Antihypertensive Peptides in Dairy Products

M Zeki Durak1* and Neslihan Atlıhan Turan2

1Department of Food Engineering, Yildiz Technical University, Turkey
2Central Research Institute of Food and Feed Control, Turkey

*Corresponding author: M Zeki Durak, Department of Food Engineering, Chemical and Metallurgical Engineering Faculty, Yildiz Technical University, Davutpasa Campus, 34210 Esenler, Istanbul, Turkey.

To Cite This Article: M Zeki Durak, Antihypertensive Peptides in Dairy Products. 2020 - 7(2). AJBSR.MS.ID.001139.
DOI: 10.34297/AJBSR.2020.07.001139.

Received: February 03, 2020; Published: February 11, 2020

Abstract

Dairy products are a fundamental source of protein. Also, they are popular foods for the intake of bioactive peptides with their sensory properties and high consumption. Among various bioactivities, antihypertensive activity has special importance due to high blood pressure, which has become a growing health problem in recent years. Milk proteins are the main precursors for ACE inhibitor peptides. The antihypertensive effect of a few bioactive peptides found in dairy products has been confirmed in vivo, and a few commercial products have shown to reduce blood pressure. This review discusses the studies about the antihypertensive specifically ACE inhibitor peptides found in milk and dairy products as an essential source of bioactive peptides.

Keywords: Bioactive Peptides; Milk; Dairy Products; ACE Inhibitor; Antihypertensive

Introduction

Milk and dairy products are rich in protein content, and main proteins in milk casein and whey proteins are the principle bioactive peptide precursors [1]. Although there are numerous studies on bioactive peptides, the first discoveries of bioactive peptides from foods are dairy products. These bioactive peptides have different functions such as antihypertensive, opioid, immune-modulatory, antimicrobial, and antioxidant [2-5]. Hypertension is a condition in which the blood vessels have persistently raised pressure [6]. It affects approximately 30% of the adult population worldwide [7]. It is a prominent risk factor for cardiovascular diseases such as coronary heart disease, peripheral artery disease, and stroke. Even though it is a controllable disease high prevalence, and serious consequences make hypertension an important comprehensive health threat [7].

ACE (peptidyldepeptide hydrolase, EC 3.4.15.1) is an exopeptidase which splits various peptides from C-terminal and forms dipeptides. ACE performs a critical role in regulating the blood pressure by the renin-angiotensin and bradykinin pathways. Angiotensin I is a decapeptide and inactive in its intact form. ACE catalyzes hydrolyzation of angiotensin I to the potent vasoconstrictor octapeptide angiotensin II [8]. Bradykinin, a vasodilator, is involved in the blood pressure system. ACE also controls the blood pressure by degrading bradykinin. Thus, inhibition of ACE results in an antihypertensive effect. Consequently, synthetic ACE inhibitors such as captopril and enalapril, are used in the treatment of hypertension and other related heart diseases [9]. However, they can cause adverse effects such as hypotension, cough, altered taste, rash and angioedema [10]. Bioactive peptides are natural and healthier alternatives to synthetic ACE inhibitors without side effects [11]. Although there are many different protein sources of ACE inhibitor peptides, milk proteins are accepted as the primary sources [12]. Chemical structures of peptides are important for binding to the catalytic sites of ACE [13,14]. The presence of aromatic or branched hydrophobic structures in the tripeptide structure at the carbon end of the peptide is necessary for binding [13,15]. Several functional dairy products are present in the market comprising antihypertensive peptides. Calpis sour milk in Japan (Calpis Co Ltd, Tokyo, Japan) cultured via Saccharomyces cerevisiae and Lactobacillus helveticus poses two potent ACE inhibitory peptides, Val-Pro-Pro and Ile-Pro-Pro [15]. These two peptides are also present in calcium-enriched fermented milk drink in Finland [16]. In short- and long-term human studies have shown that IPP and VPP peptides decrease blood pressure [4]. BioZate contains β-lactoglobulin fragments as functional bioactive peptides [16].
This paper will review studies on antihypertensive especially, ACE inhibitor peptides and their production in dairy products.

Factors Affecting Occurrence of Bioactive Peptides in Dairy Products

The bioactive peptide profile of a dairy product is tightly dependent on processes used such as thermal processes, homogenization, pressure applications, coagulation of milk, fermentation, and ripening [17]. Thermal processes are essential in the production of almost all dairy products. Reactions that occurred during thermal processes can affect the structure of proteins and the bioactive peptide content of the product [17,18]. Thermal processes affect activities of natural enzymes found in milk, thus affect the peptide profile of the last product. Caseins are hydrolyzed through the action of enzymes from different sources such as casein residue coagulants, natural milk enzymes, starter culture enzymes, enzymes of second cultures and non-starter lactic acid bacteria [5].

ACE Inhibitor Peptides Naturally Found in Milk and Dairy Products

In general, dairy products, in particular, fermented dairy products, are the most popular foods for the intake of bioactive peptides with their sensory properties and high levels of consumption favored by consumers [1]. Some of these studies summarized in Table 1. Among the dairy products, ripened cheeses contain numerous peptides, affecting the properties of the final product such as taste, odor, and texture due to the variety and complexity of the production methods. ACE inhibitor peptides in Spanish cheeses (Cabrales, Idiazábal, Roncal, Manchego, Mahón and goat’s milk) are identified [14]. In this study, researchers confirmed ACE inhibition effect of 8 synthetic peptides (VRGP, PFP, QP, DKIHP, PKHP, FP, PP, and DKIHPF). Since proteolysis and peptide formation continue during cheese ripening, the ACE inhibitor effect may alter during the cheese maturation period. Further proteolysis during ripening may cause hydrolyzation of bioactive peptides and inactivation of them. Gomez-Ruiz et al. [19] determined the ACE-inhibitor peptides in Manchego cheese. The antihypertensive activity reached the maximum level after eight months of maturation and decreased again after twelve months of maturation. Likewise, Gouda ripened for 8 months decreased more strongly the blood pressure of spontaneously hypertensive rats than 24-month-old Gouda, although they have a similar ACE inhibitor activity in vitro [3]. In view of composition rich in proteins, cheese whey can be considered as a valuable source of bioactive proteins [20]. Alongside studies on bioactivities of cheese varieties some researchers identified ACE inhibitor peptides (FVAPFPE, NLHLPLPLLQ, FVAPFPEVFG, NLHLPLPLLQ originated from αs1-casein, β-casein, αs1-casein, β-casein, respectively) in a liquid waste deriving from Ricotta cheese production [21]. Probiotic fermented milk beverage from milk of different species also have antihypertensive activity [22,23]. Caseins are the best precursors for the production of angiotensin I converting enzyme (ACE) [de Gobba et al. 2014].

| Table 1: Antihypertensive peptides found in dairy products. |
|-------------------------------------------------------------|
| **Dairy Product**                                           | **Peptide Sequence and Precursor Protein** | **Bioactivity**                          | **Reference** |
| Gouda, Emmental, Blue, Camembert, Haveriti Cheese          | RPKHPKHIQ αs1-casein (f1–9), RPKHPKHIQGLPQ αs1-casein (f1–13), YPPPGPIN β-casein (f60–68), MPFPKYPVQPF β-casein (f109–119) | ACE inhibitor activity in vivo and in vitro | 3             |
| Commercial caprine kefir                                   | PYVRYL and LVYPFTGIPPN β-casein            | ACE inhibitor effect of peptides similar after simulated gastrointestinal digestion | 23            |
| Cabrales, Idiazábal, Roncal, Manchego, Mahón and goat’s milk cheese (Spain) | VRGP, PFP, QP, DKIHP, PKHP, FP, PP, DKIHPF | ACE inhibitor activity concentrated in the permeate of molecular weight <1000 kDa | 14            |
| 39 Swiss cheeses and matured Gouda, Allgauer Limburger, Munster, Reblochon, Gorgonzola, Roquefort, Manchengo, Feta Cheese | IPP and VPP | The quantities of ACE inhibitor peptides IPP and VPP is higher in ripened cheeses produced from raw milk | 4             |
| Different types of olive cheeses from Southern Brazil and Uruguay | KEMPFPKYPVE β-casein f(122–132) | ACE inhibitor | 24            |
| Scotta (liquid waste from Ricotta cheese production)       | FVAPFPEVFG αs1-casein (f24–33), FVAPFPEVFGK αs1-casein (f23–34), YQEPVLGVRGFPPIVβ-casein (f193–209) | Known ACE inhibitor peptides identified by using peptidomics approach | 21            |
| Kefir                                                      | EMPPFPKYPVEPF, FVAPFPEVFG, KVGINYWLHAK, VAPFPEVFGK | Antihypertensive effect in vivo | 22            |
Production of ACE Inhibitor Peptides from Milk Proteins

Basically, there are two approaches to generate ACE-inhibitor peptides from milk proteins. One approach is to utilize the proteolytic enzymes of lactic acid bacteria in fermented dairy products. The other approach is to hydrolyze milk proteins in vitro by one protease or a combination of various proteases or peptidases.

Production of ACE Inhibitor Peptides with Enzymes

Most of the researches about the production of bioactive peptides with enzymes have utilized digestive enzymes, and commercial dry cheese whey, purified whey proteins or microfiltration permeates as a substrate [27]. Besides, other digestive enzymes from different sources and various milk protein preparations have been studied to generate antihypertensive peptides (Table 2). Different bioactive peptides are produced from caseins of milk from different species, which implicates the sequence and conformation of the caseins affect the bioactive peptide yield [28]. Minervini et al. [28] used a protease from Lactobacillus helveticus PR4 to obtain ACE inhibitor and antimicrobial peptides from casein of milk from six different species (bovine, sheep, goat, pig, buffalo, and human). Abdel-Hamid et al. [29] identified new peptide sequences (FPGPIP, IPPK, QPPQ) showing ACE inhibitor activity generated from buffalos’ skim milk hydrolyzed with pepsin.

Table 2: Using proteases to generate ACE inhibitor peptides from milk proteins.

| Protease                        | Substrate       | Hydrolysis and/or Fermentation Conditions | Peptides                                                                                     | Reference |
|---------------------------------|-----------------|------------------------------------------|------------------------------------------------------------------------------------------------|-----------|
| Lactobacillus helveticus PR4    | Bovine, sheep, goat, pig, buffalo, and human milk | pH 4.6, 35°C, 10 min                      | Bovine LVYPFPGPIPNSLPQNIIP; LVYPFPGPIPNSLQPNIIPP; LVYPFPGPIPNSLQPNIIPP β-casein                  | 28        |
| Protease from Bacillus sp. P7   | Sheep cheese whey | pH 8.0, temperature 45°C, 0.5,1,2,3,4,5,6 hour | LAFNPFQLEGQCHV (β-lactoglobulin, f 149-162)                                                   | 20        |
| Papain, pepsin, and trypsin     | Buffalo milk    | pH 6, 8, 2 for papain, trypsin, and pepsin, respectively, temperature 37°C, 4 hour | IPPK (k-casein), IPNP, AdFP (α1-casein), QPPQ, DMPQ, LVYPFPG, β-casein TVDQHQ TVDQH QPQ         | 29        |
| L. helveticus strain 881315 and Flavourzyme® | Skimmed milk | 37 °C, 12 h                              | IPPK (k-casein), IPNP, AdFP (α1-casein), QPPQ, DMPQ, LVYPFPG, β-casein TVDQHQ TVDQH QPQ         | 1         |
| Trypsin                         | Cheese whey     | pH 7.5-9.0, temperature 37°C-50°C, 1 hour | IPPK (k-casein), IPNP, AdFP (α1-casein), QPPQ, DMPQ, LVYPFPG, β-casein TVDQHQ TVDQH QPQ         | 27        |

ACE inhibitor and antioxidant capacity of 6 synthetic peptides (WY, WYS, WYSL, WYSAL, WYSALM, WYSALMA) deriving from β-lactoglobulin were evaluated [30]. Dipeptide WY β-lactoglobulin fragment f 19-20 showed potent ACE inhibitor activity. ACE inhibitor activity depends on the amino acid sequence in the C-terminus of the peptide, and the amino acid Ser at the C-terminus showed a potential decreasing effect on ACE inhibitor activity. Sheep cheese whey hydrolysed using protease from Bacillus sp. P7 to generate ACE inhibitor peptides [20]. ACE inhibitor activity was dependent on hydrolysis time. In a recent work, trypsin from bovine pancreas employed to hydrolyze whey from the production of panela cheese to generate bioactive peptides [27]. The researchers found a significant correlation between antioxidant and ACE inhibitor activity.

Production of ACE Inhibitor Peptides through Fermentation

In the dairy industry mainly highly proteolytic starter cultures are preferred. Bioactive peptides can be generated by the starter culture or non-starter bacteria added as a adjunct culture (Table 3).

Table 3: Obtaining ACE inhibitor peptides by using adjunct culture and fermentation.

| Microorganism                      | Dairy Product | Peptides                                                                                     | Reference |
|------------------------------------|---------------|------------------------------------------------------------------------------------------------|-----------|
| Bifidobacterium longum 1941, Lactobacillus casei 279, Lactobacillus acidophilus 4962, Bifidobacterium animalis subsp. lactis LAFTI® B94, L.caseiLAFTI®L26, L. acidophilus LAFTI® L10 | Cheddar | ARHPHPH k-casein (f 96-102), RPKHPKHQH αs1-casein (f 1-9), RPKHPKHQH αs1-casein (f 1-7), RPKHPKHQH αs1-casein (f 1-6), FVAPPEVPF αs1-casein (f 24-32), YQEPVLGPVGRPPHPHP β-casein (f 193-209) | 32        |
| L. casei 279, L. casei LAFTI PL26 | Cheddar | RPKHPKIKHQ αs1-casein (f1–9), RPKHIPIK αs1-casein (f1–7), RPKHIPIK αs1-casein (f1–6), DKIHFP β-casein (f47–52), PVAFPFPVF αs1-casein (f24–32), KKYKVPQ LLE αs1-casein (f102–110), YQEPVLGPVRGPFPFIV IV αs1-casein (f193–209) | 13 |

| Lactococcus. lactis ssp. lactis, Enterobacter faecium, L. casei, and 1:1 mixtures of Lc. lactis ssp. lactis–L. casei and Lc. Lactis ssp. lactis–E. faecium | Fresco cheese (Mexi-co) | YQEPVLGPVRGPFPFIV IV, YQEPVLGPVRGPFPFIV, YQEPVLGPVRGPFPFIV (β-casein), PVAFPFPVF, EVLNLNLRG, RPKHPIHQ–LPOYE, RPKHIPIHQ–LPOYE, EVLNLRG, PVAFP–PEVFGK, EVLNLNLRG | 33 |

| Kluyveromyces marxianus | Fermented milk | VLSRYP (κ-casein f31–36) and LRFP (αs1-casein f21–24) | 12 |

| Set of L. casei, Lactobacillus rhamnosus Lactobacillus paracasei non-starter strains | Fermented milk | IPP and VPP (quantification) | 31 |

| L. casei PRA205 and L. rhamnosus PRA331 | Yoghurt | IPP and VPP (quantification) | 34 |

| L. helveticus LH-B02 | Prato cheese | GQPVLPVRGFPFPFII β-casein (f193–206) and GQPVLPVRGFPFPFIV β-casein (b194–209) | 5 |

| L. acidophilus 2499 L. rhamnosus 489, Lactobacillus delbrueckii 490, L. casei 2639 | Dutch type cheese | - | 35 |

Ahlesh et al. [1] produced a new fermented functional dairy product with combination of L. helveticus and Flavourzyme® using a bioreactor. They have achieved to obtain an acceptable product with high ACE inhibitor activity. L. helveticus is a highly proteolytic bacterium, thus, there are many studies on both fermentation with this bacterium and hydrolysis with proteinases of this bacterium [22,28].

Similarly, researchers utilized L. helveticus LH-B02 strain in order to improve the ACE inhibitor activity in Prato cheese [5]. They observed that levels of ACE inhibitor peptides β-casein (f193-206) and β-casein (f194-209) increased while relative intensity of αs1-casein (f1-9) reduced. Gonzalez Gonzalez et al. [25] isolated highly proteolytic lactic acid bacteria from Chiapas cheese and evaluated the tendency of releasing bioactive peptides of selected strains. They employed four selected strains for fermentation of milk and observed that most proteolytic strain has lowest ACE inhibitor activity, presumably according to further breakdown peptides to inactive amino acids. Solieri et al. [31], fermented bovine milk with non-starter lactic acid bacteria (Lactobacillus casei, Lactobacillus paracasei and Lactobacillus rhamnosus strains) to evaluate their potential to produce fermented milk with enhanced ACE inhibitor activity [32-35]. They concluded that the strains used in the study especially L. casei PRA205 can produce high amounts of VPP and IPP peptides.

**Conclusion**

In recent years, the tendency to consume functional health-promoting foods has increased the interest in bioactive peptides. There are numerous studies on bioactive peptides in foods in the literature. Dairy products, which are an indispensable part of a healthy and balanced diet, are considered as ideal sources for bioactive peptides and natural alternatives to therapeutic drugs due to their high protein content and technological processes in production. However, the mechanism of action of bioactive peptides is not fully described. Molecular studies employing new technologic enhancements and peptidomics approach are necessary to understand the mechanisms of antihypertensive peptides as well as to design functional products.

**References**

1. Ahlesh FB, Stojanovska L, Apostolopulo V (2015) Processing and sensory characteristics of a fermented low-fat skim milk drink containing bioactive antihypertensive peptides, a functional milk product. International Journal of Dairy Technology 71(2): 230-239.
2. De Simone C, Picariello G, Mamone G, Stusso P, Dicitore A, et al. (2009) Characterisation and cytomodulatory properties of peptides from Mozzarella di Bufala Campana cheese whey. J Pept Sci 15(3): 251-258.
3. Saito T, Nakamura T, Kitazawa H, Kawai Y, Itoh T (2000) Isolation and structural analysis of antihypertensive peptides that exist naturally in Gouda cheese. J Dairy Sci 83(7): 1434-1440.
4. Böttikofer U, Meyer J, Sieber R, Wechsler D (2007) Quantification of the angiotension-converting enzyme-inhibiting tripeptides Val-Pro-Pro and Ile-Pro-Pro in hard, semi-hard and soft cheeses. International Dairy Journal 17(8): 968-975.
5. Baptista DF, Galli BD, Caralheiro FG, Negro E, Eberlin MN, et al. (2018) Lactobacillus helveticus LH-B02 favours the release of bioactive peptide during Prato cheese ripening. International Dairy Journal 87: 75-83.
6. WHO (2013) A global brief on Hypertension? p. 40.
7. Jäkäla R, Vapaatalo H (2010) Antihypertensive Peptides from Milk Proteins. Pharmaceuticals 3(1): 251-272.
8. Sagardia I, Iboro I, Bortza F, Bald C (2013) Quantitative structure/reactivity relationship-based screening of bioactive peptides identified in ripened cheese. International Dairy Journal 33: 184-190.
9. Alemán A, Giménez E, Pérez Santín M, Gómez Guillén C, Montero P, et al. (2011) Contribution of Leu and Hyp residues to antioxidant and ACE-inhibitory activities of peptide sequences isolated from squid gelatin hydrolysate. Food Chemistry 125: 334-341.
10. López Fandiño R, Otte J, van Camp J (2006) Physiological, chemical and technological aspects of milk-protein-derived peptides with antihypertensive and ACE-inhibitory activity. International Dairy Journal 16(11): 1277-1293.
11. Shi M, Ahlesh A, Mathai M, McAineh J, Xiao Q, et al. (2017) Effects of fermentation conditions on the potential antihypertensive peptides...
reduced from yogurt fermented by Lactobacillus helveticus and Flavourzyme®. International Journal of Food Science and Technology 52(1): 137-145.
12. Li Y, Sadiq FA, Liu TJ, Chen JC, He GQ, et al. (2015) Purification and identification of novel peptides with inhibitory effect against angiotensin-I-converting enzyme and optimization of processing conditions in milk fermented with the yeast Kluyveromyces marxianus. Journal of Functional Foods 16: 278-288.
13. Ong L, Henriksson A, Shah NP (2007) Angiotensin converting enzyme-inhibitory activity in Cheddar cheeses made with the addition of probiotic Lactococcus casei ssp. Lait 87(2): 149-165.
14. Gómez Ruiz JA, Taborda G, Amigo L, Recio I, Ramos M, et al. (2006) Identification of ACE-inhibitory peptides in different Spanish cheeses by tandem mass spectrometry. Eur Food Res Technol 223: 595-601.
15. Hernández Ledesma B, Miliales B, Amigo L, Ramos M, Recio I, et al. (2005) Identification of antioxidant and ACE-inhibitory peptides in fermented milk. Journal of the Science of Food and Agriculture 85(6): 1041-1048.
16. Korhonen H, Pihlanto A (2006) Bioactive peptides: Production and functionality. International Dairy Journal 16: 945-960.
17. Santiago López L, Aguilar Toalá JF, Hernández Mendoza A, Vallejo Córdoba B, Leuga AM, et al. (2018) Invited review: Bioactive compounds produced during cheese ripening and health effects associated with aged cheese consumption. J Dairy Sci 101(5): 3742-3757.
18. Silva RA, Lima MSF, Viana JBM, Bezerra VS, Pimentel MCB, et al. (2012) Can artisanal “Coelho” cheese from Northeastern Brazil be used as a functional food. Food Chem 135(3): 1533-1538.
19. Goméz Ruiz JA, Ramos M, Recio I (2002) Angiotensin-I-converting enzyme-inhibitory peptides in Manchego cheeses manufactured with different starter cultures. Int Dairy J 12: 697-706.
20. Corrêa APF, Daroit DJ, Fontoura R, Meira SMM, Segalin J, et al. (2014) Hydrolysates of sheep cheese whey as a source of bioactive peptides with antioxidant and angiotensin-converting enzyme inhibitory activities. Peptides 61: 48-55.
21. Somella E, Pepe G, Ventre G, Pagano F, Conte GM, et al. (2016) Detailed peptide profiling of “Scotta”: from a dairy waste to a source of potential health-promoting compounds. Dairy Science & Technology 96(5): 763-771.
22. Amorim FG, Caltinho LB, Dias AT, Priques AGF, Monteiro BL, et al. (2019) Identification of new bioactive peptides from Kefir milk through proteoepitomics: Bioprospection of antihypertensive molecules. Food Chemistry 282: 109-119.
23. Quírolo A, Hernández Ledesma B, Ramos M, Amigo L, Recio I, et al. (2005) Angiotensin-I-converting Enzyme Inhibitory Activity of Peptides Derived from Carpine Kefir. J Dairy Sci 88(10): 3480-3487.
24. Meira SMM, Daroit DJ, Hlefer VE, Corrêa APF, Segalin J, et al. (2012) Bioactive peptides in water-soluble extracts of ovine cheeses from Southern Brazil and Uruguay. Food Research International 48: 322-329.