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Angiotensin converting enzyme inhibitory peptides in dairy products

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Abstract
Bioactive peptides are protein sequences that remain inactive in the native protein primary structure, but when released may regulate many physiological functions. Bioactive peptides have been isolated from many protein sources such as soy proteins, fish proteins, maize and milk proteins but milk proteins appear to be the most important sources of bioactive peptides identified so far. These bioactive peptides can be released during fermentation with specific lactic acid bacteria or during gastrointestinal digestion. The most studied bioactive peptides derived from dairy proteins are anti-hypertensive or ACE inhibitory peptides. ACE is an enzyme that plays a crucial role in the function of the renin-angiotensin system which is an important regulator of blood pressure. Angiotensin converting enzyme (ACE)-inhibitory peptide blocks the conversion of angiotensin I to angiotensin II and thereby reduces the blood pressure. The ACE-inhibitors derived from milk proteins are attributed to different fragments of casein and whey proteins. The concentration of ACE inhibitory peptides in fermented dairy products can be increased by fermenting or co-fermenting with specific proteolytic strains of LAB. Thus, a great opportunity is available for the development of functional dairy products enriched with ACE inhibitory peptides that support heart health through blood pressure lowering effects.

Keywords: bioactive peptides, ACE inhibitory peptides, anti-hypertensive and dairy products

Introduction
Cardiovascular diseases (CVD) remain the major cause of death worldwide, with a further expected increase by 2030. (Agnes A. Fekete, 2015) [6]. High BP is one of the most important controllable risk factors of CVD, responsible for 13% of deaths in the world annually, and is a leading global risk for mortality. Hypertension is a chronic degenerative disease characterized by values of blood pressure exceeding the normal range (Chobanian, 2003) [2]. This can be asymptomatic, thus many hypertensive individuals are unaware of its existence. It affects more than 1 billion people worldwide (WHO, 2011). Moreover, it is an important risk factor for developing other cardiovascular diseases, strokes, renal failure and many other complications. Therefore, even a small reduction in BP of 2–5 mmHg is clinically significant and can have a major impact on health.

The rennin angiotensin system is the most important metabolic pathway in the control of blood pressure and vascular tone (Daien *et al*., 2012) [4]. The angiotensin-converting enzyme (ACE) plays a fundamental role in blood pressure, as it converts angiotensin I into angiotensin II, a potent vasoconstrictor; it also hydrolyzes the vasodilator peptides bradykinin and kallidin.

The benefits of the fermented dairy products in the diet are well accepted. The central role of microorganisms in fermentation, especially Lactic Acid Bacteria (LAB) is now widely acknowledged, and it is accepted that these microorganisms can exert beneficial effects through two mechanisms i.e. direct effects or indirect effects during fermentation where these microbes act as cell factories for the generation of secondary metabolites with health promoting properties. Among the latter the most important components in fermented milk are bioactive peptides released from milk proteins by members of the genera *Lactobacillus*, Bifidobacterium and other LAB.

Bioactive Peptides
Milk is an excellent source of well balanced nutrients and also exhibits a range of biological activities that influence digestion, metabolic responses to absorbed nutrients, growth and development of specific organs and resistance to disease. These biological activities are mainly due to the peptides and proteins in milk. Bioactive peptides have been defined as specific protein fragments that have a positive impact on body functions or conditions and may ultimately

influence health. Milk bioactive peptides consist of 2 to 20 amino acid residues that, besides being a valuable source of essential amino acids, possess specific biological properties. Some of these biological properties include mineral binding, antioxidant, antithrombotic, antimicrobial, immunomodulatory, opioid, and antihypertensive actions. These released peptides present in fermented dairy products are potential ingredients in health-promoting foods (Ricci-Cabello *et al*., 2012) [12].

Source of bioactive peptides

Milk is a rich source of protein. Casein and whey proteins are the two main protein groups in milk. Caseins comprises of about 80 percent of the total protein content in bovine milk and are divided into α, ß and κ caseins. Whey protein is composed of *ß*-lactoglobulin, α lactalbumin, immunoglobulins (IgGs), glycomacropeptides, bovine serum albumin, and minor proteins such as lactoperoxidase, lysozyme and lactoferrin. Each of the sub fractions found in casein or whey has its own unique biological properties.

Production of bioactive peptides

Bioactive peptides are inactive within the sequence of the parent protein and can be released in three ways:

a) Enzymatic hydrolysis by digestive enzymes
b) Food processing
c) Proteolysis by enzymes derived from microorganisms or plants.

a) Enzymatic hydrolysis

The cleavage of latent bioactive peptides from milk proteins normally occurs during digestion by pepsin and pancreatic enzymes (trypsin, chymotrypsin, carboxy and aminopeptidases), producing active peptide fragments in the gastrointestinal tract of the milk-consuming individual (Schlimme and Meisel, 1995) [15].

b) Food Processing

The structural and chemical changes that occur during the processing of food proteins may result in the release of bioactive peptides. In particular, heat and/or alkali treatment can generate additional inter and intra molecular covalent bonds that are resistant to hydrolysis. Bioactive peptides can be generated during manufacture of several milk products and may thus be ingested as food components.

c) Microbial fermentation

The starter cultures used by many industries are proteolytic to some extent. Bioactive peptides can be generated by the proteolytic activities of the strains of starter and non-starter bacteria such as *L. helveticus, L. delbrueckii* ssp. *bulgaricus*, *L. plantarum*, *L. rhamnosus*, *L. acidophilus, L. lactis, Streptococcus thermophilus* used in the manufacture of fermented dairy products.

Angiotensin converting enzyme (ACE) inhibitory peptides

The angiotensin is one of two polypeptide hormones and a powerful vasoconstrictor that functions in the body by controlling arterial blood pressure through the contraction of smooth muscles of the blood vessel (Park, 2009) [10]. ACE causes elevation of blood pressure by converting angiotensin-I to the potent vasoconstrictor, angiotensin- II, and by degrading bradykinin, a vasodilatory peptide and enkephalins. Angiotensin converting enzyme (ACE)-inhibitory peptide blocks the conversion of angiotensin I to angiotensin II and thereby reduces the blood pressure.

ACE Inhibitory peptides identified from milk products

Product	Origin	Bio functional role	Reference
Italian cheeses	*ß*-CN *f* (58–72)	ACE inhibitory / Antihypertensive	Smacchi and Gobbetti (1998)
Yoghurt type products	α_{s1}-, *ß*- and κ-CN fragments	ACE inhibitory	Yamamoto *et al*. (1999)
Gouda Cheese	α_{s1}-CN *f* (1–9), *ß* -CN *f* (60–68)	ACE inhibitory	
Festivo Cheese	α_{s1}-CN *f* (1–9), *f* (1–7), *f* (1–6)	ACE inhibitory	
Manchego Cheese	Ovine α_{s1}-, α_{s2} – and *ß*-casein fragments	ACE inhibitory	Gomez-Ruiz *et al*. (2002)
Sour milk	*ß*-CN *f* (74–76, *f* (84–86), κ-CN *f* (108–111)	ACE inhibitory	Nakamura *et al*. (1995a) [9]
Dahi	Ser-Lys-Val-Tyr-Pro	ACE inhibitory	-

Ace inhibitory peptides in dairy products

Most of the fermented dairy products have ACE inhibitory peptides, which is helpful for human health, especially to control the cardiovascular diseases.

Fermented Milk

Fermented milks with *Lactobacillus* strains were the most studied for the screening of ACEI activity. (Gonzalez-Cordova *et al*., 2011) [8] fermented milk for 24 h with different *Lactobacillus* strains; *Lactobacillus reuteri* 14171, *Lactobacillus fermentum* ACTC 11976, and *Lactobacillus johnsonii* ATCC 33200 exhibited 42.04 to 83.36% ACEI activity.

Lactic acid bacteria	Strains	Ic50	Reference
Leuconostoc mesenteroides,	356, 358	0.44 mg/ mL 0.48 mg/mL	Pihlanto *et al*. (2010)
Lactococcus lactis ssp. *Lactis*	ATCC 19435	0.5 mg/ mL	
Lactobacillus acidophilus	ATCC 4356	0.42 mg/ mL	
Lactobacillus jensenii	ATCC 25258	0.52 mg/ mL	
Lb. fermentum		21 mg/mL	Gonzale *et al*. (2011)
Lc. Lactis		13–50 µg/mL	Rodriguez *et al*. (2010)
Lc. Lactis	NRRL B-50571 NRRL B-50572	0.034 µg/mL 0.041 µg/ mL	Rodriguez *et al*. (2012)

The enzymatic digestion of casein and whey proteins generate peptides that have the ability to inhibit ACE. The best known ACE inhibitory peptides, Val-Pro-Pro (VPP) and Ile-Pro-Pro (IPP) with IC50 values of 9 and 5µ Moles respectively have

been identified from a Japanese sour milk drink (Calpis ®) fermented with *Lb. helveticus* and *Saccharomyces cerevisiae* strains (Nakamura *et al*., 1995) [9].

The antihypertensive effect of the sour milk product Calpis, which is commercialized in Japan (Calpis Co. Ltd., Japan), was tested in a clinical study with 46 borderline hypertensive men, not taking antihypertensive medication, revealed a significant decrease in systolic blood pressure (SBP) after 2 and 4 weeks of ingestion of 95 ml sour milk. This was equivalent to an ingested dose of ACE inhibitory peptides of about 2.6 mg per day. However, no significant change was observed as compared with the placebo unfermented acidified milk group. In that study, serum levels of angiotensin I and II were measured at 4 weeks, but the angiotensin I/angiotensin II ratio did not show a significant change.

Curd

Curd (*Dadhi*) is a fermented milk product, which stimulates taste bud, acts as an appetizer and used in conditions like loss of taste, dysuria, and chronic rhinitis. As it absorbs water from intestines, it is widely used to treat diarrhoea and dysentery and it has high calcium content. It is suggested that small peptides are absorbed from the gastrointestinal tract without being decomposed by digestive enzymes. Curd peptides reduced hypertension by inhibiting angiotensin converting enzyme (ACE) and serum cholesterol. Peptides vary with bacterial species and milk type used during fermentation.

In order to study the ACE inhibitory activity, Dabarera *et al*., (2015) [3] had subjected two brands of market curd samples to enzymatic digestion with pepsin, trypsin, and carboxy peptidase at their optimum pH and temperature for 24 h. The result showed that peptide with the highest ACE inhibitory activity was a pentapeptide in both brands with brand 2 having 90% inhibition and brand 1 having 73% inhibition. The sequence of the pentapeptide closely matched with Tyr-Gly-Gly-Phe-Met standard. In brand 2, four fractions gave more than 50% ACE inhibition, whereas in brand 1, two fractions gave more than 50% inhibition. Amino acid and di peptide concentrations were higher in brand 1 and penta to octapeptide concentration was higher in brand 2. Digested peptides gave a higher ACE inhibitory activity than undigested peptides. Nine fractions showed more than 70% inhibition in both brands. Highest ACE inhibition of 96% was obtained for a dipeptide. Peptide concentration was higher in brand 2 than brand 1.

Sample	ACE Inhibition %	Peptide	Peptide concentration
Brand 1	73	Amino acid and di peptide	Lower
Brand 2	90	penta to octapeptide	Higher

Cheese

Cheese contains phosphopeptides as natural constituents and extensive proteolysis during cheese ripening leads to the formation of other bioactive peptides such as ACE-inhibitory, antioxidant, antimicrobial and immunomodulatory peptides. ACE inhibitory peptides have been separated from several Italian cheese varieties characterized by short (Crescenza and Italico) and medium (Ghorgonzola) ripening period.

Cheddar Cheeses

Cheddar cheeses made with adjunct cultures *L. casei* ssp. *casei* 300 and *L. paracasei* ssp *paracasei* 22 were assessed for ACE inhibitory activity at different stages of ripening. ACE-inhibitory activity of the UF permeates of WSE of the cheeses increased significantly (P<0.05), especially after the first two months of ripening. The inhibitory activity continued to increase in the cheeses made with adjunct cultures (IC50 value 0.16 $mg.ml^{-1}$ and 0.20 $mg.ml^{-1}$) up to third month. The results indicated that cheeses with the addition of adjunct cultures had higher ACE-inhibitory activity than control cheese.

Lassi

Padghan *et al*. (2013) reported ACE inhibitory activity of caseino phosphopeptides (CPPs) in traditional lassi. The lassi contains mainly strains of *Lactobacilli* and *Lactococcus.* ACE inhibitory activity of lassi samples ranged from 38.81–453.25µg/ml. The average inhibitory activity (IC_{50}) was 152.53µg/ml.

Butter Milk

Gille *et al.* (2011) [5] reported that the angiotensin-converting enzyme (ACE) inhibiting casein-derived peptides called casokinins seem to be responsible for the anti hypertensive effect of buttermilk. 20g of hydrolysated caseins per day, which is, however, a large amount of proteins, reduced the diastolic and systolic blood pressure significantly in hypertensive and normotensive humans. A supplementation of whey proteins for 12 weeks resulted in a reduced systolic and diastolic blood pressure in comparison to the baseline levels and control group (glucose). Whey proteins also release bioactive peptides with possible antihypertensive properties.

Yoghurt

Shakerian *et al*. (2013) [16] reported that regular and probiotic yogurts showed considerable ACE-inhibitory activities. Results revealed that both probiotic and regular yogurt samples inhibited the activity of ACE enzyme with relative inhibition ranging from 72.5 % to 100 %. IC50 ranged from 0.95 to 3.46 mg/ml.

Commercially developed functional dairy products with ace inhibitory peptides.

Brand name	Manufacturer	Description of product	Peptide sequence
Ameal S	Calpis Co.(Tokyo, Japan)	Fermented skim milk by *La. helveticus* and *Sa. cerevisiae*	Val-Pro-Pro Ile-Pro-Pro
Calpis	Calpis Co.	Fermented milk by *Lb. helveticus* and *S. cerevisiae*	Val-Pro-Pro Ile-Pro-Pro
Danaten2	Danone (Paris, France)	Fermented milk by *Lb. helveticus*	
Evolus	Valio (Helsinki, Finland)	Fermented skim milk by *La. helveticus* LBK-16H	Val-Pro-Pro Ile-Pro-Pro

(Beltran-Barrientos *et al.*, 2016) [1]

Conclusion

Milk proteins are a good source of bioactive peptides, which are latent or encrypted within the native protein. Bioactive peptides can be generated by the proteolytic activities of the strains of starter and non-starter bacteria. Most of the fermented dairy products contain ACE inhibitory peptides, which can reduce the blood pressure and thereby can prevent the cardiovascular diseases. The concentration of ACE inhibitory peptides in fermented dairy products can be increased by fermenting or co-fermenting with highly specific proteolytic strains of LAB.

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