

Review Article

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Emerging Trend in Therapeutic Potential of Casein Derived Bioactive Peptides

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ABSTRACT

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Casein is a high biological value protein due to high indispensable amino acid and protein digested corrected amino acid score. Bioactive peptides enchanted many activities, such as immunoregulatory, Opioid, etc. These peptides usually consist of 2-20 amino acids and become activated after release from inherent protein following enzymatic hydrolysis either *in vivo* or *in vitro*. The most common method to produce bioactive peptides is enzymatic proteolysis of the parent protein. Casein-derived bioactive peptides possess antioxidant, antihypertensive, antidiabetic, antithrombotic, and antimicrobial activity. Nowadays, due to the increasing demand for functional foods and nutraceuticals by consumers, bioactive peptides are incorporate into food to enhance their functionality.

Introduction

Milk is an excellent source of protein with multiple therapeutic properties on health. It consists of two types of protein; one is a soluble protein known as whey protein that accounts for 20%, whereas another fraction consists of an insoluble protein known as casein, which constitutes 80% of total milk protein (Haug *et al.*, 2007; Severin and Wenshui, 2005). Casein consists of four fractions, namely α s1, α s2, β , and κ -casein (Fox *et al.*, 1998). Casein is a high biological value protein due to high indispensable amino acid and protein digested corrected amino acid score (Boye *et al.*, 2012; Schaafsma, 2000). Bioactive peptides enchanted many

activities, such as immunoregulatory, opioid, antioxidant, antihypertensive, antidiabetic, etc. Significant studies have done on the casein derived bioactive peptides. These peptides usually consist of 2-20 amino acids and become activated after release from inherent protein following enzymatic hydrolysis either *in vivo* or *in vitro*. The significant difference between drugs and bioactive peptides is that synthetic drugs are administered intentionally within the body, on the other hand, bioactive peptides may be present in humans as they may arise from the digestion of food. Examples of casein-derived bioactive peptides constitute casein phosphopeptides (CPPs) (κ -casein, f(147–153)) that helps in transportation and

absorption of specific minerals (FitzGerald, 1998); glycomacropeptides (GMP) that bind toxins (Kawasaki *et al.*, 1992); isracidin (as1-casein, f(1–23)) that possesses immunomodulatory effects (Lahov & Regelson, 1996); and casoxins (k-casein, f(58–61)) that behave as opioid antagonists (Chiba *et al.*, 1989; Xu, 1998) while casomorphins (as1-casein, f(90–95); Arg-Tyr-Leu-Gly-Tyr-Leu) may behave as opioid receptor agonists (Loukas *et al.*, 1983; Teschemacher, 2003).

Bioactive peptides

Bioactive peptides are the chains of 2-20 amino acid sequence (Korhonen and Pihlanto, 2006), encrypted within the native protein sequence. These peptides are inactive within the parent protein but get activated when cleaved from the parent chain and show biological activity. These can be produced *in vivo* by gastrointestinal enzymes and *in vitro* by specific enzymes (Miguel *et al.*, 2009).

Functionally bioactive peptides released from parent protein in the following ways:

Enzymatic hydrolysis by digestive enzymes (Kilara and Panyam, 2003; Korhonen and Pihlanto, 2003).

Fermentation of milk with the proteolytic starter culture (Fuglsang *et al.*, 2003).

Gastrointestinal digestion (Ferranti *et al.*, 2004).

Enzymatic hydrolysis

The most common method to produce bioactive peptides is enzymatic proteolysis of the parent protein. Pepsin and trypsin are the two most common enzymes used for proteolysis (Ferreira *et al.*, 2007). Pepsin, chymotrypsin, alcalase, thermolysin, pancreatin, and multiple enzymes of different

origin, and their combinations could be used, to produce bioactive peptides having an antioxidant, antimicrobial, antihypertensive, and antidiabetic activity (Kilara and Panyam, 2003).

Microbial fermentation

Many starter cultures have been used to hydrolyze the protein especially casein. Cultures like *Lactococcus lactis*, *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Lactobacillus helveticus* possess cell wall-bound protease and peptidases like dipeptidases, tripeptidases, aminopeptidases, and endopeptidases (Griffiths and Tellez, 2013). *Lactococcus helveticus* was highly proteolytic and the most commonly used starter culture in fermented dairy products (Szwajkowska *et al.*, 2011). Two renowned antihypertensive peptides, Val-Pro-Pro and Ile-Pro-Pro were isolated from fermented milk of *Lactococcus helveticus* (FitzGerald *et al.*, 2004). Ashar and Chand (2004) identified an antihypertensive peptide from fermented milk. The identified fragment had an amino acid sequence Ser-Lys-Val-Tyr-Pro-Phe-Pro-Gly-Pro-Ile that showed it was a part of β -casein. Tryptic and peptic casein hydrolysates triggers *in vitro* immunomodulatory effect on human blood lymphocytes. Moreover, bioactive peptides released by microbial enzymes could be attacked by other enzymes present in GIT and produce some other peptides (Shimizu and Ok Son, 2007; Moller *et al.*, 2008).

Gastrointestinal digestion

Numbers of functional peptides are released, during gastrointestinal digestion in the body (Ferranti *et al.*, 2004). After ingestion, food protein undergoes denaturation and assimilation by several proteolytic enzymes e.g. pepsin, trypsin, and chymotrypsin to produce functional peptides. Peptidase like carboxypeptidase, aminopeptidase, act further

on peptides and convert them into amino acids for absorption. For example, β -casomorphins and casein phosphopeptide (CPP) discharges in the gastrointestinal tract following oral consumption of dairy products (Hernandez-Ledesma *et al.*, 2004).

Bioactive peptides used in the dairy industry

Nowadays, due to the increasing demand for functional foods and nutraceuticals by consumers, bioactive peptides are incorporated in food to enhance their functionality. Sugai (1998) reported a product “casein DP peptio drink” made up of casein hydrolyzed with trypsin. 20 g of tryptic casein hydrolysate for 4 weeks resulted in lowering of blood pressure in hypertensive patients (Sekiya *et al.*, 1992). Another product named “C12 Peption” in the Netherlands made up of tryptic hydrolyzed casein, prescribed in hypertensive animals and humans to control the blood pressure (Cadee *et al.*, 2007; Karaki *et al.*, 1990). Calpis, fermented milk, cultured by using *Saccharomyces cerevisiae* and *Lactobacillus helveticus*, contained two well-known ACE inhibitory peptides, Val-Pro-Pro (VPP) and Ile-Pro-Pro (IPP) from β -casein and κ -casein, respectively (Nakamura *et al.*, 1995). The number of commercial dairy products from different countries with health claims has shown in Table. 1.

Antioxidant activity

Oxidative metabolism is one of the primary functions for cell maintenance while, failure in metabolism generates several reactive oxygen species (ROS) and free radicals, inducing oxidative stress. Free radicals like peroxide radical, hydroxyl radical, superoxide radical, and hydrogen peroxide are ordinarily known as ROS, not only cause food spoilage, also harmful to biological systems. Certain free radical species like peroxidase, catalase,

and superoxide dismutase have a damaging effect on endogenous defensive enzymes (Fang *et al.*, 2002). These enzymes damage and prompt deadly cellular outcomes (e.g., apoptosis) by oxidation of cellular proteins, lipids in the membrane, enzymes, and DNA (Harvey and Ferrier, 2011). These free radical species may additionally produce alterations at the cellular level that drive several human ailments like arthritis, hypertension, atherosclerosis, and cancer (Frenkel, 1992).

Ahmed *et al.*, (2015) reported the antioxidant activity of both casein and whey protein hydrolysate from goat milk. Pepsin-digested goat casein protein (P-GCP) and pepsin digested goat whey protein (P-GWP) showed remarkably high superoxide and DPPH radical scavenging activity as compared to the GCP and GWP. The hydrolysates of goat milk whey and casein fractions contain peptides, which acts as electron donors that could reduce the free DPPH radicals into stable molecules. Various bioactive peptides possessing antioxidant activity were e.g. α s2-casein f(82–88), α s2-casein f(141–148), β -casein f(124–137), κ -casein f(61–70), lactoferrin f(33–43), β -lactoglobulin f(38–48) and β -lactoglobulin f(113–122). Bioactive peptides having Leu residues and the Ser-Leu/Thr-Leu/Pro-Leu motifs in their sequences, particularly at N and C-terminus, seemed to have superior scavenging activities. Chen *et al.*, (1996) reported a peptide from casein, Pro-His-His that showed maximum antioxidant activity. Bioactive peptides having His were associated with hydrogen-donating ability, lipid peroxy-radical trapping, and metal ion chelating ability. Bovine casein hydrolyzed with trypsin alone and with a combination of pepsin and trypsin showed antioxidant activity. Tryptic hydrolysate showed maximum antioxidant activity (13.7%) at 4 hr of digestion. The casein hydrolysate was fraction into 10 kDa and 1 kDa through ultrafiltration. Crude

hydrolysates and the permeates from 10 kDa and 1 kDa showed radical scavenging activity 13.7%, 17.3%, and 24.4%, respectively (Irshad *et al.*, 2015). Shanmugam *et al.*, (2015) scrutinized the antioxidant activity of buffalo casein hydrolyzed with pepsin, trypsin, and chymotrypsin alone and in combinations. He found that Pepsin-trypsin digested hydrolysates showed higher antioxidative action than other hydrolysates. Kumar *et al.*, (2016) studied that α -chymotrypsin produced significantly higher DH and antioxidant activity than alcalase and papain. Nongonierma and FitzGerald (2013) reported that tryptophan was a better scavenger of DPPH radical than valine. Suetsuna and Chen (2002) stated antioxidant activity of peptides depends on the C or N-terminal position of amino acid residues. For example, Trp-Lys has a more robust antioxidant action than Lys-Trp. Table. 2 showing the antioxidant peptides derived from casein.

Antihypertensive activity

Hypertension is also known as high blood pressure. Cardiovascular disease (CVD) is the number one cause of death globally, and 17.9 million people died due to CVD in 2016, representing 31% of global death (WHO, 2017). Different interacting biochemical pathways such as the renin-angiotensin system control the risk of CVD (Sultan *et al.*, 2018). In clinical practice, vasodilators, diuretics, calcium channel blockers, angiotensin II receptor blockers, and angiotensin I-converting enzyme (ACE) inhibitors, applied to control blood pressure. These substances interfere with the different interacting biochemical pathways that control blood pressure, specifically the renin-angiotensin system, the kinin-kallikrein system, the neutral endopeptidases system, and the endothelin-converting enzyme system (FitzGerald *et al.*, 2004). The dipeptidyl

carboxypeptidase enzyme (ACE) catalyzes, the production of vasoconstrictor i.e. angiotensin II on one side and inactivation of vasodilator i.e. bradykinin on another side, hence involved in both renin-angiotensin system and kinin-kallikrein system. Thus ACE blockers played a significant role in the regulation of blood pressure. Despite, the side effects of synthetic ACE inhibitors such as hypotension, cough, increased potassium levels, decreased kidney function, angioedema, they are used extensively in hypertension (FitzGerald *et al.*, 2004). This stimulates our interest in finding natural ACE inhibitors. The natural ACE inhibitor was first time identified in snake venom (Ondetti *et al.*, 1977). The number of bioactive peptides isolated from the milk (Meisel, 2004), gelatine (Oshima *et al.*, 1979), maize, soybean (Chen *et al.*, 1996) that inhibit ACE. Casokinins and lactokinins from casein and whey protein showed ACE inhibitory property (Kitts and Weiler, 2003). The presence of aromatic or branched-chain amino acids such as tyrosine, phenylalanine, proline, and lysine at the C-terminal positions of a peptide enhances the binding of the substrate with the enzyme. The ACE enzyme is present in the lung, kidney, heart, skeletal muscle, arteries, testes, uterus, brain, and epithelial cells of the jejunum (Ondetti and Cushman, 1982).

Mao *et al.*, (2007) reported that yak milk casein hydrolysate obtained at 4 h of hydrolysis showed maximum ACE inhibitory activity. Furthermore, identified two novel peptides, Pro-Pro-Glu-Ile-Asn (PPEIN) and Pro-Leu-Pro-Leu-Leu (PLPLL) from κ -casein and β -casein, respectively. The molecular weight and IC₅₀ value of the peptides were 550 Da, 566.4 Da and 0.29 ± 0.01 mg/ml, 0.25 ± 0.01 mg/ml respectively. Perpetuo *et al.*, (2003) identified a peptide Tyr-Pro-Val-Glu-Pro-Phe-Thr-Glu from tryptic casein hydrolysates that exhibited

ACE inhibitory activity. Otte *et al.*, (2007) elucidated lower activity of the A1 than the A2 variant of β -casein on digestion with trypsin. Some di-peptides, tri-peptides and longer chain peptides such as Tyr-Gln-Glu-Pro, Val-Pro-Lys-Val-Lys, Pro-Leu-Pro-Leu-Leu, His-Leu-Pro-Leu-Pro, Tyr-Gln-Glu-Pro-Val-Leu-Gly-Pro and Ser-Leu-Val-Tyr-Pro-Phe-Pro-Gly-Pro-Ile from β -casein, Tyr-Pro, Arg-Pro-Lys and Arg-Pro-Lys-His-Pro-Ile-Lys-His from α 1-casein and Pro-Pro-Glu-Ile-Asn from κ -casein showed ACE-inhibitory activity (Ashar and Chand, 2004; Hernandez-Ledesma *et al.*, 2007; Yamamoto *et al.*, 1999). Peptides derived from casein correspond to the fraction (23-24), (23-27) and (194-199) of bovine α 1-casein, as well as fraction (177-183) and (193-202) of bovine β -casein showed ACE inhibitory activity (Maruyama and Suzuki, 1982). Karaki *et al.*, (1990) observed a significant decrease in blood pressure after oral administration of tryptic casein hydrolysate in spontaneously hypertensive rats. Camel whole milk casein and β -casein were digested with pepsin, trypsin, and chymotrypsin. Hydrolysate fraction showed higher ACE inhibitory activity compared with an unhydrolyzed fraction (Salami *et al.*, 2011). Miguel *et al.*, (2009) investigated both *in vivo* and *in vitro* effect of bovine casein hydrolysates (HBC) on blood pressure. *In vitro* study of HBC and HBC, <3 kDa showed an IC₅₀ value of 52 μ g/ml and 5.5 μ g/ml, respectively. *In vivo* study showed a significant decrease in both systolic and diastolic blood pressure when HBC and HBC <3 kDa administered in spontaneously hypertensive rats. Lopez-Exposito *et al.*, (2007) identified two chemically synthesized peptides, LKKISQ and PYVRYL from, ovine α 2-casein that exerted potent ACE inhibitory activity. Petrat-Melin *et al.*, (2015) reported maximum ACE inhibition in the A1 and B variant as compared to the A2 and I variant of β -casein hydrolyzed with pepsin and pancreatic

enzyme. Garg *et al.*, (2021) reported Both A1 and A2 casein hydrolysates manifested ACE inhibition, whereas improved activity was recorded with progress in time and degree of hydrolysis.

Antidiabetic activity

Diabetes is a chronic disease that occurs either not secretion of insulin from the pancreas or the body cannot effectively utilize the insulin that it produces. It leads to severe damage to many organs and the major cause of blindness, kidney failure, heart attack, stroke, and lower limb amputation (WHO, 2016). So, effective management of diabetes is the principal concern. Diabetes mellitus (DM) is of two types: type 1 DM is also known as insulin-dependent or juvenile or childhood-onset diabetes, owing to the damage of β cells of the pancreas and requires daily administration of insulin. Type 2 DM is also known as non-insulin-dependent or adult-onset diabetes that occurs when the body becomes resistant to insulin or does not make enough insulin. Type 2 DM is the most common type of diabetes in the world and the result of physical inactivity and obesity (WHO, 2016). According to the WHO global report on diabetes, the number of people has almost quadrupled since 1980 to 422 million adults. The dramatic rise is only due to the surge in type 2 DM. In 2012, diabetes caused 1.5 million deaths. This report calls upon governments to ensure that people can make healthy choices and that health systems can diagnose, treat and care for people with diabetes. It encourages all individuals to eat healthily, be physically active and avoid excessive weight gain (WHO, 2016). People with type 2 diabetes need to make a healthy diet plan (choose low sugar and low-fat food) and exercise daily. Type 2 diabetes accounts for about 90% of cases diagnosed. It is a principal cause of mortality, accounting for 14.5% of global mortality among adults aged

20 to 79 (International Diabetes Federation (IDF), 2015). IDF estimated that currently, 415 million people (1 in 11 adults) suffering from diabetes, and predicts that, if the present demographic growth continues, 642 million (1 in 10 adults) will be affected by this metabolic disorder by 2040. India is one of the six countries of the IDF Southeast Asian (SEA) region. Globally, 425 million people have diabetes, and 82 million people live in SEA Region; by 2045, this will rise to 151 million. There were over 72,946,400 cases of diabetes in India in 2017. Casein protein supplementation to high carbohydrate-containing meals leads to a reduction in postprandial blood glucose levels and an increase in plasma insulin concentrations in healthy and diabetic individuals. Exclusively the release of bioactive peptides upon digestion of whey protein was responsible for antidiabetic activity (Jakubowicz and Froy, 2013).

α -glucosidase is present in the brush border of the small intestine and transforming the oligosaccharides and disaccharides into monosaccharides, thus facilitates the glucose absorption process (Matsui *et al.*, 1996). Curtailing the action of α -glucosidase is considered to be an effective strategy to control postprandial blood glucose levels. Some synthetic compounds such as acarbose, miglitol, and voglibose possess α -glucosidase inhibitory activity. Ghamari *et al.*, (2013) studied the α -glucosidase inhibitory activity of aloin with and without β -casein and observed that aloin was a potent inhibitor. *In vitro* antidiabetic effect of curd supplemented with Aloe gel (AG) powder demonstrated α -glucosidase inhibitory activity. Curd with different concentrations of AG- 0.15AG, 0.20AG, and 0.25AG showed 30.7%, 40.1%, and 43.6% greater inhibition as compared to plain curd (Ramachandran and Srividya, 2014). α -amylase is an enzyme that breaks down the polysaccharides like starch into

maltose and glucose. Reducing the starch hydrolysis by inhibiting the pancreatic α -amylase enzyme is an effective approach to control type 2 DM (Pinto *et al.*, 2009) because it delays the hydrolysis and reduces the availability of glucose in the blood, resulting in the reduction of postprandial blood glucose level (Ali *et al.*, 2006). One of the disadvantages of this strategy is that excessive inhibition leads to bloating, flatulence, and other digestive issues (Kwon *et al.*, 2006). Raw and boiled sheep milk casein was hydrolyzed with pepsin, trypsin, and chymotrypsin and showed higher α -amylase inhibition. Chymotrypsin treatment showed maximum α -amylase inhibitory activity as compared to pepsin and trypsin. Peptides from boiled milk casein show reduced antidiabetic properties. Therefore, raw milk casein hydrolysates may be used as nutritional supplements for diabetic patients, as it causes significant inhibition of α -amylase (Jan *et al.*, 2016).

Antithrombotic activity

Blood clotting is a multifaceted physiological process regulated by a sequence of proteolytic reactions with extensive interactions (Davie *et al.*, 1991; Furie & Furie, 1988). During blood clotting, prothrombin gets converted to thrombin by proteolytic degradation (Bode, 2006). The principal purpose of thrombin is to transform soluble fibrinogen into insoluble fibrin and catalyze a chain of responses linked to blood coagulation. Thus the inhibitory activity of any bioactive agents against thrombin is supposed to be a pointer of antithrombotic activity. Food-derived constituents such as peptides may be suitable nominees for antithrombotic activity because of their least side effects. Food proteins can be perceived as antecedents of bioactive peptides (Shahidi & Zhong, 2008). Milk clotting proteins like chymosin and kappa casein, share similar mechanism like blood

clotting mechanism that involves thrombin and fibrinogen (Erdmann *et al.*, 2008). Peptides derived from κ -casein hydrolysates demonstrated antithrombotic activity preventing fibrinogen binding platelet (Schlimme and Meisel, 1995). These peptides are released during gastrointestinal digestion and get absorbed into the bloodstream, this mechanism supports the fact of *in vivo* antithrombotic activity. κ -casein hydrolysates

exhibited multifunctional roles like ACE inhibitory and antithrombotic. κ -casein fraction (106–116), illustrated anticoagulant activity, similar to the amino acid sequence to decapeptide from the fibrinogen Gamma-chain (fragment 400–411) (Tu *et al.*, 2017). Sheep caseins derived κ -caseinoglycopeptide (106-171) reduced thrombin and collagen-induced platelet aggregation in a dose dependent manner (Qian *et al.*, 1995).

Table.1 Commercial dairy products and ingredients with health claims based on bioactive peptides (Adapted from: Korhonen and Pihlanto, 2006)

Brand name	Bioactive peptide	Health claims	Country
Evolus	VPP, IPP from β -casein and κ -casein in calcium enriched fermented milk	Blood pressure reduction	Finland
Biozate	β -lactoglobulin from hydrolyzed whey protein	Blood pressure reduction	USA
Biopure GMP	κ -casein f(106-169)	Prevention of dental caries, influence the clotting of blood, protection against viruses and bacteria	USA
Prodiet F200/Lactium	α 1-casein (91-100) in flavoured milk and confectionery	Reduction of stress effects	France
Festivo	α 1-casein (1-9), α 1-casein (1-7), α 1-casein (1-6) from fermented low-fat hard cheese	No health claim as yet	Finland
Capolac	Casein-derived peptide	Helps mineral absorption	Sweden
Peptopro	Casein-derived peptide	Improves athletic performance and muscle recovery	Netherlands
Vivinal Alpha	Whey derived peptide	Aids relaxation and sleep	Netherlands

Table.2 Antioxidant peptides identified from casein

Protein	Treatment	Fragment	Reference
Casein	Pepsin	α s-1-casein f(144-149)	Suetsuna <i>et al.</i> , 2000
Casein	Trypsin	β -casein f(98-105) β -casein f(177-183) β -casein f(169-176) β -casein f(170-176)	Rival <i>et al.</i> , 2001
Casein	Papain	α s2-casein f(123-129) κ -casein f(61-65) κ -casein f(61-66)	Bezerra <i>et al.</i> , 2013
Casein	Pepsin	α s2-casein f(174-181) α s2-casein f(203-208) κ -casein f(28-30) κ -casein f(30-32)	Lopez-Exposito <i>et al.</i> , 2007
Casein	Fermentation with <i>Lactobacillus delbrueckii</i> subsp. <i>bulgaricus</i>	κ -casein f(96-106)	Kudoh <i>et al.</i> , 2001

Antimicrobial activity

Antimicrobial peptides are encrypted within the parent protein and are inactive, upon hydrolysis, it becomes biologically active and shows positive health impact. Anti-microbial peptides are very essential for newborn babies because these are vulnerable to a broad spectrum of pathogenic microorganisms mainly present at mucosal surfaces like small intestine and lungs. It is reported that anti-microbial effect of milk is accredited by both immunoglobulins and non-immunoglobulin proteins like lysozymes, lactoferrin, lactoperoxidase system and peptides (Jabbari *et al.*, 2012). Cationic antimicrobial peptides interact with the anionic component of teichoic acid and lipopolysaccharides (LPS) respectively, in gram-positive and gram-negative bacteria (Hancock, 1997) resulting in neutralization of the charge on the microbial surface, leads to disruption of a cellular

envelope, and increases the permeability of cell (Oren, 1999). Sometimes, antimicrobial peptides lead to the formation of pores in the cell envelope leading to leakage of cell contents and ultimately resulting in cell death (He *et al.*, 1996). Antimicrobial peptides damage the microbial cell membrane by electrostatic interaction, inhibiting protein, DNA, and RNA synthesis, or interacting with intracellular targets (Bahar and Ren, 2013). Lopez-Exposito *et al.*, (2007) reported that peptic hydrolysate of ovine α s2-casein and bovine κ -casein showed antimicrobial effect against *Escherichia coli* and *Listeria innocua*. Recio and Visser (1999) studied the antimicrobial activity of two fractions f(164-179) and f(183-207) isolated from peptic hydrolysate of bovine α s2-casein. These peptides exhibited minimum inhibitory concentration between 8-99 μ M. Antimicrobial activity is determined, by the presence and position of positive charge,

hydrophobic, and the basic amino acid residues in peptides. Bougherra *et al.*, (2017) reported a peptide from α 2-casein (SSSEESII) that showed antimicrobial activity against *Listeria innocua*, *Micrococcus luteus*, *Escherichia coli*, and *Salmonella enteritidis* due to the presence of hydrophobic amino acid in sequence, by using agar diffusion and micro-dilution method. Kumar *et al.*, (2016) investigated the antimicrobial activity of camel casein hydrolyzed with alcalase, α -chymotrypsin, and pepsin using a well diffusion method against *Listeria monocytogenes*, *Staphylococcus aureus*, *Escherichia coli*, and *Bacillus cereus*. Benkerroum *et al.*, (2004) reported antimicrobial activity of camel milk against pathogenic strains of *Escherichia coli*, *Listeria monocytogenes*, and *Bacillus cereus*. Pepsin hydrolysis of ovine α S2-CN resulted in production of four anti-microbial peptide fragments like f165-170, f165-181, f184-208 and f203-208. These fragments are potent anti-bacterial against Gram negative bacteria. Furthermore, among all these fragment f165-181 showed highest anti-microbial activity while fragment f203-208 also showed anti-oxidant and anti-hypertensive activity.

In conclusion, casein is a rich source of bioactive peptides that exhibits a multifunctional role. These bioactive peptides get active only after releasing from native protein. So more research is required in the direction of how these peptides can retain their sequence during gastrointestinal digestion, enter into the bloodstream, and act on the target site. There is an urgent need to focus on developing novel facilities including advanced proteomics approaches, recombinant enzyme technologies and microbial fermentation, to study the various impacts of bioactive peptides on expression of genes and also to optimize the nutritional and health effects of these compounds.

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