foods, or medical foods. These bioactive peptides possess very important biological activities and functionalities, including antimicrobial, antihypertensive, antioxidative, anticytotoxic, immunomodulatory, opioid, and mineral-carrying activities.

The bioactive peptides derived from a variety of dietary proteins have been reviewed by many researchers (Clare, 2003; FitzGerald and Meisel, 2003; Li *et al*., 2004). BPs are inactive within the sequence of the parent protein, and they can be released in three ways: (i) through hydrolysis by digestive enzymes, (ii) through hydrolysis of proteins by proteolytic microorganisms, and (iii) through the action of proteolytic enzymes derived from microorganisms or plants (Korhonen and Pihlanto, 2007b). A schematic representation of formation of bioactive peptides from major milk proteins is presented in Fig. 2.

Many scientists have conducted a variety of research and demonstrated many biologically active compounds especially in BPs in different dairy species such as bovine, ovine and caprine milk (Table 1 and 2). The proven functionalities of milk bioactive peptides are summarized as follows:

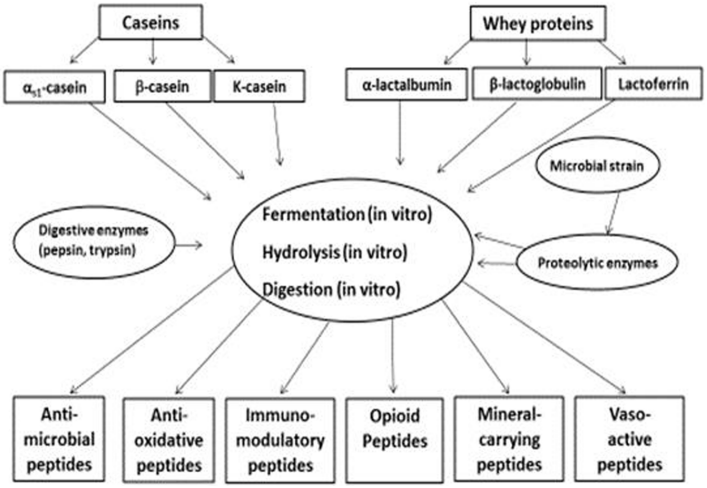

Fig. 2. Formation of bioactive peptides from major milk proteins (Korhonen and Pihlanto, 2007b).

Antihypertensive peptides

The angiotensin is one of two polypeptide hormones and a powerful vasoconstrictor that functions in the body by controlling arterial blood pressure through the contraction of smooth muscles of the blood vessel (Park, 2009a). Angiotensin converting enzyme (ACE)-inhibitory peptide blocks the conversion of angiotensin I to angiotensin II. The ACE causes elevation of blood pressure by converting angiotensin-I to the potent vasoconstrictor, angiotensin-II, and by degrading bradykinin, a vasodilatory peptide, and enkephalins (Petrillo and Ondetti, 1982).

As shown in Table 1, several ACE-inhibitory peptides were identified by in vitro enzymatic digestion of milk proteins or chemical synthesis of peptide analogs (Gobbetti *et al*., 2004). The ACE-inhibitors derived from milk proteins are attributed to different fragments of casein, named casokinins (Meisel and Schlimme, 1994), or whey proteins, named lactokinins (FitzGerald and Meisel, 2000).

Antioxidative peptides

Peptides derived from αs-casein have free radical-scavenging activity and inhibit enzymatic and nonenzymatic lipid peroxidation (Rival *et al*., 2001; Suetsuna *et al*., 2000). Proteolytic enzymes can release antioxidative peptides from caseins, soybean and gelatine by enzymatic hydrolysis (Korhonen and Pihlanto, 2003).

Low temperature-processed whey protein contained high levels of specific dipeptides (glutamylcysteine) (Bounous and Gold, 1991). These dipeptides can promote the synthesis of glutathione, which is an important antioxidant for cellular protection and repair processes.

Apart from milk peptides, Byun *et al*. (2009) analyzed fish protein hydrolysates from the rotifer *Brachionus rotundiformis,* using different proteases (Alcalase, α-chymotrypsin, Neutrase, papain, pepsin and trypsin), and found antioxidant peptides from the hydrolysates. Antioxidant activities of hydrolysates were evaluated using DPPH radical scavenging activity.

Antithrombotic peptides

These peptides reduce or inhibit the formation of blood clots. Caseinomacropeptide (CMP) is a peptide split from k-casein when the milk protein is coagulated by rennin enzyme. This CMP has functions of inhibiting the aggregation of blood platelets and binding of the human fibrinogen γ-chain to platelet surface fibrinogen receptors (Fiat *et al*., 1993). There are two reported antithrombotic peptides, derived from human and bovine k-caseinoglycopeptides, which were identified in the plasma of 5-d-old newborns after breast-feeding and feeding cow milk based formula (Chabance *et al*., 1998).

Casoplatelin, peptide derived from κ-CN (Table 1), exhibited influence on platelet function and inhibited both the aggregation of ADP-activated platelets and the binding of human fibrinogen γ-chain to its receptor region on the platelets surface (Jolles *et al*., 1986). Sheep caseins derived κ-caseinoglycopeptide (106-171) reduced thrombin- and collagen-induced platelet aggregation in a dose dependent manner (Qian *et al*., 1995).

Hypocholesterolemic peptides

Iwami *et al*. (1986) reported that a peptide having high bile acid-binding capacity can inhibit the reabsorption of bile acid in the ileum, whereby it can decrease the blood

Table 1. Major biologically active milk components and their functions[1]

Milk precursors or components	Bioactive compounds	Bioactivities observed
α-, β-caseins	Casomorphins	Opioid agonist (Decrease gut mobility, gastric emptying rate; increase amino acids and electrolytes uptake)
α-, β-caseins	Casokinins	ACE inhibitory (Increase blood flow to intestinal epithelium)
α-, β-caseins	Phosphopeptides	Mineral binding (Ca binding; increase mineral absorption, i.e., Ca, P, Zn)
α-, β-caseins	Immunopeptides Casomorphins Casokinins	Immunomodulatory (Increase immune response and phagocytic activity)
α_{s1}-casein	Isracidin	Antimicrobial
α_{s2}-casein	Casocidin	Antimicrobial
κ-casein	Casoxins	Opioid antagonist
κ-casein	Casoplatelins	Antithrombotic
κ-casein	ê-caseinglyco-	Probiotic (Growth of Bifidobacteria in GI tract) macropeptide
α-lactalbumin (α-La) β-lactoglobulin (β-La)	Lactorphins	Opioid agonist
Serum albumin	Serorphin	Opioid agonist
α-La, β-La and Serum albumin	Lactokinins	ACE inhibitory
Immunoglobulins	IgG, IgA	Immunomodulatory (Passive immunity)
Lactoferrin	Lactoferrin	Immunomodulatory (Increase natural killer cell activity, humoral immune response, thymocyte trafficking immunological development, and interleukins-6; decrease tumor necrosis factor-α) Antimicrobial (Increase bacteriostatic inhibition of Fe-dependent bacteria; decrease viral attachment to and infections of cells) Probiotic activity (Increase growth of Bifidobacteria in GI tract)
Lactoferrin	Lactoferroxins	Opioid antagonist
Oligosaccharides	Oligosaccharides	Probiotic (Increase growth of Bifidobacteria in GI tract)
Glycolipids Oligosaccharides	Glycolipids Oligosaccharides	Antimicrobial (Decrease bacterial & viral attachment to intestinal epithelial cells)
Prolactin	Prolactin	Immunomodulatory (Increase lymphocyte and thymocyte trafficking, and immune development)
Cytokines	Interleukins-1,2,6, & 10 Tumor necrosis factor-α Interferon-γ Transforming growth Factors-α, β;leukotriene B_4 Prostaglandin E_2, Fn	Immunomodulatory (Lympocyte trafficking, immune development)
Growth factors	IGF-1, TGF-α, EGF, TGF-β	Organ development and functions
Parathromone-P	PTHrP	Increase Ca^{+2} metabolism and uptake

[1]Adapted from Schanbacher *et al*. (1998), Meisel (1998), and Clare and Swaisgood (2000), Park (2009b)

cholesterol level. A novel peptide (Ile-Ile-Ala-Glu-Lys) from tryptichydrolysate of β-lactoglobulin showed hypocholesterolemic effect (Nagoaka *et al*., 2001).

The serum cholesterol-lowering activity is directly influenced by the degree of fecal steroid excretion (Nagata *et al*., 1982). Cholesterol is become soluble in bile salt-mixed micelles and then absorbed (Wilson and Rudel, 1994). Cholesterol absorption was suppressed by this peptide in Caco-2 cells in vitro, and also the peptide elicited hypocholesterolemic activity in vivo in rats after oral administration of the peptide solution. The mechanism of the hypocholesterolemic effect by these peptides has not been delineated (Korhonen and Pihlanto, 2007b), while four bioactive peptides were identified in the hydrolysate which corresponded to β-lactoglobulin f9-14, f41-60, f71-75, and f142-146.

Opioid peptides

An opioid is any chemical such as morphine that resembles opiates in its pharmacological effects. Opioids are defined as peptides (i.e., enkephalins) which have an affinity for an opiate receptor and opiate-like effects, inhibited by naloxone (Gobbetti *et al*., 2007). As shown in Table 1, many opioid peptides have been identified. Opioid pep-

Table 2. Sequence of bioactive peptides derived from ovine and caprine milk proteins

Peptide fragment	Sequence	Biological activity	Reference
Ovine α_{s1}-CN f(86-92)	VPSERYL	ACE-inhibitory	Gómez-Ruiz *et al*. (2002)
Ovine α_{s1}-CN f(102-109)	KKYNVPQL	ACE-inhibitory	Ómez-Ruiz *et al*. (2002)
Caprineα_{s1}-CN f(143-146)	AYFY	ACE-inhibitory	Lee *et al*. (2005)
Ovine α_{s2}-CN f(165-170)	LKKISQ	Antibacterial	López-Expósito *et al*. (2006)
Ovine α_{s2}-CN f(165-181)	LKKISQYYQKFAWPQYL	Antibacterial	López-Expósito *et al*. (2006)
Caprineα_{s2}-CN f(174-179)	KFAWPQ	ACE-inhibitory	Quirós*et al*. (2005)
Ovine α_{s2}-CN f(184-208)	VDQHQAMKPWTQPKT-KAIPYVRYL	Antibacterial	López-Expósito *et al*. (2006)
Ovine α_{s2}-CN f(202-204)	IPY	ACE-inhibitory	Gómez-Ruiz *et al*. (2002)
Ovine and caprineα_{s2}-CN f(203-208)	PYVRYL	Antibacterial ACE-inhibitory Antihypertensive	López-Expósito *et al*. (2006) Quirós *et al*. (2005) Recio *et al*. (2005)
Ovine α_{s2}-CN f(205-208)	VRYL	ACE-inhibitory	Gómez-Ruiz *et al*. (2002)
Ovine and caprineβ-CN f(47-51)	DKIHP	ACE-inhibitory	Gómez-Ruiz *et al*. (2005)
Ovine β-CN f(58-68)	LVYPFTGPIPN	ACE-inhibitory	Quirós *et al*. (2005)
Caprineκ-CN f(59-61)	PYY	ACE-inhibitory	Lee *et al*. (2005)
Ovine and caprineκ-CN f(106-111)	MAIPPK	ACE-inhibitory	Manso *et al*. (2003)
Ovine and caprineκ-CN f(106-112)	MAIPPKK	ACE-inhibitory	Manso *et al*. (2003)
Ovine κ-CN f(112-116)	KDQDK	Antithrombotic	Qian *et al*. (1995)
Caprineβ-Lg f(46-53)	LKPTPEGD	ACE-inhibitory	Hernández-Ledesma *et al*. (2002)
Caprineβ-Lg f(58-61)	LQKW	ACE-inhibitory	Hernández-Ledesma *et al*. (2002)
Caprineβ-Lg f(103-105)	LLF	ACE-inhibitory	Hernández-Ledesma *et al*. (2002)
Caprineβ-Lg f(122-125)	LVRT	ACE-inhibitory	Hernández-Ledesma *et al*. (2002)
Ovine and caprine LF f(17-41)	ATKCFQWQRNMRKVRGP-PVSCIKRD	Antibacterial	Vorland *et al*. (1998)
Ovine and caprine LF f(14-42)	QPEATKCFQWQRNMRKVRGP-PVSCIKRDS	Antibacterial	Recio and Visser (2000)

Park *et al*. (2007)

tides are opioid receptor ligands with agonistic or antagonistic activities (Park, 2009a).

The α_{s1}-casein-exorphin (α_{s1}-CN f90-96), β-casomorphins-7 and -5 (β-CN f60-66 and f60-64, respectively), and lactorphins (α-lactalbumin f50-53 and β-lactoglobulin f102-105) act as opioid agonists, whereas casoxins (i.e., k-CN f35-42, f58-61, and f25-34) act as opioid antagonists (Gobbetti *et al*., 2007; Meisel and FitzGerald, 2000). β-Casomorphins were found in the analoguous position of the natural proteins in cow, sheep, water buffalo, and human β-CN (Meisel and Schlimme, 1996).

In all endogenous and exogenous opioid peptides, the common structural feature of these peptides is the presence of a Tyr residue at the amino terminal end (except for α_{s1}-CN-exorphin, casoxin 6, and lactoferroxin B and C) and of another aromatic residue, Phe or Tyr, in 3rd or 4th position (Gobbetti *et al*., 2007). The hydrolysis of *Lactobacillus* GG fermented UHT milk by the pepsin/trypsin has shown to release several opioid peptides from α_{s1}- and β-CN, and α-lactalbumin (Rokka *et al*., 1997).

Antiappetizing peptides

These peptides have functions of suppressing the appetite, whereby they prevent gaining weight and obesity. Zhang and Beynen (1993) reported that the total whey protein in the diet has been associated with a lowering of LDL cholesterol, and also related to the increased release of an appetite-suppressing hormone, cholecystokinin.

The bioactive functions of total whey protein may arise from the combinations of active whey protein fractions or amino acid sequences. Regester *et al*. (1997) suggested that this physiological role of total whey protein has a great potential for processed whey products in development of new and lucrative health food markets as functional food ingredients.

Antimicrobial peptides

These peptides have bacterial membrane-lytic activities which disrupt normal membrane permeability. The total antibacterial effect in milk is greater than the sum of individual immunoglobulin and nonimmunoglobulin such as lactoferrin, lactoferricins, lactoperoxidase, lysozyme, lac-

tenin, casecudubs, etc. (Gobbetti *et al*., 2007; Park, 2009a).

Peptides exhibiting antimicrobial activities have been isolated and purified from several

Bovine milk protein hydrolysates, edible plants, fish and eggs (Clare *et al*., 2003; Gobbetti *et al*., 2004). Among antimicrobial peptides, the lactoferricins are studied the most, which are derived from bovine and human lactoferrin (Kitts and Weiler 2003; Wakabayashi *et al*., 2003). Lactoferricins have been shown to have antimicrobial activity against various Gram-positive and -negative bacteria, yeasts and filamentous fungi (Korhonen and Pihlanto, 2007b).

Lactoferricin is an amphipathic, cationic peptide with anti-microbial (Wakabayashi *et al*., 2003) and anti-cancer (Eliassen *et al*., 2002) properties. Lactoferricin can be generated by the pepsin-mediated digestion of lactoferrin. The MilkAMP database contains a total of 111 peptides (natural, synthetic and modified) comprising or derived from the complete lactoferricin (Théolier *et al.*, 2013), which displays anti-microbial and anti-carcinogenic functions.

Lactenin may have been the first antibacterial factor found in milk, which has been released from rennet hydrolysis of milk (Jones and Simms, 1930). Casecidins are a group of basic, glycosylated and high molecular weight (about 5 kDa) polypeptides, which possess bactericidal properties against lactobacilli and also against various pathogenic bacteria such as *Staphylococcus aureus*. Isracidin is another antibacterial peptide derived from α_{s1}-CN, which is hydrolyzed with chymosin (Hill *et al*., 1974).

Immunomodulatory peptides

Peptides and protein hydrolysates generated from milk caseins and major whey proteins exert immunomodulatory effects [possess immune cell functions], including lymphocyte proliferation, antibody synthesis, and cytokine regulation (Gill *et al.*, 2000). Casein derived peptides are produced during fermentation of milk by lactic acid bacteria. These peptides have become special interest to food researchers and food processing industry due to their immune cell functions. These immunomodulatory peptides have been shown to modulate the proliferation of human lymphocytes, to stimulate the phagocytic activities of macrophages, and to down-regulate the production of certain cytokines (Korhonen and Pihlanto, 2003, 2007; Matar *et al.*, 2003). Immunomodulatory peptides generated from milk include α_{s1}-CN f194-199 (α_{s1}-immunocasokinin) and β-CN f193-202, f63-68, f191-193 (immunopeptides), which are synthesized by hydrolysis with pepsin-chymosin.

The proliferation of human colonic lamina propria lymphocytes was inhibited by immunomodulatory effect of β-casomorphin-7, where the antiproliferative effect of micromolar concentrations was reversed by the opiate receptor antagonist naloxone (Elitsur and Luk, 1991). Free amino acid glutamine can be substituted by glutamine-containing peptides, where glutamine is required for lymphocyte proliferation, and it is also utilized at a high rate by immunocompetent cells for the immunomodulatory effect (Calder, 1994).

Cytomodulatory peptides

Peptides derived from caseins can modulate cell viability such as proliferation and apoptosis in different human cell cultures, inhibit cancer cell growth or stimulate the activity of immunocompetent cells and neonatal intestinal cells (Hartmann *et al*., 2000). Peptides derived from milk act as specific signals that may trigger viability of cancer cells (Gobbettti *et al*., 2007).

Casein hydrolysis by bacteria using commercial yogurt starter cultures can yield bioactive peptides which affect colon cell Caco-2 kinetics *in vitro*. Roy *et al*. (1999) also found that skim cow milk digested with cell-free extract of the yeast Saccharomyces cerevisiae showed antiproliferative activity towards leukemia cells.

Caseinophosphopeptides (CPPs) have also been reported to exhibit cytomodulatory effects. Cytomodulatory peptides obtained from casein fractions can inhibit cancer cell growth or stimulate the activity of immunocompetent cells and neonatal intestinal cells (Meisel and FitzGerald, 2003). Gobbetti *et al*. (2007) reported that peptides released from a lyophilized extract of Gouda cheese inhibited proliferation of leukemia cells.

Mineral binding peptides

Mineral-binding phosphopeptides or caseinophosphopeptides (CCPs) have the function of carriers for different minerals by forming soluble organophosphate salts, especially Ca++ ion; About 1 mol of CPP can bind 40 mol of Ca^{2+}(Meisel and Olieman, 1998; Schlimme and Meisel, 1995). The α_{s1}-, α_{s2}- and β-CN of cow milk contain phosphorylated regions which can be released by digestive enzymes. Specific CPPs can form soluble organophosphate salt and increase Ca absorption by limiting Ca precipitation in the ileum (Korhonen and Pihlanto, 2007b).

Most CPPs contain a common motif, such as a sequence of three phosphoseryl followed by two glutamic acid residues (Gobbetti *et al*., 2007). The negatively charged side chains, particularly the phosphate groups, of these amino acids of CPPs become the specific binding sites for min-

erals (Gobbetti *et al*., 2007). Chemical phosphorylation of α_{s1}- and β-CN increased the binding capacity and the stability of these proteins in the presence of Ca^{2+} (Yoshikawa *et al*., 1981).

Growth factors

Milk growth factor [MGF] is a peptide having the complete N-terminal sequence homologous to bovine TGF-β2.MGF suppresses in vitro proliferation of human T-cells, which includes proliferation induced by mitogen, IL-2 and exposure of primed cells to tetanus toxoid antigen (Stoeck *et al*., 1989). Many growth factors in milk have been identified, such as insulin-like growth factor, platelet-derived growth factor, epidermal growth factor, and transforming growth factor; lactulose from lactose, nucleotides, somatotropin for bifidus growth (Grosnvenor *et al*., 1992; Park, 2009a).

Human milk contains physiologically active levels of growth factor, whereas bovine milk has much lower levels of growth factor activity (Grosvenor *et al*., 1992; Wu and Elsasser, 1995). Colostrum of most mammals usually contains high concentrations of growth factors and others bioactive compounds, while the high levels of these growth factor compounds drop rapidly during the first 3 d postpartum (Brown and Blakeley, 1984; Denhard *et al*., 2000). Goat milk is shown to be a great source of physiologically active growth factors (Wu and Elsasser, 1995).

Unlike bovine milk, human milk contains a growth-promoting activity for *Lactobacillus bifidus* var. *Pennsylvanicus* (Gyorgy *et al*., 1974), which is responsible for the predominance of *Lactobacillus* in the bacterial flora of large intestines of breast-fed infants. Caprine milk has yet to be studied in this premise. The bifidus growth-factor activity is attributed to N-containing oligosaccharides (Gyorgy *et al*., 1974) and glycopeptides and glycoproteins (Bezkorovainy *et al*., 1979). The oligosaccharide moiety of those molecules may possess the bifidobacterium growth-promoter activity which is associated with caseins (Bezkorvainy and Topouzian, 1981).

Bioactive Peptides Uniquely Derived from Whey Proteins

There are many bioactive peptides derived from whey proteins. Some of the known bioactive peptides obtained from whey proteins include α-lactorphin, β-lactorphin, β-lactotensin, serorphin, albutensin A and lactoferricin (Table 3). Some whey proteins are known to contain bioactive peptides with weak opioid activity, including serorphin, albutensin from serum albumin fraction, lactoferroxin from lactoferrin and lactotensin from β-lactoglobulin (Shah, 2000; Tani *et al.*, 1994).

It was found that minor whey proteins such as lactoferrin, lysozyme, lactoperoxidase and immunoglobulins are believed to be antimicrobial proteins. These whey proteins generate bioactive peptides. Lactoferrin is a dominant whey protein in human milk and plays an important role in iron uptake in the intestine (Hutchens *et al.*, 1994; Vilgoen, 1995). Bovine lactoferrin is homologous to human lactoferrin. Bovine colostrum and milk contain about 1.5-5 mg/mL and 0.1 mg/mL, respectively.

Lactoferricin is a simple peptide consisting of 25 amino acid residues. A similar active peptide consisting of 47 amino acid residues has been obtained from human lactoferrin. The lactoferrin molecule is folded into two globular units, where each one is capable of binding one ferric (Fe^{+3}) ion (Shah, 2000).

Conclusions

Bovine milk and colostrum have been shown to be highly important source of natural bioactive components for

Table 3. Bioactive peptides derived from whey proteins

Precursor protein	Fragment	Peptide sequence	Name	Function
α-Lactalbumin	50-53	Tyr-Gly-Leu-Phe	α-Lactorphin	Opioid agonist ACE inhibition
	102-105	Tyr-Leu-Leu-Phe	β-Lactorphin	Non-opioid stimulatory effect on ileum
α-Lactoglobulin	142-148	Ala-Leu-Pro-Met-His-Ile-Arg	-	ACE inhibition
	146-149	His-Ile-Arg-Leu	β-Lactotensin	Ileum contraction
Bovine serum albumin	399-404	Tyr-Gly-Phe-Gln-Asp-Ala	Serorphin	Opioid
	208-216	Ala-Leu-Lys-Ala-Trp-Tyr-Gly-Phe-Gln-Asp-Ala	Albutensin A	Ileum contraction, ACE inhibition
Lactoferrin	17-42	Lys-Cys-Arg-Arg-Trp-Glu-Trp-Arg-Met-Lys-Lys-Leu-Gly-Ala-Pro-Ser-Ile-Thr-Cys-Val-Arg-Arg-Ala-Phe	Lactoferricin	Antimicobial

Adapted from Korhonen *et al.* (1998).

human nutrition and health. Bioactive peptides are liberated during gastrointestinal digestion and fermentation of food materials by lactic acid bacteria. Research have proven that these peptides exhibit a wide variety of physiological functionalities, including antimicrobial, antihypertensive, antithrombotic, antioxidative, opioid, anti-appetizing, immunomodulatory, mineral-binding and growth promoting activities.

The myriad of innate bioactive peptides and biologically and physiologically active milk compounds from casein, whey proteins and other components in milk have been discovered. They present an excellent source of natural ingredients for different applications in functional foods. Industrial or semi-industrial scale processing techniques are available for fractionation and isolation of major proteins from colostrum and milk. In the near future, several break-through products based on these ingredients will be launched on worldwide markets. These bioactive peptides and milk components could be targeted to the development of functional food products for infants, elderly and immune-compromised people as well as to improve performance and prevent diet-related chronic diseases.

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