

***In silico* analysis of bioactive peptide of goat milk based on beta-casein protein**

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Abstract. Goat milk peptidomics focuses on the composition, interactions, and properties of bioactive peptides present in the most abundance milk protein, CSN2 gene. Goat milk peptidome is considered a valuable source of a number of biologically active peptides along with health promoting activities. In silico analysis on bioinformatic approach has led to enhancements of global knowledge regarding the health benefits of dairy products, through data mining for bioactive peptide selection associated with current bioactive peptide data algorithm resulted in bioactive peptides profiles and visualization of three-dimensional protein structure. Biological potentials are identified using these techniques, to increase goat milk bioactive peptides commercialization as a functional food and promising treatments.

1. Introduction

Goat milk is an essential food source with critical value in improving digestibility, buffering capacity, alkalinity, therapeutic values and lower allergenicity that can be used to improve human health [5][11]. Unlike goat's milk, cow's milk, the world's most popular and mass-produced dairy product, has been associated with some health issues such as allergy [16]. Switching cow milk intake to goat milk provides many health benefits, linked to lower allergic reactivity, polypeptides with antioxidant and ACE inhibitor properties, and the prevention of atherosclerosis and cardiovascular disease. Goat milk protein is more digestible and its constituent amino acids are more efficiently absorbed than cow milk protein [5].

Enzymatic proteolysis during gastrointestinal digestion of milk processing releases and activates the bioactivities of peptides milk protein [21, 22]. During gastrointestinal digestion, bioactive peptides of milk protein are synthesized in the form of large peptides, which are cleaved and modified to give active products in small intestines. Bioactive peptides produced play important roles in physiological functions and pathogenesis [5].

Bioactive milk peptides, the sequences between 2-20 amino acids have gained numbers of popularity in recent years [6]. Proteomic studies on protein bioactive peptides focused on the discovery of new dairy bioactive peptides, often using advanced analytical techniques including in silico study to discover the ability to promote as a functional food [2]. Therefore, the objective of this study was to evaluate potential bioactive peptides based on beta casein protein of goat milk using *in silico* analysis.

2. Materials and methods

2.1. Proteomic identification

Current proteomic method harnesses sequencing technologies, in the form of widely and easily accessible nucleotide sequence data, to maximize the potential of characterizing bioactive peptides at the protein level, in *Capra hircus* CSN2 gene [23]. Peptide sequences derived from amino acid sequence annotated using BIOPEP-UWM database of bioactive peptides [14]. The profile of a potential biological activity is defined as the type and location of bioactive fragments in a protein or a peptide chain. The calculations run to obtain two quantitative parameters that characterize proteins as potential precursors of bioactive peptides: the frequency of bioactive fragments occurrence in a protein sequence and a potential biological activity of protein fragments.

2.2. Protein structure modeling

To analyze the virtual protein sequence fragments of caprine milk CSN2 amino acid sequence NC_030813.1 is visualized by prediction of 3-dimensional protein structures modeled using the I-TASSER web server [3, 9, 24]. The result in computational prediction shown in Figure 1 below.

3. Results and discussion

3.1. Goat milk potential

Research in dairy products has exponentially grown recently, changing the way its considered and not just a simple source of energy for the body, but it provides components with specific functions and nutritional properties, which also include potential benefits as well as possible detrimental effects on health. Functional compounds include high compounds of high level of vitamin a, vitamin d, vitamin b12, vitamin c, also biotin and minerals: potassium, calcium, chloride, phosphorus, selenium, zinc and copper [20]. Goat milk is also made up from protein and peptides that make up one of the main groups of food bioactive components, their new emerging field, namely nutritional proteomics as the nutritional value.

Goats are the earliest domesticated animals and their milk has been used for consumption since ancient times. Goat's milk can be categorized as a functional food because of its friendly characteristic intolerance even for those with cow's milk protein allergy. Consumption of functional goat's milk as a daily nutritional intake should receive more attention, especially for infants and children because it is way better in nutrient uptake efficiency, especially because they contain prebiotic supplements and ultra nourishing properties [11].

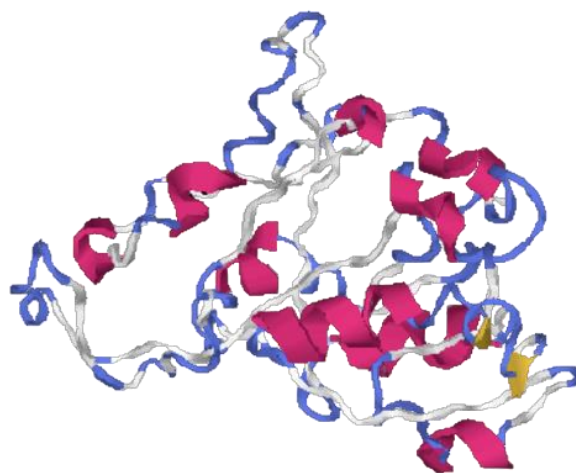


Figure 1. CSN2 protein structure prediction

Goat milk is a complex biological matrix, and its proteins and their primary sequences are the main sources of Bioactive Milk Peptides (BMP). These peptides are potential ingredients of health-promoting functional foods [19] attributed to exhibiting ACE inhibitor, antioxidative, and antibiotic. Recent substantial improvements and innovations in bioinformatic in silico study have led to the development of a large variety of analytical techniques to be able to carry out the analysis of extremely complex mixtures, detecting different types of analytes over a wide range of concentrations [1].

3.2. CSN2 3D protein structure

Proteins include both genotype and phenotype information, and allows proteomics methods to identify biological samples and characterize the conditions that produce these proteins. Proteomic analysis observes the interactions within protein to describe the structures and higher-order complexes. Several amino acids that were conserved across the genus and the potential location of CSN2 conserved amino acid motifs in these proteins was determined. The tertiary structure of a CSN2 protein refers to the overall three-dimensional arrangement of its polypeptide chain in space.

3.3. Goat milk bioactive peptides discoveries

The most investigated matrices for peptidomic studies are milk and its derivative products. The precursors of milk and derivative product bioactive peptides are casein which consist 43.6% proteins, with β -CN level with the most abundant 29.6% [20]. BIOPEP using a bioinformatic approach through data mining for bioactive peptide selection associated with current bioactive peptide data algorithm resulted in bioactive peptides profiles by using in silico study.

Table 1. Bioactivity of milk-derived peptides and health promoting activities

ID	Name of peptide	Activity	AA Sequence
2606	PEP inhibitor	Antiamnestic	HLPLPL
3461	Prolyl endopeptidase inhibitor	Antiamnestic	GP
2622	ACE inhibitor	ACE inhibitor	PQNILP, LPQNILP, ILP, VLP, PLP, LPLP, HLPLP, LHLPLP, LHLPLP, PQR, LY, IY, MF, KVLVPVQ, TPVVVPFLQP, VYP, EMPFPK, LSP, LQP, YP, VPK, KVLVPV, GPV, HLPLPL, IP, AF, LA, VP, RA, VG, HL, FG, GV, MG, GK, GE, AI, LG, TG, KF, AR, EI, IE, VE, TE, LQ, LN, PT, TQ, PP, PQ, EK, KE, HP, HK, LTLTDVE, KVLILA, VPP, AFL, TESQSLT, LVY, AFL, LVQ, TP, DM, FQ, IL, WM, TGP, RG, LTQTPVVVPF, SLPQN, VVVVPF, YQEPVL, FPPQS, QEPVLGPVRGPF, LGPVRGPF, DKIH, VRGP, PFP, QP, VM, LPL, LP
9771	Antibacterial peptide	Antibacterial	EMPFPK
3283	Antithrombotic peptide	Antithrombotic	GP
2847	Cytochrome P-450	Opioid	YPFT
2870	opioid peptide	Opioid	YPF
9852	Neocasinomorphin	Opioid	YPVEPF
3355	Stimulating vasoactive substance release	Stimulating	SSS, LLL
8320	Glucose uptake stimulating peptide	Stimulating	VL, LV, IL, LI, II, LL

8329	Stimulating vasoactive substance release	Stimulating	EE, SE
9745	Peptide stimulating mucin secretion	Stimulating	EMPFPK, YPVEPF, EPFTESQ
10111	Wheylin-1	Neuropeptide	MH
2753	peptide regulating the stomach mucosal membrane activity	Regulating	GP
9955	Regulator of phosphoglycerate kinase activity	Regulating	SL
8318	Dvl protein binding	Anticancer	VVV
3305		Antioxidative	LH
3317		Antioxidative	HL
7879	peptide from b-casein (ovine and caprine milk)	Antioxidative	YQEPVLGP
7888	antioxidative peptide	Antioxidative	EL
7995	synthetic peptide	Antioxidative	LHL
8224	antioxidative peptide	Antioxidative	VY
8479	Antioxidant peptide from b-CN (183-188)	Antioxidative	RDMPIQ
9355	Antioxidative peptide	Antioxidative	LAC, CLV, SVL, HKEMPFPKYPVEPFESQ, VVPPFLQPE, EDELQDKIHPP, LPL, YPVEPF, LLY
3751		Bacterial permease ligand	KK
9538	Anti-inflammatory peptide	Anti-inflammatory	VPP
10188	Zinc binding peptide	Binding	HKEMPFPKYPVEPF
10078	Hypotensive peptide	Hypotensive	LHLPLPL
10097	Hypotensive peptide	Hypotensive	LPLP
4005		Activating ubiquitin-mediated proteolysis	RA
4006	Ubiquitin-mediated proteolysis activating peptide	Activating ubiquitin-mediated proteolysis	LA
3169	Dipeptidyl peptidase IV inhibitor (DPP IV inhibitor)	Dipeptidyl peptidase IV inhibitor	PP, MP, VA, LA, FA, LP, VP, LL, VV, IP, TP, SP, FP, HP, YP, IA, RA, EP, QP, FL, HL, EK, AL, SL, FLQP, VLGP, VR, LPL, LPLPL, PL, WM, LQP, AF, AS, EI, ES, ET, FQ, GE, GV, HI, HS, IH, II, IL, IM, IN, IQ, KE, KF, KH, KI, KK, KV, KY, LH, LI, LN, LT, LV, MF, MG, MH, MK, MV, NQ, NT, NV, PF, PI, PK, PN, PQ, PS, PT, PV, QA, QD, QE, QI, QN, QQ, QS, QT, RG, SI, SV, SW, TD, TE, TG, TH, TI, TL, TM, TQ, TV, VE, VG, VK,

9548	Alpha-glucosidase inhibitor	Alpha-glucosidase inhibitor	VL, VM, VQ, VY, YF, YK, YQ, GPV, GPFILV, YPVEPF YP, PP, VE, PE
9480	DPP-III inhibitor	Dipeptidyl peptidase III inhibitor	YF, YK, GE, HL, HK, HP, IH, LA, FA, FL, PE, PF, WM, VY
8249	CaMPDE inhibitor	CaMPDE inhibitor	KF
2835	Renin inhibitor	Renin inhibitor	FT, KF, LY, LPL
9685	Antidiabetic peptide from goat milk β -casein, f30–40	Antidiabetic	SLSSSEESITH, QEPVLGPVRGPF

Milk-derived BPs are made up of short amino acid sequences, inactive inside the parent protein, but that can be released by endogenous proteases or during gastrointestinal digestion, food processing, and storage or by in vitro hydrolysis by specific proteolytic enzymes. Bioactive peptides contain 2–20 amino acid residues per molecule or more than 20 and classified into small peptides (less than 7 AAs, the most active but difficult to analyze by conventional proteomics approaches), medium peptides (7–25 AAs) and large peptides (more than 25 AAs) [1].

Food-derived constituents represent important sources of several classes of bioactive compounds analyzed of short amino acid sequences and endogenous peptides [1]. Several functionalities for bioactive peptides have been described and considered as novel and potential dietary ingredients to promote human health, that can be naturally occurring, produced in vitro by enzymatic hydrolysis, and formed in vivo during gastrointestinal digestion of proteins.

3.4. Cardiovascular system

Goat milk peptides have strong facilities in Antithrombotic, Anti Inflammatory, Hypotensive, Antioxidative from synthetic peptide and antioxidant peptide, renin inhibitor, and mostly ACE inhibitor. ACE inhibitor role in human health to alter the metabolism of a number of other vasoactive substances. ACE inhibitors decrease systemic vascular resistance without increasing heart rate as an effective in the treatment of hypertension, and reduce mortality in congestive heart failure and left ventricular dysfunction after myocardial infarction [7].

3.5. Nervous system

Several studies have suggested that bioactive peptides might be beneficial for nervous system especially in opioid treatment, neuropeptide and activating ubiquitin-mediated proteolysis. Opioid treatment is strongly advised for pain relief in and associated with its role as analgesic class for drugs for cancer management [4]. Neuropeptides are signaling molecules mainly secreted from neurons that act as neurotransmitters or peptide hormones to affect physiological processes and modulate behaviours [13]. In humans, neuropeptides are implicated in Schizophrenia [12]. Physiological role for the ubiquitin-proteasome pathway (UPP) was first established in progression of cell cycle following by the discoveries that connected ubiquitin-proteasome-mediated proteolysis to synaptic plasticity. In the nervous system, the UPP has been connected to several diseases such as Alzheimer's, Parkinson's, and Huntington's diseases [8].

3.6. Metabolic Health

DPP-3 is a cytoplasmic zinc-binding metallopeptidase, and its activity is associated with protein metabolism, blood pressure regulation and pain modulation [18]. DPP-4 cleaves a wide range of

substrates, including growth factors, chemokines, and peptides in addition to its major role in glucose metabolism are a class of oral diabetic medications through incretin hormones, which are gut hormones responsible for glucose homeostasis after oral food intake approved by the FDA to treat type 2 diabetes mellitus in adults [17]. Besides the major role by dipeptidyl peptidase IV inhibitor and dipeptidyl peptidase III inhibitor, milk peptide also contains biological potentials for CaMPDE inhibitor, antidiabetic, stimulating vasoactive substance release, glucose uptake stimulating peptide, and peptide stimulating mucin secretion also regulating peptide in stomach mucosal membrane activity, regulator of phosphoglycerate kinase activity.

3.7. Immune system

Besides oral consumption, peptides can also be engineered as a vaccine base. The number of short peptide-based vaccines with other biological therapeutics in clinical trials has grown significantly over the last decade against cancers [15]. Antimicrobial peptides (AMPs) are expressed in many cell types in response to the activation of the Toll-like receptor (TLR) signaling pathway and predominantly comprised of two types of amino acids residues-cationic residues such as Arg, Lys, and His, and hydrophobic residues (mainly aliphatic and aromatic) [or glycine usually have a linear structure. HDPs have a wide range of antibacterial activities, and low susceptibility to antimicrobial resistance. The widely developed peptide-drug research has led to the introduction of around 60 FDA approved peptide therapeutics in the market [10].

4. Conclusions

The main challenges to commercialization of BPs are due to the little attention paid to their bioavailability and biodistribution after ingestion and also to inadequate clinical evidence of bioefficacy. Despite the progress, the information available is mainly related to in vitro data and there is limited clinical evidence to justify the production and the use of BPs from goat milk as a health promoting or functional food component. Nevertheless, the development of the study of BPs is of great interest for pharmaceuticals to control high sugar levels and preventions from producing angiotensin II that narrows blood vessels, baseline for pain relief in cancer management drugs, treatments for type 2 diabetes mellitus in adults, biological therapeutics of short peptide-based vaccines against cancer and antibacterial with low resistance. Although the potential of goat milk proteins and peptides for the formulation of functional foods has demonstrated, further efforts are needed to increase their commercialization, with the development of economically feasible methods for the large-scale production of bioactive milk components.

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