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Food Production, Processing and Nutrition (2025) 7:6
https://doi.org/10.1186/s43014-024-00280-2

Food Production, Processing and Nutrition

REVIEW

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Milk-derived bioactive peptides

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Abstract

Bioactive peptides are the functional concept that refer to fragments of proteins that provide both functional and nutritional benefits as a functional component. Milk has been widely exploited as a source of diverse bioactive peptides. Bioactivity of peptides depends on amino acid composition and sequence specific to bioactivity. The production of bioactive peptides can be carried out through classical and bioinformatics approaches. In the classical approach, enzymatic hydrolysis, simulated digestion and hydrolysis by microbial enzymes are included. In the bioinformatics approach, databases, QSAR modeling, molecular docking and design of experiments (DoE) with response surface methodology are employed for identification and characterization of bioactive peptides from milk proteins. Milk-derived peptides have been identified to be antihypertensive, anti-diabetic, cytomodulatory, antioxidative, antimicrobial, and mineral binding peptides with wide applications as nutraceuticals in commercial products and food preservatives in food industry. Hence, this review has attempted to provide insight into the approaches and applications of milk- derived bioactive peptides with nutraceuticals properties in the development of functional foods.

Keywords Bioactive Peptides, Milk Protein, Enzymatic Hydrolysis, Simulated Digestion, Microbial fermentation, Bioinformatics Approach, Applications of Peptides

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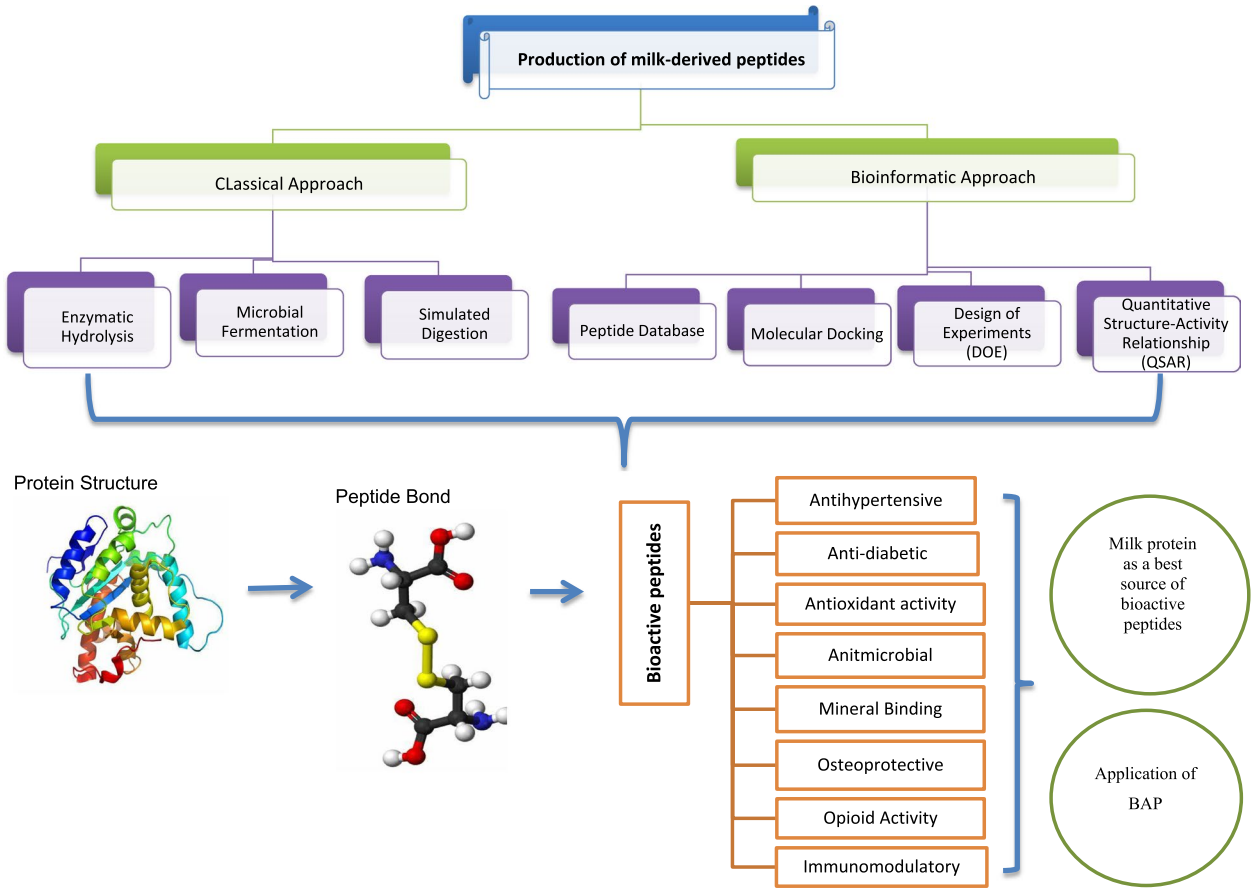
Graphical Abstract



Introduction

Bioactive peptides are functional aspect of proteins that denotes the attainment of both health and nutritional benefits rather than the conventional utility of proteins for basic nutritional functions alone (Bhandari et al., 2020). Bioactive peptides (BAP) are fragments of proteins with the appropriate peptide sequence that exhibit bioactivities to enhance human health (Clare & Swaisgood, 2000). In the production of bioactive peptides, the structure of the proteins at four levels are broken down so that even interiorly encrypted peptides are released to the surface in such a way that all the peptides will be accessible to exhibit bioactivity than the bioactivity from a structurally whole protein (Abdel-Hamid et al., 2017; Daliri et al., 2017a, 2017b). Hence, the exploration of bioactive peptides from various food proteins is the recent thrust area of the functional food science from a public health perspective.

Among food proteins, milk proteins have been widely explored for the production of bioactive peptides. Milk proteins contain 80 percent of casein, including αs_1-casein, β-casein and κ-casein and 20 per cent of whey proteins including α-lactalbumin, β-lactoglobulin and lactoferrins (Davoodi et al., 2016). The production of peptides could be carried out through classical approaches and bioinformatics approaches (Abdel-Hamid et al., 2017). Classical approach refers to hydrolysis of proteins by proteolytic enzymes, whereas bioinformatics approach refers to database based method employed in the optimization of hydrolytic parameters and characterization of peptides with bioactivities (Aguilar-Toalá et al., 2017; Daliri et al., 2017a, 2017b; García-Nebot et al., 2010). Milk proteins have been investigated in the production of peptides with bioactivities such as antihypertensive, anti-diabetic, cytomodulatory, antioxidant, antimicrobial, and mineral binding effects (Daliri et al., 2017a, 2017b), which

could counteract the recent high prevalence of chronic diseases such as hypertension, diabetes mellitus, oxidative stress and minerals deficiencies, deteriorating human health (Bhandari et al., 2020; Chaudhury et al., 2017). For example, the pepsin hydrolyzed peptide YFYPEL from bovine casein was proven to exhibit antioxidant activity (Suetsuna et al., 2000). Similarly, chymosin digested peptide Casecidin α_{s1} has been reported for antimicrobial activity (Lahov & Regelson, 1996). In the emerging research on BAPfrom animals, plants, and microbes, the bioactivity has been linked to the ability to inhibit protein–protein interaction because of their small size and specificity (Clare & Swaisgood, 2000; Daliri et al., 2017a, 2017b). Since there is a need for more exploration of the production and mechanism of action of BAP, this review attempts to explore the approaches and applications of peptides with various activities from milk protein.

Production of bioactive peptides

Milk bioactive peptides can be derived through classical and bioinformatics approaches as illustrated in Fig. 1.

Classical approach

Table 1 depicts the hydrolytic factors for the optimization and pretreatments specific to enzymes in the production of milk-derived bioactive peptides from classical approaches.

1(a) Enzymatic hydrolysis

BAP are obtained through the enzymatic hydrolysis of milk protein at optimum pH, temperature, and substrate ratio, specific to enzymes. Gastrointestinal enzymes, namely pepsin and trypsin have been investigated for the production of peptides. Other enzymes such as protease, papain, pancreatin, chymotrypsin and thermolysin, have also been studied in the hydrolysis of proteins into peptides. (Bhandari et al., 2020; Korhonen & Pihlanto, 2003; Mohanty et al., 2016). Enzymatic hydrolysis is generally preferred due to its precision, shorter time of reaction, and adaptability. Hydrolysates containing short-chain peptide sequences can be produced by using a single or mixture of enzymes with the addition either simultaneously or sequentially (Korhonen & Pihlanto, 2006).

The selection and use of proteases affect the properties and outcome of the milk proteins. Trypsin and chymotrypsin are used owing to their specificity for cleaving peptide bonds. Trypsin is specific for lysine and arginine residues, while chymotrypsin is specific for aromatic amino acid chains like tryptophan, phenylalanine, and tyrosine. Pepsin is used for acidic hydrolysis as it cleaves peptide bonds at the carboxyl side of aromatic amino

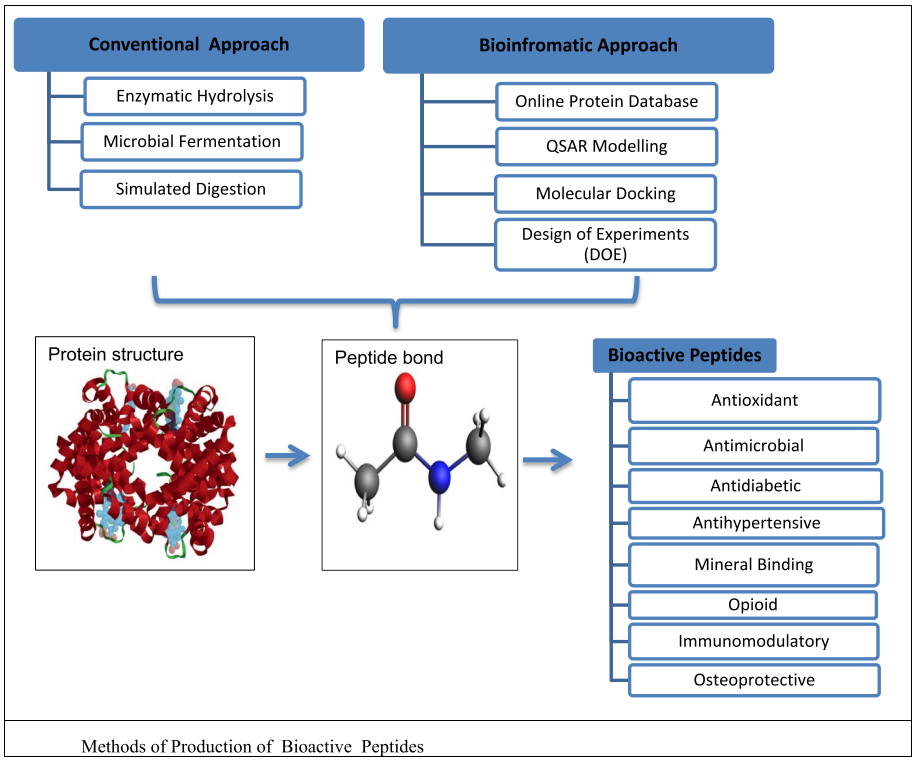


Fig. 1 Methods of Production of Bioactive Peptides

Table 1 Hydrolytic factors and pre-treatments for proteases in the production of milk bioactive peptides

Production	Proteases	Optimization	Pre-treatments
Enzymatic Hydrolysis	• Trypsin • Chymotrypsin • Pepsin • Protease mixtures	• Enzyme or protease concentration • Substrate concentration • Reaction pH • Temperature • Reaction time	• Heating of milk to denature the milk proteins • Adjustments of milk pH, • Ultrafiltration to concentrate the milk proteins • Pre-incubation of protease enzyme before adding to milk proteins,
Microbial Fermentation	• Alcalase • Protamex • Neutrase	• Selection of the microbial protease • Optimal pH, temperature, and time • Substrate concentration • Control of microbial growth	• Heat Treatment • pH Adjustment • Enzyme Activation • Homogenization
Simulated Digestion	• Pepsin • Trypsin • Chymotrypsin	• Selection of preotease • Optimization of Digestion Conditions: • Simulation of Digestive Environments	• Heat Denaturation • Adjustment of pH • Enzyme Activation

acids. Also, a mixture of proteases is sometimes used to achieve a broader spectrum of hydrolysis. Factors such as enzyme or protease concentration, substrate concentration, pH, temperature, and time are adjusted to optimize hydrolysis. Protein hydrolysis can be controlled by adjusting the concentration of protease. Although a higher concentration facilitates increased hydrolysis, it is limited due to enzyme cost and side effects. Adjustment of pH can maximize the protease activity; similarly, with an increase in temperature, the enzyme activity is increased to a certain point. More extensive hydrolysis is usually achieved with longer reaction times, but beyond a certain point, the benefits of longer reaction times diminish. Pretreatments are done to enhance the efficiency and effectiveness of the hydrolysis by altering the milk protein properties to make them more accessible for enzymatic breakdown. Several methods, such as heating of milk to denature the milk proteins, adjustments of milk pH, ultrafiltration to concentrate the milk proteins, and pre-incubation of protease enzyme before adding to milk proteins, have been employed for the pretreatments of milk protein hydrolysis to increase the rate of hydrolysis potentially (Bhandari et al., 2020; Mohanty et al., 2016).

1(b) Microbial fermentation

Microbial fermentation refers to protein hydrolysis by microbial protease from microorganisms like bacteria and fungi during fermentation. Microbial protease can efficiently break down milk proteins into peptides and amino acids. While optimizing Protein hydrolysis in microbial fermentation, factors to be considered include: i) selection of the microbial protease based on the efficiency and specificity of cleaving peptide bond as each protease has varying substrate specificity, optimum pH range, and temperature. Commonly used proteases include Alcalase, protamex, and Neutrase; ii) optimal pH, temperature, and time as discussed in the enzymatic hydrolysis, optimal conditions vary based on the type of protease and microbial strain used; iii) maintenance of appropriate substrate concentration will ensure effective utilization of microbial protease and maximizing the degree of protein hydrolysis; iv) control of microbial growth by maintaining the nutrient supply, oxygen supply, and the fermentation conditions is necessary to avert excess protease production or undesirable by-products (Daliri et al., 2017a, 2017b; Korhonen & Pihlanto, 2006).

Microbial fermentation refers to hydrolysis of protein by enzymes in microorganisms during fermentation. Lactic acid bacteria (LAB) such as *Lactococcus lactis* and *Lactobacillus helveticus* were studied to contain peptidases such as endo-peptidase, di-peptidase, tripeptidase, and amino-peptidase in fermented milk and milk products (Anusha & Bindhu, 2016; Mohanty et al., 2016; Widyastuti et al., 2014) and the release of types of BAPsuch as ACE- inhibitory (Gobbetti et al., 2004), antioxidative (Korhonen & Pihlanto, 2006), and antimicrobial and immunomodulatory (Mohanty et al., 2016) with respect to the types of microbial proteases used in fermentation of proteins. *Lactobacillus sp. and Lactococcus sp.* were revealed to hydrolyze more than 40% of α1 and β casein peptide bonds (Anusha & Bindhu, 2016; Choi et al., 2012). Similarly, *Lactobacillus helveticus* strains were found to release antihypertensive peptides, wherein VPP and IPP (ACE-inhibitory tripeptides) were the best known and been further studied in rat and human studies (Aihara et al., 2005; Mann et al., 2017; Mizushima et al., 2004; Seppo et al., 2003).

1(c) Simulated digestion

Strategies for optimizing protein hydrolysis in simulated digestion include: i) selection of proteases based on their specificity ensures efficient protein digestion. Pepsin can

initiate digestion in acidic environments like the stomach, then trypsin and chymotrypsin continue peptide hydrolysis in alkaline conditions such as in the small intestine; ii) optimal pH, temperature, and digestion time mimicking the physiological conditions enhance the protease activity and facilitates efficient protein hydrolysis such as in the maintenance of acidic condition for pepsin activity in the stomach followed by the alkaline condition for trypsin and chymotrypsin activity such as in the small intestine (Mohanty et al., 2016).

Bioactive peptides are obtained through in vitro digestion of proteins in the presence of pepsin, trypsin and chymotrypsin at their respective required pH, simulating the gastrointestinal environment of human beings (Korhonen & Pihlanto, 2006; Mohanty et al., 2016). BAP, such as anti-hypertensive, antibacterial, opioid and immunomodulatory were obtained during the gastrointestinal digestion of casein and /or whey proteins (FitzGerald et al., 2004; Gobbetti et al., 2002, 2004; Meisel & FitzGerald, 2003; Mohanty et al., 2016; Yamamoto et al., 2003). Pepsin and trypsin, in combination with proteolytic enzymes like alcalase and thermolysin stimulate gastrointestinal digestion to release BAPs such as CCP (McDonagh & FitzGerald, 1998), antibacterial (Mohanty et al., 2014), antioxidative (Suetsuna et al., 2000), ACE -inhibitory (Vermeirssen et al., 2004) and opioid peptides (Korhonen & Pihlanto, 2006).

Although enzymatic hydrolysis and simulated digestion are employed for protein hydrolysis, their purpose and approach differ. Simulated digestion mimics the natural gastrointestinal process by exposing food proteins to digestive enzymes and adjusting pH conditions. It aims to understand how food proteins are broken down, release bioactive peptides, and evaluate their physiological effects. Enzymatic hydrolysis uses specific enzymes to break down food proteins into smaller peptides and amino acids, optimizing conditions like enzyme concentration, pH, temperature, and reaction time. These peptides can have desired functionalities like antioxidant, antihypertensive, antimicrobial, or immunomodulatory activities, making them applicable in food, pharmaceuticals, and nutraceuticals. Despite the benefits of simulated digestion as a natural way for the production of bioactive peptides, enzymatic hydrolysis is still commonly used for several reasons: i) control of conditions: enzymatic hydrolysis ensures consistent and reproducible results by precise control of conditions during bioactive peptide production; ii) yield and efficiency: compared to simulated digestion, it gives higher yield in a shorter time. Bioactive peptides with high purity and activity can be produced through enzyme specific conditions and parameters; iii) large-scale production: bioactive peptides can be produced in large quantities through enzymatic hydrolysis for commercial applications; iv) flexibility of targeted design: Using specific enzymes and substrates, enzymatic hydrolysis can be used to modify and design bioactive peptides, this flexibility enables researchers to customize the characteristics and functions of bioactive peptides for specific applications (Anusha & Bindhu, 2016; Daliri et al., 2017a, 2017b; Korhonen & Pihlanto, 2006; Mann et al., 2017).

Bioinformatics approach

In the classical approach, choice of substrate and enzyme, screening of bioactive potential, fractionation of active peptide and peptide sequence identification followed by in vivo validation is time consuming and non-targeted. Bioinformatics or in silico approach is database based and provides faster results, cost effective due to a lower consumption of chemical-reagents in conventional approach (FitzGerald et al., 2020). Several in silico methods are now employed in the identification of milk-derived bioactive peptide:

1. Website based databases can be used for the selection of appropriate substrate for bioactive peptide formation by parameters like; rate of occurrence of BAP, frequency of peptides released and the effectiveness of the protein substrate (Han et al., 2019).
2. Elucidation of structure- activity relationship among BAP and biological activities by Quantitative Structure- Activity Relationship (QSAR) modeling (Nongonierma et al., 2018).
3. Application of Molecular Docking to virtually screen peptide sequencing for the determination of interactions between peptides and enzyme active site (FitzGerald et al., 2020).
4. Optimization of enzymatic and fermented hydrolysates by Design of Experiments (DOE) coupled with Response Surface Methodology (Nongonierma et al., 2018).

2(a) Website based databases

Website based Databases can be used for processing proteolytic enzyme specific hydrolysates. Uniprot (https://www.uniprot.org/) and NCBI (The National Center for Biotechnology Information) (https://www.ncbi.nlm.nih.gov/) are used for screening primary milk proteins, peptide sequences from different milk are available in BIOPEP-UWM (University of Warmia and Mazury) (https://biochemia.uwm.edu.pl/) and PeptideLocator (https://bio.tools/). Publicly available online databases include substrate-specific databases like the Milk Bioactive Peptide Database (MBDB) https://mbpdb.nws.oregonstate.edu/ and bioactivity specific like the Collection of Antimicrobial Peptides

(CAMP) http://www.camp.bicnirrh.res.in/ and the Antimicrobial Peptide Database (APD) https://aps.unmc.edu/ (Nielsen et al., 2017).

Furthermore, the BIOPEP-UWM database includes enzyme cutter tools such as Peptide Cutter. 'Enzyme(s) activity' can predict the peptides to be produced on hydrolysis of certain dietary protein by specific hydrolytic enzymes (Lin et al., 2018).

In silico approaches offer advantages for the release of specific BAPs from the substrate of milk protein, but limitations can be found in its widespread usage. Alternative strategies are employed to rank protein substrates based on their potential as sources of BAPs, but some potency indices may overestimate bioactive potency by not considering overlapping sequences within specific protein regions (Nongonierma & FitzGerald, 2017). Enzyme cutters, commonly used for predicting peptide release, have limitations as they are bound to a limited number of hydrolytic activities and may not include enzymes with specificities required for releasing certain peptide sequences. Moreover, enzyme cutters do not overlook functional variables like pH, substrate properties, incubation conditions, and ionic effects on enzyme activity (Cheison & Kulozik, 2017). These limitations should be taken into account for the selection of substrates and enzymes for the production of targeted BAPs.

2(b) QSAR modeling for peptide identification

QSAR is a computational method employed to study the association of structural and physicochemical properties of chemical compounds and their biological activity. Recently, it has been employed for BAP identification and analysis of BAP (FitzGerald et al., 2020). QSAR modeling for peptides involves the following steps:

1. Data collection and dataset selection: A comprehensive data of peptides is compiled from literature and databases such as BIOPEP-UWM, UniProt, etc., which provides information on peptide structures, amino acid sequences, hydrophobicity and bioactivity. Based on the biological structure and features, a dataset is selected for further analysis; The Minimum Analogue Peptide Set (MAPS) approach may be employed, containing a minimal number of analogue peptides with diverse structural features (FitzGerald et al., 2020).
2. Descriptor Selection: Peptides are further classified depending on the structural features of the molecular descriptors (Lin et al., 2018). These descriptors can range from 1 to 4D, representing primary sequence, secondary structure, tertiary structure, and multiple conformations. Commonly used descriptors include hydrophilicity, size, charge, electronic properties, and van der Waals interactions (Nongonierma & FitzGerald, 2016).
3. QSAR Modeling: The dataset is subjected to QSAR Modeling that uses mathematical equations and computational simulations such as artificial neural networks (ANN), principal component analysis (PCA), multiple linear regression (MLR) and partial least square regression (PLSR). These models are trained using known peptide sequences and validated using excluded peptides (Fukunishi et al., 2017).
4. Validation and Confirmation: QSAR model accuracy is evaluated by comparing predicted bioactivities with experimental results. Peptides with the highest predicted bioactivities are synthesized and evaluated in vivo or in vitro to validate the predictions (Du et al., 2023).

2(c) Molecular docking for bioactive peptide analysis

Molecular docking is a technique employed to analyze the interaction and range of molecules with different biological activity, it can be used for the identification of interaction between specific peptides and enzymes. By using advanced techniques like fourier transform infrared (FTIR), nuclear magnetic resonance (NMR) spectroscopy, X-ray crystallography and circular dichroism (CD) along with molecular docking can be employed to understand the molecular structure (FitzGerald et al., 2020).

Molecular docking software like AutoDock, GRIDock, ICM DOT, GOLD, Tag-Dockare and HEX are used for the prediction of the mode of their interaction and binding energy between the BAP and their targeted enzymes (Pagadala et al., 2017).

Mass spectrometry is employed for determining the amino acid composition of peptides through the analysis of the mass-to-charge ratio (m/z) of the peptide ions. Electrospray ionization (ESI) or matrix-assisted laser desorption/ionization (MALDI) are commonly used for the peptide ionization which are then introduced into mass spectrometer which separates them based on their m/z values. Based on the mass of the peptide ions, the mass spectrometer develops a mass spectrum, representing the distribution of ions based on their mass. The mass spectrum is then compared with a known database of amino acids to determine the amino acid composition of the peptide (Zhang et al., 2010).

2(d) Optimization of BAP through Design of Experiments (DoE) and Response Surface Methodology (RSM)

Multifactorial Design of Experiments is applied to analyze the effect of parameters on the outcomes to optimize a specific process (FitzGerald et al., 2020).

The DOE process involves the elucidation of the research scope and objectives and the targeted output that is to be

optimized such as mineral binding activity, antimicrobial activity; the selection of factors influencing the output such as incubation time, pH and temperature and designing the experiments accordingly (Nongonierma et al., 2018). DOE allows for efficient data collection by reducing the number of experiments while providing the best possible outcome. Experiments predicted by the DOE are performed and the actual data is analyzed using a mathematical model like Multiple Linear Regression (MLR) and Response Surface Methodology (RSM) analysis. The predicted conditions are then validated through in vitro and in vivo studies (Nongonierma & FitzGerald, 2017).

Classification of bioactive peptides

3(a) Antihypertensive peptides

Hypertension can substantially increase the risk of multiple disorders including heart and renal diseases as well as stroke (Lee & Hur, 2017; Zaky et al., 2022). In hypertension, the systolic and diastolic blood pressure are constantly at a value of ≥ 140 mg Hg and ≥ 90 mmHg, respectively. With the increase in age, blood pressure also increases. Therefore, persons over 60 years old having blood pressure of 150/90 mmHg are recommended for treatment (Daliri et al., 2017a, 2017b). Antihypertensive peptides have the potential to regulate the renin-angiotensin system in order to regulate blood pressure in human body. With the use of γ- Zein, α-lactalbumin, β-lactaglobulin-like enzymes, the ACE inhibitory peptides isolated from sunflower, barley, garlic, etc. have been known to exhibit a similar effect (Bhandari et al., 2020).

Renin-angiotensin system (RAS) and kinin-nitric oxide system (NO) physiologically control the blood pressure, among which, scientific interest in the RAS has been increasing (Bhandari et al., 2020; Daliri et al., 2017a, 2017b). Angiotensin-I converting enzyme (ACE), an essential component of RAS catalyzes the transition of angiotensin-I to angiotensin-II resulting in the vasoconstriction. ACE inhibitory peptides inhibit the ACE conversion thereby causing the blood vessels to dilate and hence control hypersensitivity (Bhandari et al., 2020).

VPP and IPP are tripeptides isolated from L. helveticus and Saccharomyces cerevisiae fermented sour milk inhibited ACE. According to According to Tuomilehto et al., (2004), Tripeptides (VPP and IPP) derived from milk lowered blood pressure effectively in patients with hypertension. (Silva & Malcata, 2005) reported peptides from αs_1-casein (RPK and RPKHPIKH) and β-casein (VPKKVK, and YQEPVLGP and YQEP) derived from ovine milk, manufactured with proteases from *Cynara cardunculus* exhibited ACE inhibitory activity. In most cases, human and bovine caseins are used to isolate these peptides. Beta Lactoglobulin and α-lactalbumin, α-lactorphin and β-lactorphin, have been identified to exhibit ACE inhibitory activity (Maruyama et al., 1987; Mohanty et al., 2016). Enzymatic digestion of the sour milk proteins such as αS1 and β-CN produced casein-derived ACE inhibitory peptides such as β-casein and κ-casein (Mohanty et al., 2016).

Studies show that consumption of this fermented sour milk decreased the blood pressure moderately. VPP and IPP isolated from casein are known to significantly reduce high blood pressure in humans. It has been demonstrated that a single oral administration of IPP and VPP over long period prevented the development of hypertension and significantly reduced blood pressure in rats. (Nakamura et al., 2011) reported the tripeptides, VPP and IPP, form fermented β-casein fermented with Saccharomyces cerevisiae and Lactobacillus helveticus CP790. A potential ACE inhibitory peptide DPYKLRP derived from lactoferrin fermentation with Kluyveromyces marxianus demonstrated a reduction in systolic blood pressure (10 mg/kg body weight) in spontaneous hypertensive rats. In a double-blind parallel group intervention study conducted, 89 hypertensive subjects administered with 5 mg fermented milk containing IPP and VPP for 12 weeks and an increased dose of 50 mg/day for another 12 weeks reported a decrease in arterial stiffness resulting in decrease in blood pressure (Davoodi et al., 2016; Mohanty et al., 2016).

3(b) Antimicrobial peptides

Antimicrobial peptide (AMP) inhibits the proliferation of pathogenic or infectious microorganisms without affecting the host. The primary target of AMPs is the microbial cell wall comprised of lipoproteins that are responsible for their antimicrobial activity. Antimicrobial peptides mostly comprise of α-helical structure that is cationic and amphipathic and some hydrophobic helical peptides. The cationic portion of the peptides interacts with negatively charged phospholipids causing the formation of pores in the bacterial cell membrane culminating in the disruption of the ionic balance. This imbalance in the cell membrane leads to cell lysis. Furthermore, these peptides can also bind and integrate into the cell membrane surface (Corrêa et al., 2023).

Milk is a rich source of antimicrobial peptides having synergistic activity between the defense proteins (Lf, lysozyme and lacto peroxidase) and the peptides that are naturally present (Clare et al., 2003; Mann et al., 2017). Milk peptides can inhibit Gram + ve and Gram-ve pathogens such as *Aeromonas hydrophila* ATCC7966, *Bacillus cereus* atcc10702, *Escherichia coli* mtcc83, *Salmonella typhi* MTCC3216, *Salmonella typhinurium* SB300, *Salmonella. enteritidis* 125,109 and S*taphylococcus aureus* MTCC96 (Mann et al., 2017; Mohanty et al., 2014, 2016). Lactoferricins derived from bovine and lactoferrin (Lf)

from human are the most studied antimicrobial peptides (Kitts & Weiler, 2003; Mann et al., 2017). Peptides from β-lactoglobulin (β-Lg) and α-lactalbumin(α–La) revealed inhibitory effect against Gram-positive bacteria (Brandelli et al., 2015). Derivatives of β-Lg (VAGTWY) exhibited antimicrobial activity against Gram-positive bacteria while α-La derivative (EQLTK) exhibited a weaker antimicrobial activity against Gram-negative bacteria. Caseicidin peptide derived from chymosin digested casein inhibited the growth of S*taphylococcus spp., Sarcina spp., Streptococcus pyogenes* and *Bacillus subtilis* (Lahov & Regelson, 1996; Mohanty et al., 2016). Peptide fragments isolated from casein f183-207 and f164-179 exhibited antimicrobial activity and casein displayed antimicrobial activity against *Escherichia coli and Staphylococcus Carnosus* (Mohanty et al., 2016; Recio & Visser, 1999; Zucht et al., 1995). A lactoferrin fragment, lactoferrampin exhibited antimicrobial activity against *Escherichia coli, Bacillus Subtilis, Streptococcus mutans and Pseudomonas aeruginosa* (Marieke et al., 2005). Isracidine, a recently recognized peptide isolated by chymosin digestion of bovine αs_2- CN, displayed inhibitory activity against *Streptococcus pyogenes, Staphylococcus aureus*and *Listeria monocytogenes* (Lahov & Regelson, 1996).

Recent studies suggest that Caseinomacropeptide (CMP) and Glycomacropeptide (GMP) isolated from casein by chymosin hydrolysis may hinder the growth of *Streptococcus mutans* and *Escherichia coli* while GMP assists in gut micro flora regulation (Lopez-Fandino & Manso-Silvan, 2004). Isfracidin and lactoferrin B exhibited inhibition against *Candida albicans* (Mohanty et al., 2016). Derivatives of Lactoferrin exhibited in vitro antibacterial activity against pathogens such as *Helicobacter pylori, Candida Albicans, Clostridium perfrinfens, Listeria Monocytogenes, Vibrio cholera, Salmonella enteritidis* and anti-viral activity against *HIV-1, Poliovirus, rotavirus, and Hepatitis G, B and C virus.* Lactoferrin B coupled with Azole antifungal agents inhibited the growth of *Candida albicans* and also against several filamentous fungi and dermatophytes (Clare & Swaisgood, 2000).

3(c) Anti-diabetic peptides

Diabetes is a chronic disease that occurs either when the pancreas does not produce enough insulin or when the body cannot effectively use the insulin it produced". Hormone insulin regulates blood sugar in the body (Widyastuti et al., 2014). It is characterized by hyperglycemia which alters the carbohydrate, protein, and fat metabolism due to a defect in the action or secretion of insulin or both (Daliri et al., 2017a, 2017b).It is divided into Type-I and Type-II categories. Type-I diabetes, also known as insulin-dependent or juvenile diabetes, develops when the pancreas is unable to produce enough insulin, necessitating daily insulin injection. Type II diabetes (non-insulin-dependent) is brought on by an imbalance between the release of insulin and the uptake of blood sugar (Chaudhury et al., 2017).

At present, synthetic antidiabetic drugs are known to induce effects including hypoglycemia, gastrointestinal problem and increased risk of pancreatitis (Meier & Nauck, 2014). Hence, food derived anti-diabetic peptides are essential and a promising prospect as an alternative without side effects (Daliri et al., 2017a, 2017b).

Studies have shown the relation between the milk-derived BAP and serum glucose regulatory properties (Lacroix & Li-Chan, 2014a, 2014b; Mann et al., 2017). These mechanisms include incretin secretagogue action, insulinotropic response and metabolic enzymes like dipeptidyl peptidase IV (DPP-IV), amylase and α -glucosidase that are associated with the serum glucose regulation in humans (Lacroix & Li-Chan, 2014a). A clear explanation for milk protein hydrolysates insulinotropic activity in humans is yet to be found. There are speculations that a combination of peptides of branch-chain amino acids (BCAA) and peptides isolated from milk components make up the active components (Mann et al., 2017; Morifuji et al., 2010). Various studies and literature states that milk -derived components having insulinotropic properties are associated with whey derived peptides (Mann et al., 2017; Oseguera-Toledo et al., 2014). On the contrary, both whey protein hydrolysates (WPH) and casein hydrolysates (CNHs) have exhibited insulinotropic properties in humans. Several of the peptide hydrolysates derived from milk evaluated are proprietary products. However, a recent study on humans has been conducted using WPI Hydrolysates with flavourzyme (Goudarzi & Madadlou, 2013).

Geerts et al., (2011) indicated that hydrolysates of milk protein with carbohydrates in the presence or absence of an amino acid, Leu, which is known to have insulinotropic properties. In addition to their insulinotropic activity (Akhavan et al., 2010; Geerts et al., 2011), WPH may increase plasma concentration of free amino acids (Leu, Phe, Arg, Ile, Thr, Tyr, Val, Ala and Lys). Branched chain amino acids (BCAA) contain dipeptides such as Leu-Leu, Ile-Leu and Val-Leu and also cyclic dipeptides (Morifuji et al., 2010). On the other hand, Power, Hallinhan & Jakeman (2009) contradict the analog between the consumption of insulinotropic plasma BCAA and whey protein hydrolysates (Power et al., 2009). After the consumption of 10 g of commercial whey protein hydrolysates, there was no reduction in glucose level post-meal inspite of increase in insulin secretion (Akhavan et al., 2010). Lacroix & Li- chan (2014a) have identified two fragments of β-lg such as LKPTPEGDL and LKPTPEGDLEIL isolated from pepsin treated bovine whey protein, having potent DPP-IV inhibition activity.

DPP-IV inhibits insulin secretion from pancreatic beta cell by inhibiting the activity of glucagon- like peptide (GLP), glucagon-like peptide-1 (GLP-1) and incretin hormones, leading to hyperglycemia. However, DPP-IV inhibitors control the blood sugar level by blocking the DPP-IV action. Moreover, inhibiting the enzymes, α-glucosidase and α-amylase, which are involved in the digestion of complex carbohydrate is known to effectively lower blood sugar levels. α-amylase interacts with hydrophobic amino acids like Leu, Met and Pro at the terminal of the peptide sequence, inhibiting α-glucosidase Pro, Phe, Leu and Met and DPP-IV inhibited by both hydrophobic (Ala, Met, Gly, Leu, Pro and Trp) and hydrophilic amino acids (Arg, His, Gln, and Ser). Novel peptides namely, ELKDLKGY, ILDKVGINY, KILDK, LKPTPEGDL, LKPTPEGDLEIL, LDQWLCEKL, RNAVPITPTLNR, TKVIPYVRYL, FALPQYLK and YLGYLEQLLR with potential inhibition against α-glucosidase, α-amylase and DPP-IV have been identified (Koirala et al., 2023).

DPP-IV inhibitory peptide isolated from bovine whey α-lactalbumin (f 115–123), LDQWLCEKL, by trypsin hydrolysis exhibited a potency for the prevention of type 2 diabetes. ELKDLKGY and ILDKVGINY also isolated from bovine α-lactalbumin by alcalase hydrolysis exhibited inhibition against DPP-IV. α-glucosidase inhibitory peptides isolated from *Dregea sinensis* hydrolyzed milk have been identified. Peptides from αS1 and αS2-casein demonstrated potential α-glucosidase inhibition activity by binding to α-glucosidase active sites like Arg387, Arg428, Arg727, Arg799, Arg801 and Trp710 through hydrogen bonds, preventing formation and α-glucosidase glycosylation with the substrate. Antidiabetic peptides, ACGP, PLMLP, HLPGRG, MFE, QNVLPLH and GPAHCLL isolated from bovine casein by hydrolysis with alcalase and pronase demonstrated inhibition against α-glucosidase, α-amylase and DPP-IV (Koirala et al., 2023).

3(d) Osteoprotective peptides

Osteoprotective peptides help in the protection of bones in our body. Calcium intake is required for the prevention and cure of osteoporosis. Bone metabolism is increased by whey components in milk. These components are called milk-based proteins promoting mineral density, bone formation, fracture strength, ostocalcium content, but suppress bone reabsorption, pitting and urinary deoxypyridindine levels in animal and human models (Toba et al., 2000). Lactoferrin is identified as having functional role in bone healing, repair and growth and plays a therapeutic role in bone disorders namely osteoporosis (Bouhallab & Bouglé, 2004; Naot et al., 2005). A diet with adequate amounts of casein and whey protein is effective against osteoporosis (Bhandari et al., 2020; Bouhallab & Bouglé, 2004). A study conducted on ovariectomized (OVR) osteoporotic rat model showed that by suppressing inflammation levels and improving bone formation markers, whey derived antioxidative peptide (YVEEL) demonstrated greater osteoprotective efficacy than ACE inhibitory peptide (Pandey et al., 2018). Another study evidenced that buffalo casein derived peptide (NAVPITPTL) showed osteoprotective activity in ovariectomized rats (Reddi et al., 2019).

3(e) Antioxidantative peptides

Researchers have investigated antioxidant peptides and mechanism of action and also identified several factors that contribute to antioxidant activity. These peptides are believed to chelate transition metal ions, inhibit lipid peroxidation and scavenge free radicals. However, the precise mechanism underlying their antioxidant activity remains incompletely understood. It has been observed that antioxidative peptides can enhance the intracellular conversion of cysteine into glutathione, a potent antioxidant which protects cells from oxidative damage. The antioxidant properties of peptides are affected by their structure, composition and hydrophobicity. Additionally, the SH group in cysteine plays a significant role in antioxidant action by directly interacting with radicals (Mann et al., 2017).

Amino acids like glutamic acid, cysteine, methionine, proline, phenylalanine, histidine and tyrosine are known to contribute to antioxidative activity. Depending on the type of amino acid, peptides contribute to antioxidative activity (Zaky et al., 2022). Aromatic amino acids like tyrosine and phenylalanine bind pro-oxidant metal ions or scavenge OH radical to exhibit antioxidant activity (Yousr & Howell, 2015). Generally, antioxidants peptides contain 4–16 amino acids residue with 0.4–2 kDA molecular mass. The molecular size of the peptides can have an increased effect on the antioxidants pathway activity in vivo by influencing the pathways to target site and in the gastrointestinal digestion (Toba et al., 2000). Peptides containing cysteine, histidine and tryptophan exhibit antioxidant activity through single electron transfer while peptides like tyrosine exhibit by hydrogen atom transfer (Esfandi et al., 2019). Milk isolated antioxidative peptides consist of 5 to 11 hydrophobic amino acids such as proline, histidine, tyrosine or tryptophan, are derived from hydrolysates of caseins by proteolytic enzymes (Kitts & Weiler, 2003; Mohanty et al., 2016).

Bovine derived peptides namely EMPFPK, PYPQ, PQSV and YFYPE and buffalo casein derived peptide namely EQL, MEDNKQ, RELEE and TVA by alcalase and trypsin hydrolysis have been identified to exhibit antioxidant activity. EQL, RELEE and TVA showed potential in ROS binding in ABTS•+radical inhibition. Peptides, LLY, YFPQL and YQEPVLGPVR derived from

buffalo casein also exhibited significant free radical scavenging capacity. Based on study by (Abdel-Hamid et al., 2017), buffalo milk hydrolyzed by papain having peptide sequence residues with Pro, Phe, Try/His, or Pro/Phe exhibited significant antioxidant activity. Furthermore, according to (Srivastava et al., 2022) two milk-derived peptides AGWNIPM (73.45%) and YLGYLEQLLR (64.46%) demonstrated a significantly high antioxidant activity in ABTS•+radical inhibition. Peptide VKEAMAPK, YLGYLEQLLR, and YIPIQYVLSR isolated from Milk fermented by *Lactobacillus brevis* CGMCC15954, *Lactobacillus plantarum* A3, and *Lactobacillus reuteri* WQ-Y1 demonstrated effective DPPH free radical scavenging activity.

3(f) Mineral binding peptides

Mineral binding peptides such as S-P:S-P:SP:EIVPN from αS1-casein can bind with calcium, phosphate, in the intestines. These peptides exhibit a high anionic character ensuing to form soluble complexes and resist further breakdown by enzymes. This prevents insoluble complexes formation of minerals. Due to the stabilizing ability of milk caseins to calcium and phosphate, various phosphopeptides have been revealed from the enzymatic breakdown of milk proteins, including fragments from αS1-casein (f43–f58, f59–f79 & f43–f58) αS2-casein (f1–f24 and f46–f70) and β-casein (f1–f25, f1–f28, f2–f28 and f33–f48) (Reynolds, 2017). Caseinophosphopeptides (CPPs) are released from the polar domain containing phosphorylated serine residues near the N-terminus on digestion of milk protein by trypsin. These CPPs contribute in the interaction between Calcium, Phosphorus and casein, leading to casein micelles formation. These phosphorylated peptides can increase the bioavailability by binding with the calcium and phosphate ions (Meisel & FitzGerald, 2003).

Adequate amount of calcium is required for the growth and development of bones in humans (Anusha & Bindhu, 2016). For increased vitamin D-independent bone calcification, casein-derived phosphorylated peptides were suggested among rachitic infants. Low intake of calcium is prevalent among premature babies, young and postmenopausal women and the aged having low calcium absorption and individuals undergoing osteoporosis treatment or prevention. The phosphorylated portions of caseinophosphopeptides (CPPs), which are produced from α- and β-casein can combine to form soluble organophosphate salts that act as calcium's mineral carriers (Anusha & Bindhu, 2016; Korhonen & Pihlanto, 2006). CPPs are known to exhibit mineral binding capacity, which can result in improved metal ion- bioavailability (Bouhallab & Bouglé, 2004). A study observed improved iron bioavailability with the addition of CPP in fruit beverages (FitzGerald, 1998).

Anti-plaque, demineralization prevention and remineralization of teeth, was demonstrated by CPP-ACP (amorphous calcium phosphate) whey peptides which are known to possess mineral binding properties (Iijima et al., 2004). Bovine casein derived peptides by pepsin hydrolysis: AMKPWIQPKTKVIPYVRYL, KVIPYVRYL, KPWIQPKTKVIPYV, RYL, LKKISQRYQKFALPQY, VYQHQKAMKPWIQPKT and VYQHQKAMKPWIQPKTKVIPYVRY having calmodulin-binding property exhibited inhibition against CaM-dependent phosphodiesterase (CaM-PDE) (Sánchez & Vázquez, 2017).

According to a study by (Kim et al., 2007), affirmed that hydrolysis of whey protein by various protease such as alcalase, esperase, flavourzyme, neutrase, papain, pepsin, pancreatin and trypsin effected the binding with minerals mainly iron. The study recorded that alcalase hydrolysates (97.6%) had the highest iron-binding capacity with higher content of Ala, Phe and Lys. (Caetano-Silva et al., 2015) reported 28 1 kDa β-Lg fractions of iron-binding WPHs by pancreatin hydrolysis with Glu, Asp and Glu/Asp present in the peptide sequence. Another study reported the WPC hydrolyzed with neutrase exhibited a potential action of iron absorption in Caco-2 cell model due to the presence of amino acids: Asp, Cys, His, Pro, Glu and Gly and peptides with MW of 10 kDa (Ou et al., 2010).

3(g) Opioid activity

Opioid peptides are short sequences of amino acids demonstrating functional roles in the brain and have similar effects to opiates. They bind to opiate receptors and perform various activities, including influencing social behavior, enhancing pain relief, stimulating hormone secretion (Anusha & Bindhu, 2016). Opioid peptides have pharmacological properties like that of opium. In an opioid peptide sequence, YGGF are generally present at the N-terminal end of peptides (Tyagi et al., 2020).

Opioid receptor ligands have specific characteristics that ensure the peptide and receptor are the perfect fit for the particular target activity, such as an N-terminal tyrosine, Tyrosine residue, or other aromatic amino acid in the 3rd and 4th positions from the N-terminal end, as well as another Phe (Anusha & Bindhu, 2016). These peptides are opioid receptor ligands that were in vitro synthesized by human and bovine casein enzymes. They are present in endocrine, immune and nervous systems. These peptides associate with exogenous and endogenous ligands and can influence the release of various neurotransmitters of the nervous system involved in the decreased appetite, and increase gastrointestinal transient time subsequently inhibits intestinal peristalsis and motility, body temperature control and hypotension (Meisel & FitzGerald, 2000; Mohanty et al., 2016; Molina & Abumrad, 1994). In milk, β-casomorphins, a casein opioid peptide,

present in Sheep, buffalo and human milk were among the earliest BAP discovered. Exogenous opioids isolated from milk α-casein, bovine α_1- casein and casoxins can act as opioid antagonists (Anusha & Bindhu, 2016). Opioid antagonists inhibit the agonist activity of enkephalin (Mohanty et al., 2016). In a recent study, two agonist opioids; casoxin C and serophine have been derived by enzymatic hydrolysis from bovine κ-CN receptor: f (399–404) and bovine serum albumin respectively. Based on numerous evidences, the antagonistic effectiveness of opioid receptors casoxins A and B are reported to be relatively lower. Casoxins A is analogous to f (35–41) (YPSYGLN) of κ-casein, casoxins B is analogous to f (58–61) (YPYY) of κ-casein and casoxins C is analogous to f (25–34) (YIPEYVLSR) of κ-casein, which have high biological opioid antagonist peptide potency (Mohanty et al., 2016; Xu, 1998).

Recent studies suggest that casomorphins exhibit anti-secretory properties as opioid ligands (Daliri et al., 2017b). It influences the endocrine activity of somatostatin and insulin secretion as well as stimulating analgesic behavior (Meisel & FitzGerald, 2000). Bovine derived α-casein peptide RYLGYLG demonstrated narcotic activity (Akbarian et al., 2022).

α-lactorphin, whey protein derived peptide, with potent activity can enhance secretion and gene expression of mucin in human colonic goblet-like cells. (Martínez-Maqueda et al., 2012). Trypsin β-Lg hydrolysate and β-lactorphin, likely through an opioid pathway showed similar effects, suggesting that WPHs with mucin production modulation could enhance gastrointestinal protection (Brandelli et al., 2015).

3(h) Immunomodulatory peptides

Immunomodulatory peptides suppress or stimulate the immune function of the body. The immune system defends the body against pathogens through antibodies and lymphatic system. Nutrition is a key factor in regulation of the immune system. BAPs, which are found in many sources, are known to modulate the immune system by producing antibodies, regulation of cytokine, and induced reactive oxygen species. Immune-stimulating peptides are used for overall immune strength of the body, while immunosuppressive peptides are used in autoimmune disorders, transplantation and grafting (Anusha & Bindhu, 2016).

Immunomodulatory peptides stimulate the humoral immune functions and suppress cell-mediated immunity. These peptides include glycopeptides, immunoglobulin peptide fragments and hormones. There are a lot of BAP that have been extracted from milk and milk products and those peptides assist in the production of antibody and regulation of cytokine and induced reactive oxygen species in immune function (Anusha & Bindhu, 2016; Mohanty et al., 2016; Tidona et al., 2009).

β-casomorphin derived from human milk β-casein corresponds to Val-glu-pro-Ile-pro-tyr is found to have immunostimulatory effect (Anusha & Bindhu, 2016). Several bovine β-casein peptides have been validated to exert phagocytizes in vivo against *Klebsiella pneumoniae* in mice and in vitro phagocytizes and murine macrophages in humans (Eriksen et al., 2008). Cytomodulatory peptides isolated from casein may stimulate the activity of immune competent cells and hinder the growth of cancer cells (Meisel & FitzGerald, 2003). Peptide fragments of $\alpha_1$casei (1–23) and β- casein (193–209) possessing immunomodulatory activity were released from bovine casein by chymosin hydrolysates. Oral administration of CMP enhanced the mucosal immunity in mice (Anusha & Bindhu, 2016). Glycomacropeptide (GMP) and its fragments have immune-suppressing effects on IgG antibody formation (Mohanty et al., 2016). Lactoferrin B from lactoferrin shows opsonin like activity by directly binding to neutrophils. Bovine peptide fragments such as casein f (63–680) and f (191–193) exhibited phagocytic action in human in vitro (Migliore-Samour & Jollès, 1988). However, α-lactalbumin and κ-casein peptides are used for immunotherapy of viral infection (Mohanty et al., 2016). Caseinomacropeptitde (CMP) inhibits enteric infection by promoting the growth of *bifidibacteria* or *lacobacilli* (Brück et al., 2003). Studies on rats and mice show that dietary intake of whey is beneficial for ulcer reduction and increases IgA in the gut supporting its protective function against intestinal disorders and small intestine perioperative damage in patients (Kume et al., 2014; Nakamura et al., 2011).

In vitro research on the immunomodulatory effects of whey from cows and goats hydrolyzed with corolase pp, pepsin, and human gastric and duodenal juices was conducted. Both the whey proteins exhibited inhibition dependence on human peripheral blood mononuclear cells (Eriksen et al., 2008). Studies suggest that the production of activation signals was affected by either intact or hydrolyzed peptide fragments due to its inhibition of lymphocyte proliferation (Mann et al., 2017) (Daliri et al., 2017a, 2017b).

Milk protein as the best source of bioactive peptides

Milk proteins provide several benefits and advantages in the preparation of peptides as compared to other protein sources like cereal, egg and soy as shown in Table 2. As discussed earlier, milk proteins contain numerous bioactive peptides which confer various health benefits. Milk proteins especially whey protein and caseins are known

Table 2 Comparison of bioactive peptides from milk proteins with other food proteins

Source	Peptide sequence	Activity	References
Bioactive peptides derived from Marine sources			
Clam	VRK	ACE inhibitory	(Tsai et al., 2008)
Mussel	HFGBPFH	Antioxidant	(Rajapakse et al., 2005)
Oyster	VVYPWTQRF	ACE inhibitory	(Wang et al., 2008)
Shrimp	FCVLRP, IFVPAF, KPPETV, YLLF, AFL	ACE inhibitory	(Hai-Lun et al., 2006)
Salmon Skin Gelatin	GPAE	Anti-diabetic activity	(Li-Chan et al., 2012)
Bioactive peptides derived from plant sources			
pecan meal	LAYLQYTDFETR	Antioxidant	(Hu et al., 2018)
rice bran	YLAGMN	Antioxidant	(Mirzaei et al., 2018)
Ginkgo biloba Seeds	TNLDWY, RADFY, RVFDGAV	Antihypertensive	(Ma et al., 2019)
Sunflower (Helianthus Annuus L.)	FVNPEAGS	Antihypertensive	(Megías et al., 2004)
Chia Seed (Salvia hispanica L.)	GDVIAIR	Antihypertensive	(Aguilar-Toalá et al., 2020)
Soy Protein	YDFYPSSTKDQQS	Antihypertensive	(Aiello et al., 2018)
Bioactive peptides derived from meat and meat derivatives			
Spanish dry-cured ham)	SAGNPN	Antioxidative	(Escudero, Mora, Fraser, Aristoy, & Toldrá, 2013)
	GLAGA	Antioxidative	(Escudero, Mora, Fraser, Aristoy, & Toldrá, 2013)
	AAATP	Antihypertensive	(Escudero, Mora, Fraser, Aristoy, Arihara, et al., 2013)
	KAAAAP	Antihypertensive	(Escudero et al., 2014)
Egg (white protein)	RVPSLM	Anti-diabetic activity	(Yu et al., 2011)
Egg (Yolk Protein)	YINQMPQKSRE, YINQMPQKSREA, VTGRF-AGHPAAQ	Anti-diabetic activity	(Zambrowicz et al., 2015)
Bioactive peptides derived from milk sources			
Fermented milk	VPP and IPP	**Antihypertensive**	(Tuomilehto et al., 2004)
αs_1-casein ovine milk	RPK and RPKHPIKH		(Silva & Malcata, 2005)
β-casein ovine milk	VPKKVK, and YQEPVLGP and YQEP		(Silva & Malcata, 2005)
Bovine milk	DKIHPF, YQEPVL		(Mohanty et al., 2016)
casein f183-207 and f164-179	VAGTWY	**Antimicrobial activity**	(Mohanty et al., 2016)
Isfracidin	EQLTK		
lactoferrin B			
bovine whey protein	LKPTPEGDL and LKPTPEGDLEIL	**Anti-diabetic**	(Lacroix & Li-Chan, 2014a)
bovine whey α-lactalbumin	ELKDLKGY, ILDKVGINY, KILDK, LKPT-PEGDL, LKPTPEGDLEIL, LDQWLCEKL, RNAVPITPTLNR, TKVIPYVRYL, FALPQYLK and YLGYLEQLLR		(Koirala et al., 2023)
Bovine casein	ACGP, PLMLP, HLPGRG, MFE, QNVLPLH and GPAHCLL		(Koirala et al., 2023)
Whey protein	VAGTWY		(Agarkova et al., 2020)
whey	YVEEL	**Osteoprotective**	(Pandey et al., 2018)
buffalo casein	NAVPITPTL		(Reddi et al., 2019)
Bovine	EMPFPK, PYPQ, PQSV and YFYPE	Antioxidant activity	(Srivastava et al., 2022)
buffalo casein	EQL, MEDNKQ, RELEE and TVA		
buffalo casein	LLY, YFPQL and YQEPVLGPVR		
fermented Milk	VKEAMAPK, YLGYLEQLLR, YIPIQYVLSR		(Srivastava et al., 2022)
Cheddar cheese	EMPFPK, KEMPFPK, SDIPNPIGSENSEK		(Yang et al., 2021)
αS1-casein	S-P:S-P:SP:EIVPN	Mineral binding	(Reynolds, 2017)
Bovine casein	AMKPWIQPKTKVIPYVRYL, KVIPYVRYL, KPWIQPKTKVIPYV, RYL, LKKISQRYQK-FALPQY, VYQHQKAMKPWIQPKT and VYQHQKAMKPWIQPKTKVIPYVRY		(Sánchez & Vázquez, 2017)

Table 2 (continued)

Source	Peptide sequence	Activity	References
	YGGF	**Opioid activity**	(Tyagi et al., 2020)
κ-casein	YPYY YPSYGLN YIPEYVLSR		(Xu, 1998)
human milk β-casein	VEPIPY	**Immunomodulatory**	(Anusha & Bindhu, 2016)Anusha & Bindhu, 2016)
bovine casein	of $\alpha_1$casei (1–23) and β- casein (193–209)		(Sharma et al., 2011)
bovine casein	casein f (63–680) f (191–193)		(Migliore-Samour & Jollès, 1988)

to have higher biological value protein due to their balanced amino acid profile. Whey proteins and caseins contain all essential amino acids making them a valuable substrate in peptide production (Tidona et al., 2009). Cereal protein derived from wheat, corn and rice contain incomplete amino acid profile and may lack certain essential amino acids. While trypsin inhibitors such as phytates and lectins which can inhibit the protein digestion and nutrient absorption are present in soybean protein Whey proteins are easily digestible and highly soluble in water facilitating ease in incorporation into wide range of foods and beverages. In addition to their rapid digestion, their rapid absorption in the gastrointestinal tract enhances the release of bioactive peptides, their bioavailability and efficacy. Meanwhile, proteins from sources like plants and egg, they impart a distinct flavor and texture which may not always be desirable depending on its application. Proteins from soybean may have a strong and bitter flavor limiting its use in certain food. Milk is considered as a valuable substrate for peptide production due to the presence of abundant amount of amino acid (Akbarian et al., 2022; Daliri et al., 2017a, 2017b). Milk proteins have lower allergenicity as compared to proteins of egg and soybeans allowing them to be incorporated with other food product including those targeted for sensitivities and diet restricted individuals. Egg proteins, specifically egg white derived peptides such as Ovalbumin-derived peptide YAEERYPIL and Beta-conglycinin-derived peptide VPVPFSQPL from soybeans are known to trigger and cause severe allergic reaction in some individuals. Wheat proteins due to the presence of gluten can cause adverse reactions to individuals with gluten sensitivity or celiac diseases. The availability and accessibility of egg may be limited in certain regions as compared to milk thus, limiting their use in peptide production. Milk can be processed in numerous products namely, isolates, hydrolysates, fractions and concentrates through ultrafiltration, microfiltration and enzymatic hydrolysis. Due to this versatility and flexibility, the peptides can be produced having various bioactivities and functional properties, different molecular weights and tailored to specific applications as well. On the other hand, extraction and isolation of peptides from other sources like egg and plant sources can be more challenging and labour- intensive as in cereals due to their complex matrix and low protein content. In the case of soy protein, the processing and extraction methods can result in undesirable textures and flavors (Akbarian et al., 2022; Koirala et al., 2023; Tidona et al., 2009).

Hence, Milk proteins offer numerous advantages in peptide preparation as compared to other protein sources making them highly desirable for peptide production in food and nutraceuticals applications due to their diverse range of bioactive peptides.

Application of BAP

Milk-derived BAPs in commercial products are gaining significant interest due to its potential applications in various fields as shown in Table 3. Dairy BAP have been confirmed to hold health benefits like antimicrobial, antioxidant, anti-inflammatory, antihypertensive and immunomodulatory activities in the field of health and nutrition,. These peptides are incorporated into nutraceuticals, dietary supplements and functional food enhancing the nutritive value and health benefit of the product (Bhandari et al., 2020). Hydrolysates from whey and casein have been analyzed to enhance the performance and muscle aid recovery in sports and exercise. These peptides have the potential to enhance the synthesis of muscle protein, mitigate muscle soreness, damage and boost exercise endurance (König et al., 2021). Casein peptides are used in the formulation of infant formula to emulate the breast milk benefits. These peptides facilitate the growth and development and boost the immunity of the infants (Zhang et al., 2022).

Milk-derived peptides have shown significant potential in the field of skin care and cosmeceuticals as they possess anti-aging, skin-protective and moisturizing properties and exploited as a significant ingredient in the

Table 3 Bioactive peptides in Commercial products compiled from Korhonen & Pihlanto, 2006 and Anusha & Bindhu, 2016

Product	Protein	Peptide sequence	Bioactivity	References Website/ manufacturer
PeptoPro®	Milk proteins	Dipeptide and tripeptides	Boosts muscle protein synthesis	http://www.dsm.com/markets/foodandbeverages/en_US/ products/nutraceuticals/ peptopro.html DSM, Netherlands
Calpis	α1, β-Casein	IPP and VPP	Hypotensive	www.calpis.net/ CalpisCo. Japan
Caploc	casein	CPP	Mineral absorption	www.arlafoodsingredients.com/ Arla Foods, Denmark
Calpico/ Calpis AMEAL s	β and κ-casein	IPP and VPP	Hypotensive	www.calpis.net/ Calpis Co., Japan
Peptigen® IF-3087/3090/3012/3080	Whey protein	WPH	Mineral binding	www.arlafoodsingredients.com/ products/whey-hydrolysates/ infant-nutrition/Arla foods, Denmark
Lacprodan® DI-3071/3021 Lacprodan® HYDRO.milk	Whey protein	WPH	Vitamins and minerals uptake; oral hygiene improvement	www.arlafoodsingredients.com/ products/whey-hydrolysates/ sport-nutrition/ Arla foods, Denmark
PROTARMOR™ 80	Casein	Casein hydrolysates	Weight loss Sports nutrition	http://www.armorproteines.com/en/products/ protarmor-80/ Armor Proteines, France
Kotsu calcium	Casein	CPP	Mineral absorption	www.asahiinryo.co.jp/ Asahi Soft Drinks Co. Ltd, Japan
Tekkotsu Inryou	Casein	CPP	Mineral absorption	www.suntory.com/softdrink/ Suntory, Japan
Evolus	b-casein and j-casein	IPP and VPP	Antihypertension	Valio Oy, Finland
BioPURE-GMP		k-Casein f(106–169) (Glycomacropeptide)	Antithrombotic antimicrobial	Davisco, USA

formulation of anti-aging products, lotions and creams (Kazimierska & Kalinowska-Lis, 2021). In the field of pharmaceuticals, these peptides have demonstrated in therapeutic applications as they can serve as an alternative or supplements to the conventional use of drug for treatment in ailments such as obesity, hypertension, CVD and diabetes. Theses peptides are known to exhibit anti-thrombotic, anti-hypertensive properties and assist in the management of diabetes. Cytomodulatory peptides inhibit the growth of cancer cell and increase immune activity (Zaky et al., 2022). Milk-derived peptides have been shown for regulating blood pressure and consumption of food in the reduction of risk of metabolic syndrome. Antioxidant peptides isolated from milk is said to be a good additive for meat preservation. A mixture of beef paste and 2.0% casein calcium peptide is found to prevent odors in meat and inhibit lipid peroxidation by 70% and extend the shelf life (Zaky et al., 2022). Antioxidant effect of casein calcium peptides on lipid oxidation have been shown in beef homogenate. Furthermore, peptides from whey protein have been shown to act as functional components in meat products. Pigments added with homogenates of whey protein (2%) in cold storage have been found to reduce oxidative degradation and cooking loss (Peña-Ramos & Xiong, 2003). Peptides from whey of mozzarella cheese demonstrated the inhibition of growth of cancer in human colorectal cell line (Simone et al., 2009). Currently, due to the lack of suitable technologies, milk-derived peptides have been limited for commercial production. Membrane separation technique is used for peptide enrichment in a specific molecular weight range (Kitts & Weiler, 2003). For the production of casein and whey protein hydrolysate peptides, ultra filtration and nano filtration are used in industries. These techniques are used for the production of confectionary, chewing gum, dairy and fruit-based drinks. These products may contain mineral binding antihypertensive peptides. Commercial product Calpis® and Evolus® contain ACE inhibitory tripeptides VPP and IPP, respectively. Evolus®, a Finnish product is fermented by *Lactobacillus helveticus* LBK-16H strain (Korhonen, 2009) and Calpis®, a Japanese fermented product is produced by using *Lactobacillus helveticus* and *Saccharomyces Cerevisiae* (Takano, 2002).

The application of milk-derived peptides is still an ongoing research revealing their potential use and promising benefits in diverse fields.

Conclusion

The interest in BAP isolated from milk proteins has been growing as a functional food due to its various biological and nutritional properties. Classical and bioinformatics approaches are widely exploited in the production of various kinds of milk-derived peptides. Milk contains bioactive peptides that can assist the regulatory functions, including hormone secretion (casomorphins), nutrient uptake (casomorphins and phosphopeptides), immune defense (casokinins, immunopeptides and casomorphins) and neurotransmission (casokinins). Despite its growing interest, limited studies have been done on the interaction of BAP derived from milk in the gastrointestinal tract, their stability, efficacy and bioavailability. Clinical studies are needed for determining the health claims and safety of these BAPs.

Abbreviations

BAP	Bioactive peptides
LAB	Lactic acid bacteria
ACE	Angiotensin-I converting enzyme
CPP	Caseinophosphopeptides
QSAR	Quantitative Structure- Activity Relationship
NCBI	National Center for Biotechnology Information
MBDB	Milk Bioactive Peptide Database
CAMP	Collection of Antimicrobial Peptides
APD	Antimicrobial Peptide Database
MAPS	Minimum Analogue Peptide Set
ANN	Artificial Neural Networks
PCA	Principal Component Analysis
MLR	Multiple linear regression
PLSR	Partial least square regression
FTIR	Fourier transform infrared
NMR	Nuclear magnetic resonance
CD	Circular dichroism
DOE	Design of Experiments
RSM	Response Surface Methodology
RAS	Renin-angiotensin system
AMP	Antimicrobial peptide
CMP	Caseinomacropeptide
GMP	Glycomacropeptide
CNH	Casein hydrolysates
OVR	Ovariectomized
ACP	Amorphous calcium phosphate
Leu	Leucine
Phe	Phenylalanine
Arg	Arginine
Ile	Isoleucine
Thr	Threonine
Tyr	Tyrosine
Val	Valine
Ala	Alanine
Lys	Lysine
A	Alanine
R	Arginine
N	Asparagine
D	Aspartic acid
C	Cysteine
E	Glutamic acid
Q	Glutamine
G	Glycine
H	Histidine
I	Isoleucine
L	Leucine
K	Lysine
F	Phenylalanine
P	Proline
S	Serine
T	Threonine
Y	Tyrosine
V	Valine
WHP	Whey protein hydrolysates

Acknowledgements
None.

Authors' contributions
PK: conceptualization and writing. KD: review, editing, and supervision.

Funding
Not applicable.

Data availability
Not applicable.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
All authors give their consent for publication of this manuscript.

Competing interests
The authors declare that they have no competing interests.

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Received: 20 November 2023 Accepted: 20 June 2024
Published online: 08 January 2025

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