

Review on Bioactive Peptides in Milk and Dairy Products, and Their Health Benefits

Daniel Asfaw Kitessa
Department of Food Science and Nutrition, Wollega University, Ethiopia

Abstract
Bioactive peptides (BAPs) are defined as peptides with hormone or drug like activity that bind to specific receptors leading to induction of physiological responses. The objective of this seminar is to summarize literature reports on the bioactive peptides in milk and dairy products, and their health-benefits on human body. Milk and milk products are important source of bioactive peptides. The bioactive peptides in milk are encrypted in the structure of the parent proteins; therefore it requires enzymatic hydrolysis or food processing to make them active. Different BAPs released from milk protein have different physiological functions which improve the problem of different chronic diseases.
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Introduction

Bioactive peptides (BAPs) are defined as peptides with hormone or drug like activity that bind to specific receptors leading to induction of physiological responses with a positive impact on body functions and health (Atanasova andIvanova, 2010; Balgir and Sharma, 2017). They are short chains of amino acids (AAs) monomers linked by peptide bonds. BAPs comprise 2–20 amino acid residues with molecular weights of less than 6 kDa which are joined by covalent bonds known as amide or peptide bonds (Di Bernardini *et al*., 2011; Anusha and Bindhu, 2016).

Although some BAPs exist free in its natural sources, the vast majorities of known BAPs are encrypted in the structure of the parent proteins and are released mainly by enzymatic processes. Currently, more than 1500 different BAPs have been reported in a database named 'Biopep' (Singh *et al.*, 2014). Nowadays, researchers revealed many bioactive peptides in milk and milk products with their health benefits. Therefore, the objective of this review is to summarize literature reports on the bioactive peptides in milk and dairy products and their health benefit on human body.

Milk Derived Bioactive Peptides

Milk of all mammalian species contains a heterogeneous mixture of lacteal secretion which contains numerous components such as protein, lipids, carbohydrate (lactose), minerals and vitamins. It also naturally contains an array of bioactivity due to lysozyme, lactoferrin, growth factors, and hormones. Colostrum is especially rich in nutrients and provides protection against pathogens.

Milk and colostrum of bovine and other dairy species are considered as the most important source of natural bioactive components such as high biological value proteins, essential vitamins and minerals that are highly significant for several biochemical and physiological functions (Minieri *et al*., 2018; Park and Nam, 2015; Taj *et al*., 2019). It is needed for the optimal growth and development of humans and a very interesting food for adults, and is recommended as part of a balanced daily diet. It can be defined as a functional food, because it adapts to the definition "any food or food ingredient that can provide a health benefit over the traditional nutrients it contains" (Minieri *et al*., 2018).

Bioactive peptides derived from milk proteins have been a subject of growing interest as health-supporting foods due to their varied nutritional and biological properties (Haque *et al*., 2009). They have shown to possess multiple physiological and physicochemical properties and are therefore regarded as very important constituents for incorporation in functional and novel foods, dietary supplements and even pharmaceuticals with the purpose of targeting specific diseases (Chavan *et al*., 2015). Some of the known bioactive peptides obtained from milk proteins include α-lactorphin, β-lactorphin, β-lactotensin, serorphin, lactoferricin, casomorphins, phosphopeptides, casocidin and casokinins (Korhonen *et al*., 1998; Park and Nam, 2015; Schanbacher *et al*., 1998). However, nowadays, there are plenty of bioactive peptides are generated from milk through enzymatic hydrolysis and food processing.

Different BAPs show different functional activities. Factors to evaluate their functional activities are; molecular mass of peptides, presence and intensity of aromatic, and hydrophobic amino acid and amino acid sequence (Zohaib *et al*., 2018). Bioactivities of BAPs, which depend on constituent AAs and the sequences are; mineral binding, opioid, angiotensin-converting enzyme (ACE) inhibition, immuno-modulatory, antibacterial and antithrombotic (Anusha and Bindhu, 2016).

Generation of Bioactive Peptides from Milk Protein

Bioactive peptide sequences, encrypted within proteins, are liberated *in vivo* during gastrointestinal digestion or *in vitro* by fermentation with proteolytic starter cultures or using proteases (Alamdari *and* Ehsani, 2017; Anusha and Bindhu, 2016; Arrutia *et al.*, 2016b; Atanasova andIvanova, 2010; Korhonen and Pihlanto, 2006). Once the AA sequence is known, it is also possible to synthesize BAPs through the chemical methods, enzymatic synthesis and/or by recombinant DNA technology (Pihlanto and Korhonen, 2003).

The casein fractions (α_s and β-casein) were subjected for enzymatic modification (trypsin), the optimum degree of hydrolysis (*i.e.* ~15 percent DH) was achieved at an Enzyme: Substrate (E: S) ratio of 1:25 at a given concentration of the enzyme, the higher degree of hydrolysis was observed and the yield of β-casein hydrolysate (75-92%) was higher compared to the yield of BAPs of α_s-casein hydrolysate (Devaraja *et al*., 2018). When a whey protein concentrate was treated with trypsin and the obtained peptides were separated using membrane ultrafiltration/nanofiltration, β-lactoglobulin peptides predominated in the hydrolysate (Arrutia *et al.*, 2016b).

The proteolytic activity not only generates the free amino acids needed by the bacteria, but also a large variety of peptides, some of which are endowed with biological activities (Raveschot *et al*., 2018). The proteolytic activity of lactobacilli is exerted in a strain- and species-dependent manner: each species exhibits different proteinase content, leading to a large variety of proteolytic activities. This underlines the high potential of *Lactobacillus* strains to produce novel hydrolysates and BAPs of major interest. Three different strategies were described to produce BAPs using *Lactobacillus* strains: (i) production and functionalization of dairy products by fermentation; (ii) extraction or purification of peptides from a fermented broth; and (iii) utilization of partially purified cell envelope proteinases (CEPs) for protein hydrolysis (Raveschot *et al*., 2018).

Studies have exhibited that, LAB proteases especially from *Lactococcus* sp. and *Lactobacillus* sp. could hydrolyse ≥40% of peptide bonds of α_{s1} and β caseins resulting in the release of various oligopeptides that are further hydrolysed by complex peptidases (Choi *et al*., 2012; Dziuba and Dziuba, 2014). Peptide profiling of bovine kefir, which is a fermented milk beverage, revealed 236 unique peptides that were released from caseins during its production by kefir grains (Ebner *et al*., 2015). The fermentation processing of goat milk caused peptides of protein milk became hydrolyzed and denaturized because of the presence of lactic acid bacteria (Mahdi *et al*., 2017). They also reported that the total of peptides protein bands of fermented milk was more than peptides protein band of fresh milk.

Fermentation is used to produce antibacterial bioactive peptides compounds from different milk sources (Aguilar-Toalá *et al*., 2016**;** Khan *et al*., 2018). Aguilar-Toalá *et al*., 2016 results showed that multifunctional role of peptides derived of fermented milk by the action of specific *L. plantarum* strains. They also reported that the crude extract or their fractions showed the higher anti-inflammatory (723.68–1,759.43 μg/mL of diclofenac sodium equivalents), antihemolytic (36.65 74.45% of inhibition), and antioxidant activity [282.8–362.3 μmol of Trolox (Sigma-Aldrich, St. Louis, MO) equivalents of them. Also, anticancer peptides liberated from milk proteins can be identified from fermented dairy products (Sah *et al*., 2015).

Ozturk and Akin, (2018) reported that the number of peptides increased as the ripening days progressed due to proteolysis. They also reported that, the antioxidant activity of peptides increased as the ripening days progressed. However, the highest antioxidant activity of peptide extracts was found in goat milk cheese than cow milk cheese. In this report the highest Iron (II) binding activity of peptide extracts was determined in goat milk cheese on the 60th day. However, peptide extracts obtained from goat milk cheese on the 90th days demonstrated an inhibitory effect against *Salmonella typhimurium* ATCC 14028.

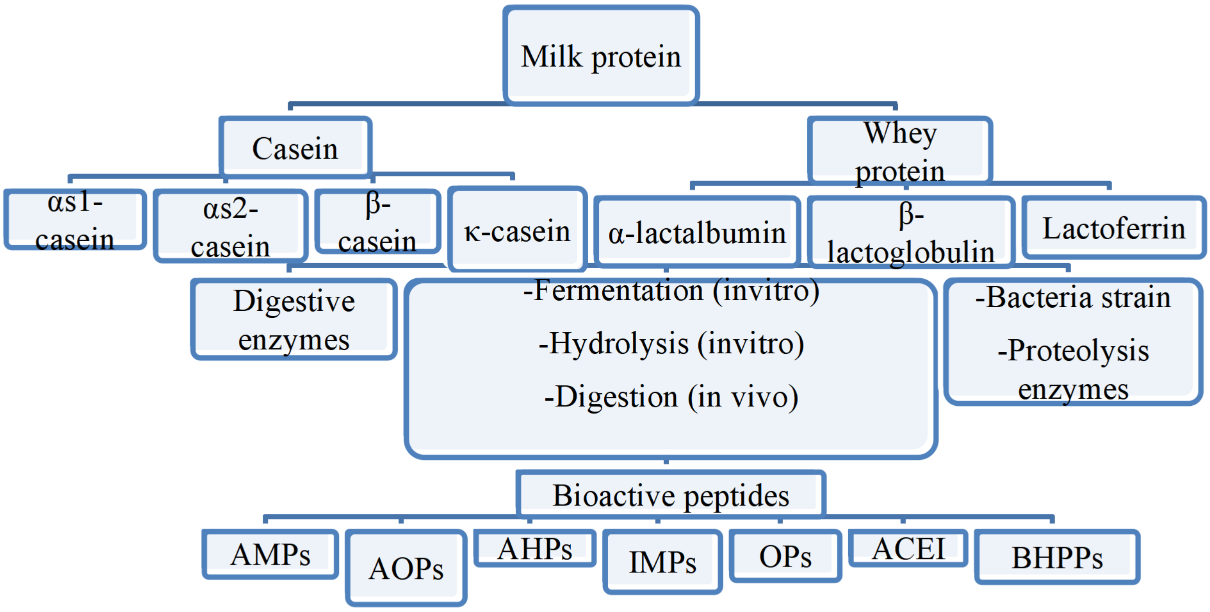


Figure 1. Generation of bioactive peptides from milk source

Table 1. Ways of generating BAPs from milk sources and their AA sequences

Sources	Ways of generation	Peptide sequence	References
Goat milk	Fermentation	TQTPVVVPPFLQPEIM GVPKVKE, TLTDVEKL, VLPVPQKAVPQ, REQEELNVVGE, VLPVPQKVVPQ	Zohaib *et al*., 2018
Goat milk	Fermentation	LYQEPVLGPVRGPFPI, YQEPVLGPVRGPFPIL, VQSWMHQPPQPLSPT	Mahdi *et al*., 2017
Goat milk	Food processing	IATLLLAR, DTEEC, TSETK, IATLLLAR, TSETK, YLGYLEQLLR, TNAIPYVR and NMAIHPR	Sharma *et al*., 2017
Bovine milk	By digestive enzymes	EPMIGVNQELA, TDAPSFSDIPNPIGSENSEK, SLTLTDVENLHLPLPL, WMHQPHQPLPPTVM, WMHQPHQPLPPTVMFPPQSV, WMHQPHQPLPPTVMFPPQSVL, HQPHQPLPPTVM, SLPQNIPPLTQTPVVVPPFLQPEVM, PVVVPPFLQPEV, NQDKTEIPTINT and ALINNQFLPYPY	Nielsen *et al*., 2019
β-casein and α_{s1}-casein	Lactobacillus GG enzyme + pepsin and trypsin	Tyr-Pro-Phe-Pro-Ala-Val-Pro-Tyr-Pro-Gln-Arg, Thr-Thr-Met-Pro-Lue-Trp	Dziuba and Dziuba, 2014
k-Casein	Synthetic	YPSY	Patten *et al*., 2011
α-s2-casein	Hydrolysis with chymotrypsin	TKKTKLTEEEKNRL, QKALNEINQF	Srinivas and Prakash, 2010
β-lactoglobulin	Proteolytic enzymes	VAGTWY HIRL	Pihlanto-Leppala *et al*., 2000

Sources	Ways of generation	Peptide sequence	References
Yak milk casein	Fermentation + alcalase hydrolysis	PLPLL	Mao et al., 2007
β-casein, κ-casein	Lactobacillus helveticus, Saccharomyces cerevisiae	Val-Pro-Pro, Ile-Pro-Pro	Nakamura *et al.* (1995)
β-casein, α_{s1}-casein	Lactobacillus GG enzymes+pepsin & trypsin	Tyr-Pro-Phe-Pro, Ala-Val-Pro-Tyr-Pro-Gln- Arg, Thr-Thr-Met-Pro-Leu-Trp	Rokka *et al.* (1997)
β-casein	Lb. helveticus CP90 proteinase	Lys-Val-Leu-Pro-Val Pro-(Glu)	Maeno *et al.* (1996)
Whey proteins	Lb. helveticus CPN 4	Tyr-Pro	Yamamoto *et al.* (1999)
β-casein	Lb. delbrueckii subsp. bulgaricus IFO13953	Ala-Arg-His-Pro-His-Pro-His-Leu-Ser-Phe-Met	Kudoh *et al.* (2001)
β-casein	Lb. rhamnosus +digestion with pepsin and Corolase PP	Asp-Lys-Ile-His-Pro-Phe, Tyr-Gln-Glu-Pro- Val-Leu	Herna´ ndez-Ledesma *et al.* (2004a)
β-casein	Lb. delbrueckii subsp. Bulgaricus	Ser-Lys-Val-Tyr-Pro-Phe-Pro-Gly Pro-Ile	Ashar and Chand (2004)
β-casein	Streptococcus thermophilus+Lc. lactis subsp.	Ser-Lys-Val-Tyr-Pro	Ashar and Chand (2004)
Skim milk hydrolysate	Lb. helveticus ICM 1004 cell free extract	Val-Pro-Pro, Ile-Pro-Pro	Pan *et al.* (2004)

Effect of Processing on Bioactive Peptides

Processing methods that may reduce the activity of one peptide may enhance the activities of others. For instance, the antibacterial activity of α-lactalbumin and lysozyme increased after they were denatured by heat while other peptides may lose their activity after heating. The effects of boiling on the activity of biopeptides may depend on the enzyme formulation as well as the treatment conditions of the parent protein (Jan *et al.*, 2016). It has been shown that hydrolyzing raw casein using chymotrypsin yielded anti-diabetic peptides with higher bioactivity than those release from boiled casein. In addition, sonication and hydrostatic pressure treatments of raw proteins have separately resulted in enhanced hydrolysis and release of potent BAPs.

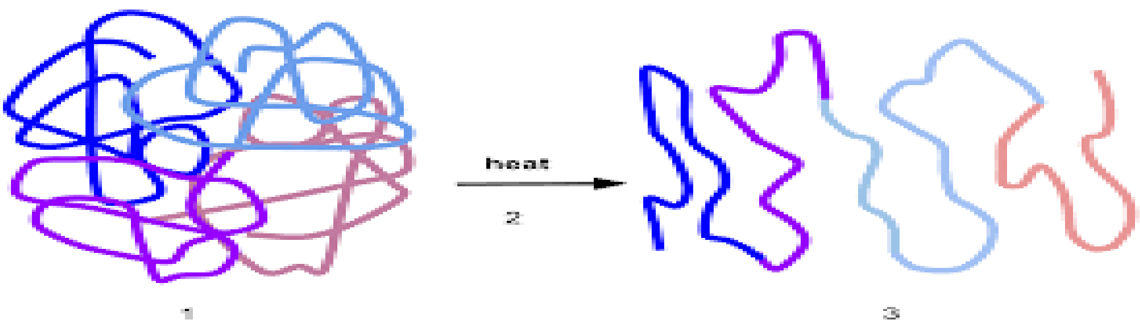


Figure 2. Effect of heat on protein structure

Dairy Products Containing Bioactive Peptides

Milk is a rich source of bioactive peptides which may contribute to regulate the nervous, gastrointestinal and cardiovascular systems as well as the immune system, confirming the added value of dairy products that, in certain cases, can be considered functional foods (Tidona *et al.*, 2014). Bioactive peptides can be produced from milk proteins through fermentation of milk, by starters employed in the manufacture of fermented milks or cheese (Korhonen and Pihlanto, 2003; Korhonen and Pihlanto, 2006). Fermented dairy products, such as yogurt, cheese, and sour milk, are gaining popularity worldwide, and are considered excellent source of dairy peptides. Peptides with various bioactivities have been identified in several dairy-products, such as milk protein hydrolysates, fermented milks and many cheese varieties (Gobbetti *et al.*, 2002; Korhonen and Pihlanto-Leppälä, 2004). These dairy products are associated with lower risks of hypertension, coagulopathy, stroke, and cancer insurgences (Korhonen and Pihlanto, 2003; Sultan *et al.*, 2018).

Table 2. Commercial dairy products and ingredients with health or function claims based on bioactive peptides (Korhonen and Pihlanto, 2006).

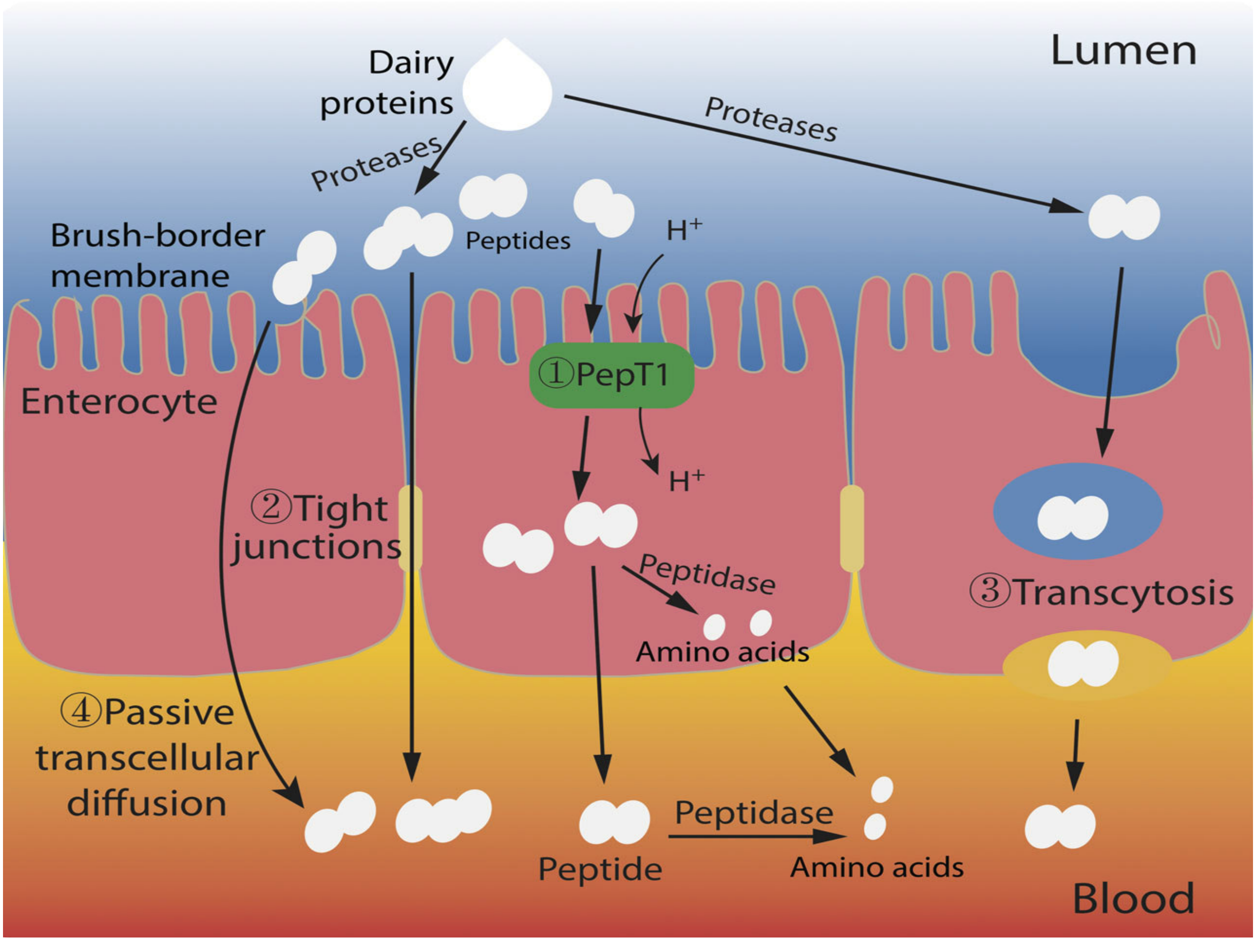
Type of product	Claimed functional bioactive peptides	Precursor protein	Health/function claims	Manufacturers
"Calpis" Sour milk	Val-Pro-Pro, Ile-Pro-Pro	β-casein and κ–casein	Reduction of blood pressure	Calpis Co., Japan
"Evolus" Calcium enriched fermented milk drink	Val-Pro-Pro, Ile-Pro-Pro	β-casein and κ–casein	Reduction of blood pressure	Valio Oy, Finland
"BioZate" Hydrolysed whey protein isolate	β-lactoglobulin fragments	β-lactoglobulin	Reduction of blood pressure	Davisco, USA
"BioPure-Alphalactalbumin" Whey protein isolate	α-lactalbumin	α-lactalbumin	Helps sleep and memory	Davisco, USA
"BioPure-GMP" Whey protein isolate	f(106–169) (Glycomacropeptide)	κ-casein	the clotting of blood, protection against microrganisms	Davisco, USA
"Prodilet F/200 Lactium" Flavoured milk drink, confectionery, capsules	f (91–100) (Tyr-Leu-Gly Tyr-Leu-Glu-Gln-Leu-Leu-Arg)	α_{s1}-casein	Reduction of stress effects	Ingredia, France
"Festivo" Fermented low-fat hard cheese	f (1–9), f (1–7), f (1–6)	α_{s1}-casein	No health claim as yet	MTT Agrifood Research Finland
"Cystein Pepetide" Ingredient/ hydrolysate	Milk protein derived peptide	Milk protein	Aids to raise energy level and sleep	DMV International, The Netherlands
"C 12" Ingredient/ hydrolysate	Casein derived peptide	Casein	Reduction of blood pressure	DMV International, The Netherlands
"Capolac" Ingredient	Caseinophosphopeptide	Milk protein	Helps mineral absorption	Arla Foods Ingredients, Sweden
"PeptoPro" Ingredient/ hydrolysate	Casein derived peptide	Casein derived peptide	Improves athletic performance and muscle recovery	DSM Food Specialties, The Netherlands
"Vivinal Alpha" Ingredient/hydrolysate	Whey derived peptide	Whey derived peptide	Aids relaxation and sleep	Borculo Domo Ingredients (BDI), The Netherlands

Bioavailability of Bioactive Peptides

BAPs bioavailability is the ability of peptides to exert physiological effects in vivo after oral ingestion. Thus, it is of crucial importance that milk-derived BAPs remains active during the gastrointestinal digestion and absorption and reaches the target site intact. The bioavailability of peptides depends on a variety of structural and chemical properties, i.e., resistance to proteases, charge, molecular weight, hydrogen bonding potential, hydrophobicity, and the presence of specific residues (Pauletti *et al.*, 1996; Pauletti *et al.*, 1997; Xu *et al.*, 2018). Indeed, proline- and hydroxyproline-containing peptides are relatively resistant to degradation by digestive enzymes (Mizuno *et al.*, 2004; Savoie *et al.*, 2005).

Some dairy protein-derived bioactive peptides can escape the degradation of peptidase and reach the bloodstream at concentrations of micromolar range, and keep intact for several minutes to hours (Xu *et al*., 2018). They also reported that the presences of brush-border peptidases at the site of absorption and other peptidases in the blood, along with the peptide properties, such as molecular size and weight, stability to proteases, hydrophobicity and charge, determine their bioavailability.

Dairy bioactive peptides generated are transported across the intestinal brush-border membrane into the bloodstream via one or more of the following routes: (i) peptide transport 1 (PepT1)-mediated route, (ii) paracellular route via tight junctions (TJs), (iii) transcytosis route and (iv) passive transcellular diffusion (Horner *et al*., 2016; Xu *et al*., 2017; Matsui, 2018).



Source: (Xu *et al*., 2018)
Fig. 4 Possible transport routes of dairy protein-derived bioactive peptides (DBPs) across the intestinal brush-border membrane into bloodstream.

Health Benefits of Bioactive Peptides

The importance of milk and its products is due to the presence of protein, bioactive peptides, conjugated linoleic acid, omega-3 fatty acid, Vitamin D, selenium, and calcium (Khan *et al*., 2019). These constituents are present in milk product, play a key role in the physiological activities in human bodies, and act as anti-inflammatory, anti-tumor, antioxidant, hypocholesterolemic, immune boosting, and antimicrobial activities. Recent scientific evidence suggests that food proteins not only serve as nutrients, but can also modulate the body's physiological functions. These physiological functions are primarily regulated by some peptides that are encrypted in the native protein sequences (Chakrabarti *et al*., 2018). These bioactive peptides can exert health beneficial properties and thus are considered as a lead compound for the development of nutraceuticals or functional foods.

BAPs are considered the new generation of biologically active regulators; they can prevent oxidation and microbial degradation in foods and also improve the treatment of various diseases and disorders, thus increasing the quality of life (Sanchez and Vazquez, 2017). Also, due to various disadvantages of pharmaceutical antihypertensive drugs such as high fever, cough, dizziness, chills, sweating, blurred vision, hypoglycaemia, depression etc., antihypertensive bioactive peptides are used for the treatment of hypertension (Balgir and

Sharma, 2017).

Milk-derived bioactive peptides can be released during gastrointestinal digestion, food processing or by enzymatic and bacterial fermentation and are considered to promote diverse beneficial effects such as lipid lowering, antihypertensive, immune-modulating, anti-inflammatory and antithrombotic effects (Marcone *et al*., 2017). They play important role in boosting natural immune protection by reducing the risk of chronic diseases (Mohanty *et al*., 2016). They are considered as potent drugs with well-defined pharmacological residues and also used to formulate health-enhancing nutraceuticals.

The health promoting, functional and performance enhancing ability of milk derived bioactive peptides have been established by various researchers which include anti-hypertensive, cytomodulatory, anti-appetizing, antithrombotic, opioid, anti-microbial and lactoferrin (Nielsen *et al*., 2019; Jayathilakan *et al*., 2018). Due to their physiological and physico-chemical versatility, milk peptides are regarded as greatly important components for health promoting foods or pharmaceutical applications (Park and Nam, 2015). Peptides derived from the milk of cow, goat, sheep, buffalo and camel exert multifunctional properties, including anti-microbial, immune-modulatory, anti-oxidant, inhibitory effect on enzymes, anti-thrombotic, and antagonistic activities against various toxic agents (Mohanty *et al*., 2016). Majority of those regulate immunological, gastrointestinal, hormonal and neurological responses, thereby playing a vital role in the prevention of cancer, osteoporosis, hypertension and other disorders.

Milk-derived proteins and peptides have the potential to act as co-adjutants in conventional therapies, addressing cardiovascular diseases, metabolic disorders, intestinal health, and chemo-preventive properties (Hsieh *et al*., 2015). Glycomacropeptide (GMP) is a milk-protein-derived peptide that, in addition to its nutritional value, retains many biological properties and has therapeutic effects in several inflammatory disorders (Córdova-Dávalos *et al*., 2019). It was shown under in vitro and in vivo conditions to exert a number of activities that regulate the physiology of important body systems, namely the gastrointestinal, endocrine, and immune systems (Córdova-Dávalos *et al*., 2019; Moughan *et al*., 2004). Hydrolysed bioactive peptides of caprine milk proteins are exhibit various physiological effects in the body, such as gastrointestinal, cardiovascular, endocrine, immune and nervous systems (Devaraja *et al*., 2018).

Camel milk has been used to reduce cholesterol levels in the blood, to avoid psoriasis disease, to heal inflammation in the body, to assist patients with tuberculosis, helping to strengthen the human immune system, to reduce the growth of cancer cells and to cure autism (Kaskous, 2016). Camel milk has a powerful effect in reducing blood glucose levels and insulin requirement, and it limits diabetic complications such as elevated cholesterol levels, liver and kidney diseases; decreased oxidative stress; and delayed wound healing (Bakr, 2015). It is safe and efficient in improving long-term glycemic control and can provide a significant reduction in the dose of insulin required by type one diabetic patients (Bakr, 2015).

The unique bioactive peptides found in goat milk play role in health benefit of human (Sharma *et al.*, 2017). Oligodendrocyte Transcription factor 2 peptide shows an effective role in repairing of the central demyelinating after injury in other organisms. Interleukin-12 subunit alpha precursor peptide shows an association with primary biliary cirrhosis and many other autoimmune diseases. Also, dipeptidyl-peptidase 1 precursor (IATLLLAR) plays a potential role in activating granzymes A and B which tends to activate the apoptosis in mitochondria. Sheep and goat milk proteins are important sources of bioactive ACE inhibitory peptides and antihypertensive peptides (Atanasova andIvanova, 2010).

Milk-derived antibacterial peptides (ABPs) have several useful properties important for human health, comprising a significant antibacterial effect against various pathogens, but contain toxic side-effects (Khan *et al*., 2018). These ABP compounds are produced by organic synthesis, fermentation, hydrolysis by food grade enzymes and in vivo by enzymatic hydrolysis, bacteria in gastrointestinal tract and recombinant DNA techniques (Albenzio *et al*., 2017; Bhat *et al*., 2015; Cleveland *et al*., 2001; Khan *et al*., 2018; Kim and Wijesekara, 2010).

Antimicrobial peptides are recognized as an important component of innate immunity, particularly at mucosal surfaces such as the oral cavity, lungs and small intestine that are constantly exposed to a range of potential pathogenic bacteria (Liu, 2017). The peptides derived from α_{s1}-casein, κ-casein, and specific regions of β-casein of bovine milk hydrolysed with gastrointestinal digestive enzymes have the ability to stimulate cell migration of intestinal epithelial cells in vitro, which is an important step in gastro-intestinal wound healing (Nielsen *et al*., 2019). Several peptides, putatively released from the C-terminal part of camel α_{s2}-CN isoforms after *in silico* digestion by proteases from the digestive tract, which were predicted to display anti-bacterial and antihypertensive activities (Ryskaliyeva *et al*., 2019).

Curd has ACE inhibitory peptides and activity increases after digestion (Chathurika *et al*., 2016). As they reported, in curds from two brands thirteen fractions were obtained, where nine fractions showed more than 70% inhibition with 96% ACE inhibition for a di-peptide. Bioactive peptides in kefir product have many different physiological effects, including ACE-inhibitory, immune-modulatory, antimicrobial, opioid, mineral binding, antioxidant and antithrombotic activity (Liu, 2017).

The bioactive peptides of goat milk fermented had the ability to decrease hypercholesterolemia (Mahdi *et* *al*., 2018). Goat's milk yoghurt therapy at a dose of 600 mg/kg has been able to provide the best therapeutic effect in lowering levels of malondialdehyde and therapeutic dose of 900 mg/ kg give the best therapeutic effect in reducing the expression fat accumulation liver rat animal model of hypercholesterolemia. Greater quantity of hydrophobic aliphatic amino acid and aromatic amino acid like Proline, Leucine, Alanine, Metionine and Isoleucine, in a sample may increase the ACE-inhibitory activity (Chalé *et al*., 2014; Zohaib *et al*., 2018). On the basis of *in vitro* study, the buffalo and cow milk cheddar cheese water-soluble peptides extract can protect intestinal epithelium against oxidative stress due to their antioxidant activity (Huma *et al*., 2018). Mozzarella Cheese obtained from Buffalo milk contains bioactive peptide that possesses anti-inflammatory effects both in vitro and in vivo (Tenore *et al*., 2019).

Table 3. Physiological function of BAPs in milk and dairy products

Sources	**Peptide (AA sequence)**	**Bioactivity**	References
Lactobacillus rhamnosus fermentation + digestion with pepsin	Asp-Lys-Ile-His-Pro-Phe, Tyr-Gln-Glu-Pro-Val-Leu	ACE inhibitory	Mohanty *et al.* (2016)
Lactobacillus helveticus fermentation	Val-Pro-Pro, Ile-Pro-Pro	ACE inhibitory	Mohanty *et al.* (2016)
Lactobacillus delbrueckii subsp., bulgaricus IFO13953 fermentation	Ala-Arg-His-Pro-His-Pro-His-Leu-Ser-Phe-Met	Antioxidant activity	Mohanty *et al.* (2016)
Kluyveromyces marxianus var. fermentation	Tyr-Leu-Leu-Phe	ACE inhibitory	Mohanty *et al.* (2016)
β-Casein derived peptides	Lys-Val-Leu-Pro-Val-P(Glu)	ACE inhibitory	Mohanty *et al.* (2016)
Buffalo milk	NAVPITPTL	Bone health-promoting	Reddi *et al.* (2016)
Buffalo milk	VLPVPQK	Bone health-promoting	Mada *et al.* (2017)
Fermented goat milk	TQTPVVVPPFLQPEIMGVPKVKE	ACE inhibitory activity	Bolin *et al*., 2018
Yoghurt of goat milk	LYQEPVLGPVRGPFPI, YQEPVLGPVRGPFPIL and VQSWMHQPPQPLSPT	Antioxidant activi ty	Mahdi *et al*., 2018
	VECYGPNRPQF	Antioxidant activi ty	Ricci-Cabello *et al.* (2016)
	WYSLAMAASDI	Antioxidant activi ty	Nongonierma and Fitzgerald (2015)
	EQLTK	Antimicrobial	Wada and Lönnerdal (2014)
α_{s1}-casein, κ-casein, and specific regions of β-casein of bovie milk	EPMIGVNQELA, TDAPSFSDIPNPIGSENSEK, SLTLTDVENLHLPLPL, WMHQPHQPLPPTVM, WMHQPHQPLPPTVMFPPQSV, WMHQPHQPLPPTVMFPPQSVL, HQPHQPLPPTVM, SLPQNIPPLTQTPVVVPPFLQPEVM, PVVVPPFLQPEV, NQDKTEIPTINT and ALINNQFLPYPY	Gastro-intestinal wound healing	Nielsen *et al*., 2019
β-casein and α_{s1}-casein	Tyr-Pro-Phe-Pro-Ala-Val-Pro-Tyr-Pro-Gln-Arg, Thr-Thr-Met-Pro-Lue-Trp	Opioid	Dziuba and Dziuba, 2014
Lactoferin	Lactoferin	Inhibition of cytokine production	Haversen *et al*., 2002
Whey protein	Lactoferricin	Anti-catabolic and anti-arthritis	Yan *et al*., 2013a; Yan *et al*., 2013b

Sources	**Peptide (AA sequence)**	**Bioactivity**	References
Casein	RYLGY, RYFYPEL, YQKFQY	Antioxidant	Contreras *et al*., 2009
Casein	RYLGY, RYFYPEL, YQKFQY	ACE inhibitor	Contreras *et al*., 2009
Yak milk casein	PLPLL, LTLTDVE	ACE inhibitor	Mao *et al*., 2007
Casein	YPFPGPI	Opioid	Cie´sli ´ nska *et al*., 2012
β-casein	YPVEP	Immunomodulatory	Kitazawa *et al*., 2007
β-casein	EPVLGPVRGPFP	ACE-inhibition	Hayes *et al*., 2007
α-s2-casein	QKALNEINQF	Antimicrobial	Srinivas and Prakash, 2010
α-s2-casein	TKKTKLTEEEKNRL	ACE inhibition	Srinivas and Prakash, 2010
k-Casein	YPSY	Opioid agonist	Patten *et al*., 2011
Ovine and caprineαs2-CN	PYVRYL	ACE-inhibitory	Quirós *et al*., 2005
Ovine α_{s2}-CN	LKKISQ, LKKISQYYYQKFAWPQYL, VDQHQKAMKPWTQPKTNAIPYVRYL, PYVRYL	Antibacterial activity	López-Expósito *et al*., 2006

Conclusion

Bioactive peptides are short chains of amino acids residues with low molecular weight. Most of the bioactivities of peptides are encrypted within the primary sequence of the native protein and peptides require to be released through hydrolysis by digestive enzymes, enzymatic cleavage by proteases derived from microorganisms or plants and food processing. Different microorganisms and enzymes produce different bioactive peptides of different physiological functions. Milk-derived bioactive peptides are considered to have diverse beneficial effects such as lipid lowering, antihypertensive, immune-modulating, anti-inflammatory, antioxidant, antimicrobial, opioid, mineral binding, antithrombotic and etc.

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