

**BOVINE MILK DERIVED PEPTIDES: A COMPREHENSIVE REVIEW****Dr. Girish M. Bhopale***

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ABSTRACT

Bovine milk proteins are rich source of biologically active peptides. These peptides demonstrated to have major activities such as Antihypertensive, antimicrobial and anticancer in human. The present review highlights methods of production of milk peptides and their Impact on human health.

KEY WORDS: Antihypertensive Peptides, Antimicrobial Peptides, Anticancer Peptides, Milk Proteins, Human Healthcare.

INTRODUCTION

Bovine milk is an excellent source of protein required by the human body.^[1] The biological activities of bovine milk are mainly due to the presence of peptides in it. Nowadays bovine milk peptides have received increasing attention as potential ingredients of human health promoting foods.^[2, 3] Some bovine milk peptides have also been demonstrated to have Antihypertensive, antimicrobial and anticancer activities. In view of above the present review highlights important protein contents of bovine milk, methods of production of peptides, physiological effects of bioactive peptides, commercially available milk peptide products and further research needed.

Important protein contents of bovine milk

Dairy protein consumed by human is predominantly from bovine milk. Bovine milk is rich in approximately 80% casein and 20% whey proteins [Table 1].^[4,5] The main component of milk proteins can give rise to number of bioactive peptides [2-20 amino acids residues]^[5] and their activities are based on the amino acid composition and sequence. Whey protein contains a higher proportion of branched chain amino acids leucine, Isoleucine and valine compared to casein.^[6] Casein contains a higher proportion of histidine, methionine, Phenylalanine and valine than whey protein.^[6]

Methods of production of bioactive peptides

Bioactive peptides can be produced from bovine milk proteins by different methods [Table 2].^[7,8] The peptides are inactive within the sequence of the parent protein molecule and can be liberated by gastrointestinal digestion of milk, fermentation of milk with proteolytic starter cultures or hydrolysis by proteolytic enzymes. In many studies, combination of enzymatic hydrolysis by digestive enzymes and fermentation of milk with proteolytic starter cultures or enzymatic hydrolysis by

digestive enzymes and proteolysis by enzymes derived from microorganisms has been proven effective in generation of peptides.

Physiological effects of bioactive peptides in human:

Bovine milk peptides are affecting major cardiovascular system of human. Hypertension is know risk of factor for cardiovascular diseases including coronary heart disease, peripheral artery disease and stroke.^[9] Angiotensin converting enzyme [ACE] is located in many tissues and plays an important role in blood pressure regulation and in turn hypertensive. ACE, a part of the rennin angiotensin system, converting angiotensin I to a potent vasoconstrictor, angiotensin II which induces the release of aldosterone and therefore increase the blood pressure further.^[9] Evidence from some epidemiological studies suggest that greater consumption of dairy products associated with lower risk of cardiovascular diseases.^[6] In most of the human studies moderate or significant reduction of blood pressure has been observed after consumption of specific milk. Reduction of systolic blood pressure of 10-12 mmHg and diastolic blood pressure reduction of 5 mmHg may reduce the risk of stroke by 40%, coronary heart disease by 16% and all cause mortality by 13%.^[9] The summary of effects of selected investigated peptides is depicted in Table 3.^[4, 10]

Antimicrobial bioactive peptides derived from bovine milk have been reported to inhibit many Gram positive and Gram negative pathogens.^[11] Lactoferrin showed the antibacterial activity and antiviral activity.^[12] Biologically active antibacterial peptides are released during the digestion of β -lactoglobulin with trypsin. Such peptides demonstrate activity against food pathogens [*Staphylococcus aureus*, *Listeria monocytogenes*, *Salmonella spp* and *Escherichia coli*].^[12] Hydrolysis of α_{s2} - casein results in releasing casocidin which showed antibacterial properties.^[12] Another casein derived

peptide isracidin is encoded in the native sequence of α_{s1} -casein and also release by of chymosin. It shows antibiotic properties against *Staphylococcus aureus* and *Candida albicans*.^[12]

The anticancer peptides from milk can be generated during proteolytic activities such as gastrointestinal digestion or food processing including fermentation.^[10] Proline and lysine respectively dominate at various positions in anticancer peptides obtained from caseins and whey proteins. Some of the bovine milk derived peptides have been shown anticancer activity [Table 3]. However an increased in consumption of dairy foods has been reported with increased prostate cancer, hepatocellular carcinoma and ovarian cancer risks.^[10,13] Conversely higher consumption of dairy food was observed in association with a reduction of bladder cancer breast cancer and colorectal cancer.^[14,15,16]

Commercially available bioactive peptides

Recently few commercial food products supplemented with milk protein derived bioactive peptides have been available in limited country [Table 4]. These milk products contain peptides with antihypertensive, anticarcinogenic and antimicrobial properties. Calpis is a soft drink manufactured by Calpis Co. Ltd., Japan. The beverage has a light somewhat milky and slightly acidic flavor, similar to plain or vanilla favored yogurt or yakult. Its ingredients include water, nonfat dry milk and lactic acid and produce by lactic acid fermentation. Evolus is fermented low-fat, low-lactose milk drinks flavored with juice preparations made from various berries or fruits. The products contain the two tripeptides which are released from milk proteins during fermentation with a *Lactobacillus helveticus* LKB-16H starter culture. The BioZate is whey protein isolated from cheese whey to over 90% protein and hydrolyzed using a

proprietary process. It is heat-stable with its high amino acid bioavailability. BioZate proteins can be incorporated into bars and sheeted or extruded snacks without introducing firm textures, and they can be blended with other proteins for optimal effect. AmealIBP Calpis proprietary milk protein is derived from milk casein with lactotripeptide supplement that helps to healthy cardiovascular function. Since it is purified and contains no lactose, it is safe for people who are lactose intolerant or have milk allergies. C12 is derived from milk caseins helps in lowering blood pressure.

Future prospects: Bovine milk peptides appear to have the specific human health benefits and therefore studies are required the applications of chromatographic methods for isolation of peptides and mechanisms by which the peptides exert their activities. Further molecular studies are required to clarify the mechanisms by which the bioactive peptides exert their activities. Now an important task for these peptides is to enhance their bioavailability in dairy products.

Further work is also required to synthesize modified peptide sequences in order to find pharmacologically active peptides with higher potency and longer duration of action. Chemical modifications of peptide have been used to increase the stability of peptides in biological fluids.

New delivery strategies employing nanotechnology have been developed not only to protect peptides from enzymatic degradation but also to improve bioactive peptide delivery to target tissues.

Table 1. Important protein contents of bovine milk.

Caseins proteins [80%]	Derived peptides effects	Whey proteins [20%]	Derived peptides effects
α_{s1} -casein [40%]	Antihypertensive, Antibacterial, Immunomodulatory, Antioxidant, Mineral carries, Anti-caries	β -lactoglobulin [52%]	Antihypertensive
α_{s2} -casein [10%]	Antihypertensive, Antibacterial, Immunomodulatory, Mineral carries, Anti-caries	α -lactoalbumin [17%]	Antihypertensive, immunomodulatory
β -casein [36%]	Antihypertensive, Immunomodulatory, Mineral carries, anti-caries	Lactoferrin [1.5 %]	Antiviral, Antitumor, Antihypertensive, Immunomodulatory
k-casein [14%]	Antihypertensive, Antithrombotic effect, Immunomodulatory, Antibacterial		

- The contents of proteins are expressed as percentage [%] of Casein proteins and whey proteins in the total milk proteins and also Whey proteins as a percentage of total whey proteins and caseins as percentage of total caseins.

Table 2. Production of bioactive peptides from bovine milk.**Microbial fermentation.**

Many industries utilizing dairy starter culture which are highly proteolytic. Variety of naturally formed peptides have been found in fermented dairy products such as yoghurt, sour milk and cheese. These peptides can be generated by proteolysis activities of certain strains of bacteria.

Food processing.

The structural and chemical changes that occur during the processing of food proteins may result in the release of bioactive peptides. Such peptides can be generated during manufacture of several milk products and may thus be ingested as food components. Cheese contains phosphopeptides as natural constituents and secondary proteolysis during cheese ripening leads to formation of various ACE inhibitor peptides.

Enzymatic hydrolysis by digestive enzymes

The most common way to produce bioactive peptides is through enzymatic hydrolysis of whole protein molecules. The most prominent enzymes are pepsin, trypsin and chymotrypsin that have been shown to release a number of peptides.

Table 3. Antihypertensive, Antimicrobial and Anticancer activity of peptides derived from bovine milk.

Peptides [name]	Origin	Fragments	Structure	Function
Casokinins	α_{s1} -casein	23-24	FF	ACE inhibitor
		23-27	FFVAP	
		23-34	FFVAPFPEVFGK	
		146-147	YP	
α_{s2} -casein	174-179	FALPQY	ACE inhibitor	
	198-202	TKVIP		
β -casein	74-76	IPP	ACE inhibitor	
	84-86	VPP		
	88-90	LQP		
	193-202	YQEPVLGPVR		
k-casein	108-110	IPP	ACE inhibitor	
Isracidin	α_{s1} -casein	1-23	RPKHPIKHQGLPQEVLNENLLRF	Antibacterial-activity, immunomodulatory effects
Casocidin-l	α_{s2} -casein	165-203	KKISQRYQKFALPQYLKTVYQHQAAM KPWIQPKTKYVIPY	Antibacterial activity
Lactokinin	β -lactoglobulin	142-148	ALPMHIR	ACE inhibitor
β -lactosin	β -lactoglobulin	142-145	ALPM	ACE inhibitor
	β -casein	63-68	PGPIP	Antitumor activity
	β -casein	41-45	INKI	Antitumor activity
	α_{s1} -casomorphin	158-162	YVPPF	Antitumor activity
	Lactoferrin	17-41	FKCRRWRMKKLGAPSITCVRRAF	Antitumor activity

Table 4. Commercially bovine milk derived peptide products for Antihypertensive effects.

Product	Type of product	Bioactive peptide	Description of the study	Dose	Duration of treatment	Effect in systolic blood pressure	Product manufacture
Calpis	Sour milk	VPPIPP, derived from β -casein and k-casein	Single blind -30 Subjects with mild mild hypertension	95 ml /day [1.1mg IPP+1.5 mg VPP]	8 weeks	-14.1 mm Hg	Calpis Co., Japan
			Double blind-randomized trial in 46 hypertensive men.	160g/day [1.2mg IPP + 2.0 mg VPP]	4 weeks	-5.2 mm Hg	

Evolus	Fermented Milk	VPPIPP, derived from β -casein and k-casein	Double blinds - randomized trial in 94 subjects with mild hypertension	2X 150 ml/day 22mg IPP + 30 mg VPP	10 weeks	-4.1mmHg	Valio Oy, Finland
BioZate	Hydrolysed whey protein	β -lactoglobulin fragments	30 borderline hypertensive subjects	20 g/day	6 weeks	-8mm Hg	Davisco, USA
Cysteine Peptide C12	Ingredient / Hydrolysate	Peptides from caseins	10 hypertensive Subjects	100 or 200mg C12 With 877or 1754 mg Alginic acid	Single dose	-9mmHg	DMV International, Netherlands

CONCLUSION

From all these information it is observed that bovine milk is a rich source of bioactive peptides. These peptides have been shown to exert beneficial effects on human health. There are already a few commercially dairy products available with supplemented bovine milk derived bioactive peptides for human health benefits. The future applications of these bioactive peptides look promising in the field of food industry as food preservatives and nutraceuticals and also as natural drug in pharmaceutical industry.

REFERENCES

- Dziuba B., Dziuiba M. Milk proteins- derived bioactive peptides in dairy products: molecular, biological and methodological aspects. *Acta Sci. Pol., Technol. Aliment.* 2014; 13: 5-25.
- Korhonen H. Milk derived bioactive peptides: from science to application. *J. Functional foods.* 2009; 177-187.
- Mohanty D.P., mahapatra S., Misra S., Sahu P.S. 2015. Milk derived bioactive peptides and their impact on human health –A review. In press. <http://dx.doi.org/10.1016/j.sibs.2015.06.005>
- Atrym J. and Zimecki M. Milk derived proteins and peptides in clinical trials. *Postepy High Med Dosw.*, 2013; 67: 800-816.
- Jabbar S. Hasani R , Kafilzadeh F. and Janefeshan S. Antimicrobial peptides from milk proteins: A prospectus. *Annals of Biological research*, 2012; 3: 5313-5318.
- McGregor RA. And Poppitt SD. Milk protein for improved metabolic health: a review of the evidence. *Nutrition & metabolism* 2013; 10: 46.
- Korhonen H. and pihlanto A. Bioactive peptides: Production and functionalty. *International Dairy J.* 2006; 16: 945-960.
- <https://www.dairyscience.info/index.php/exploitation-of-anti-microbial-proteins/111-milk-protein-derived-bioactive-peptides.html>
- Jakala P. and Vapaatalo H. Antihypertensive peptides from milk proteins. *Pharmaceuticals* 3, 2010; 251-272.
- Sah B.N.P., Vasilijevic T., McKechnie S. and Donkor O.N. 2015. Identification of anticancer peptides from bovine milk proteins and their potential roles in management of cancer: A critical review. 2015; 14: 123-138.
- Mohanty D.P. Tripathy P., Mohapatra S. and Samantaray D.P. Bioactive potential assessment of antibacterial peptide produced by *Lactobacillus* isolated from milk and milk products. *Int. J. Curr. Microbio App. Sci*, 2014; 3: 72-80.
- Szwajkowska M., Wolanciuk A. Barlowska , Krol J. Litwinczuk Z. Bovine milk proteins as the source of bioactive peptides influencing the consumer's immune system – A review. *Animal Sci paper and Reports* 2011; 29: 269-280.
- Qin L.Q., Xu J.Y., Wang P.Y., Tong J., and Hoshi K. Milk consumption is a risk factor for prostate cancer in Western countries: Evidence from cohort studies. *Asia Pac Clin Nutr.* 2007; 16: 467-476.
- Larsson S.C., Andersson S.O., Johansson J.E. and Wolk A. Cultured milk, yogurt and dairy intake in relation to bladder cancer risk in a prospective study of Swedish women and men. *Am J Clin Nutr.* 2008; 88: 1083-1087.
- Dong J.Y., Zhang L., He K. and Qin L. Q. Dairy consumption and risk of breast cancer: a meta analysis of prospective cohort studies. *Breast Cancer Res Treat.* 2011; 127-23-31.
- Aune D., Lau R., Chan D.S., Vieira R., Greenwood D.C., Kampman E and Norat T. Dairy products and colorectal cancer: A systematic review and meta-analysis of cohort studies. *Ann Oncol* 2012; 23: 37-45.