Milk proteins and hypertension

GÜLFEM ÜNAL*, A. SİBEL AKALIN
*Corresponding author
Ege University Faculty of Agriculture
Department of Dairy Technology
Bornova, İzmir, 35100, Turkey

INTRODUCTION

In recent years, the role of proteins in the diet as physiologically active components has been increasingly acknowledged (1, 2). Milk proteins are currently the main source of a range of biologically active peptides (3). Enzymatic hydrolysates of milk proteins produce various physiologically functional peptides such as opioid peptides, immunostimulating peptides, and angiotensin-I-converting enzyme inhibitors (4). Angiotensin-I-converting enzyme (ACE) is a key enzyme in the regulation of blood pressure. ACE, a dipeptide-liberating carboxypeptidase (peptidylpeptide hydrolase), classically associated with the renin-angiotensin system, converts angiotensin I into angiotensin II, a highly potent vasoconstrictor molecule. Therefore it helps to reduce hypertension by lowering blood pressure (5, 6, 7, 11, 21). Dairy products such as fermented milk, yoghurt and cheese contain bioactive peptides having ACE inhibitory activity. Although chemical and physical treatments may have an influence, proteolysis by naturally-occurring enzymes in milk-by exogenous enzymes and by enzymes from microbial starters such as lactic acid bacteria (LAB)- is mainly responsible for the generation of bioactive peptides during dairy processing (8, 9). Some studies have demonstrated the beneficial effects of lactic acid bacteria and certain dairy foods on hypertension reduction (10, 12). The proteolytic system of lactic acid bacteria, such as Lactococcus lactis, Lactobacillus helveticus and Lactobacillus delbrueckii subsp. bulgaricus, consists of a cell wall-bound proteinase and several intracellular peptides. Depending on the proteolytic activity, the type of lactic acid bacteria starter used is one of the main factors that influences the synthesis of hypotensive peptides in dairy products (3, 25).

Besides in vitro studies, various in vivo studies were held to determine the antihypertensive effect of milk protein-derived peptides (13, 14, 15). This article will review the current knowledge about ACE inhibitory activity of milk protein-derived peptides and their production, with emphasis on their effect in dairy products.

PRODUCTION OF ACE INHIBITORY PEPTIDES

There are a number of methods by which ACE inhibitory peptides can be produced from milk proteins. The most common ones are (a) enzymatic hydrolysis with digestive enzymes, (b) fermentation of milk with proteolytic starter cultures and action of enzymes derived from these microorganisms.

Enzymatic hydrolysis of proteins has been the most common way to produce bioactive peptides. Pancreatic enzymes preferably trypsin, have been used for the chemical characterization and identification of many known bioactive peptides (1, 22). It has been reported that enzyme source, enzyme concentration and hydrolysis time affect ACE inhibiting activity (23). To generate functional peptides from milk proteins, bovine casein is an excellent source for the processing of these peptides. There are some trials to isolate ACE inhibitory peptides from casein molecules by some endopeptidase (proteinase) and exo-peptidase digestions. Study of ACE inhibitory peptide from whey proteins is limited because β-lactoglobulin, a major component of whey, has rigid structure and shows resistance to digestive enzymes (4, 24). However, some ACE inhibitory peptides produced from whey proteins by enzymatic digestion were reported (6, 9). Many industrially used dairy starter cultures are highly proteolytic. Dairy industry benefit from this property which contribute to the typical flavor, aroma and texture of the products. Besides these properties, physiologically active peptides are also obtained from the proteolysis (1). Proteolytic system of lactic acid bacteria has been studied in recent years. Lactic acid bacteria have extracellular proteinase and can release bioactive peptides from milk proteins (4, 10). The proteolytic system of lactic acid bacteria provides transport systems specific for amino acids, di- and tripeptides and oligopeptides of up to 18 amino acids. Longer oligopeptides which are not transported into the cells can be a source for the liberation of bioactive peptides in fermented milk products when further degraded by intracellular peptidases after cell lysis (1). Production of ACE inhibitory peptides by microbial fermentation has been reported in many
studies (26, 27, 28). The hydrolysate of casein by the extracellular protease demonstrated antihypertensive activity in spontaneously hypertensive rats (SHR) at a dosage of 15 mg / kg oral administration, but control typic digested peptides did not show the effect (18). A potent antihypertensive peptide, Lys-Val-Leu-Pro-Val-Pro-Gln, was purified and identified from casein hydrolysate produced by a protease from \textit{L. helveticus} CP790 (39).

Nakamura et al. (15, 16) identified two ACE inhibitory peptides (Val-Pro-Pro, Ile-Pro-Pro) in milk fermented with a starter culture composed of \textit{L. helveticus} and \textit{Saccharomyces cerevisiae}. Pihlanto-Leppälä et al. (17) studied the potential formation of ACE inhibitory peptides from cheese whey and caseins during fermentation, with various commercial lactic acid starters used in the manufacture of yoghurt and sour whole milk. ACE inhibition activity was observed only after the digestion of proteins with pepsim and trypsin. The inhibition rate for whey proteins was 35-61 percent whereas it was 86 percent for caseins. Yamamoto et al. (14, 18) obtained antihypertensive peptides from both casein and whey proteins by extracellular protease from different strains of (CPN4, CP790) \textit{Lactobacillus helveticus}. Mullahly et al. (19, 20) investigated enzymatic hydrolysis of bovine \textit{β}-lactoglobulin, \textit{α}-lactalbumin and whey protein concentrate for their ACE inhibitory activity. The unhydrolysed substrates gave very low ACE inhibitory activity whereas hydrolysis of whey proteins by pepsim, trypsin, chymotrypsin resulted in high ACE inhibition indices.

**ANTIHYPERTENSIVE EFFECTS OF MILK PROTEIN HYDROLYSATES AND DAIRY PRODUCTS**

Although chemical and physical treatments may have some influence, proteolysis by naturally occurring enzymes in milk, exogenous enzymes, and enzymes from microbial starters such as lactic acid bacteria can potentially generate bioactive sequences from milk protein precursors during dairy processing (29). The antihypertensive potential of milk protein-derived peptides is dependent on the ability of these peptides to reach their target site without being degraded and as a consequence inactivated by the action of intestinal or plasma proteases (5). Besides dairy products, milk protein hydrolysates are also used as functional food ingredients because of being source of hypotensive peptides, and took their places in the market. These hydrolysates are either casein or whey protein hydrolysate and manufactured by different companies (43). There are some studies that hypotensive effects of milk protein hydrolysates have been demonstrated with human and SHR trials. In these studies hydrolysates have been reported to reduce both diastolic and systolic blood pressure when certain amount of hydrolysate was consumed per day. Furthermore according to the results of \textit{in vivo} studies anti-hypertensive effects were attributed to casokinins. ACE inhibitory peptides may be released during proteolysis of milk proteins by lactic acid bacterial cell-bound proteases and intracellular peptidases during the production of fermented milks or cheese ripening (43). ACE inhibitory peptides obtained from dairy products have been studied. Nakamura et al. (16) purified ACE inhibitory peptides from 'Calpis', a Japanese fermented sour milk drink made from skim milk fermented by \textit{L. helveticus} and \textit{Saccharomyces cerevisiae}. It was reported that these peptides had produced during fermentation. Gobberti et al. (29) produced ACE inhibitory peptides in fermented milks started by \textit{Lactobacillus delbrueckii} subsp. \textit{bulgaricus} SS1 and \textit{Lactococcus lactis} subsp. \textit{cremonis} FT4. The study showed the certainty the production of casein-derived ACE inhibitory peptides by \textit{Lactobacillus delbrueckii} subsp. \textit{bulgaricus} and \textit{Lactococcus lactis} subsp. \textit{cremonis}. The antihypertensive activity in SHR was investigated with casein-enriched milk unfermented and fermented by the two strains of \textit{L. helveticus}. Caseinate-enriched milk fermented by both strains showed higher proteolysis and ACE inhibitory activity, indicating that ACE inhibitory peptides are probably released from caseins during milk fermentation. Significant decreases in mean arterial blood pressure in SHR were also measured following oral administration of caseinate-enriched milk unfermented at certain body weight and dose (30).

In a randomized placebo-controlled study, 39 hypertensive patients received 150 ml / d of either \textit{L. helveticus} LBK-16H fermented milk or a control product for 21 weeks. \textit{L. helveticus} LBK-16H fermented milk containing bioactive peptides in normal daily use showed a blood pressure-lowering effect in hypertensive subjects (13). So it was reported that this fermented milk is thus potentially useful in the dietary treatment of hypertension. Nakaya et al. (31) investigated the effects of \textit{L. helveticus} fermented milk whey and its components on bone cells \textit{in vitro} and found that these components and peptides formed, increased osteoblastic bone formation. This positive effect is attributed to the ACE inhibitory peptides produced from \textit{L. helveticus} fermented milk whey. Suitability of soy yoghurt as a system for delivering probiotics and other bioactive compounds was assessed by fermenting soy milk using starter culture containing \textit{Lactobacillus delbrueckii} subsp. \textit{bulgaricus} Lb1466, \textit{Streptococcus thermophilus} St1342, and probiotic organisms (\textit{Lactobacillus acidophilus} LAFTI L10, \textit{Bifidobacterium lactis} LAFTI B94, and \textit{Lactobacillus paracasei}, LAFTI L261). The use of probiotic strains as a part of starter culture in soy yoghurt resulted in a substantial increase in \textit{in vitro} ACE inhibitory activity compared with the control produced by yoghurt culture only. This improvement of ACE inhibition in soy yoghurt is attributed to higher proteolytic activity of probiotics (12).

ACE inhibitory peptides were also obtained from kefir made from caprine milk. The low molecular mass peptides released from caseins during fermentation were found mainly responsible for this activity. Some of these peptides were resistant to the incubation with pepsim followed by hydrolysis with Corolase PP. The ACE inhibitory activity after simulated digestion was similar to or slightly lower than unhydrolyzed peptides, except for peptide \textit{l-casein A}(47-52) (DKIHF), which exhibited an activity 8 times greater after hydrolysis (32). Muguerza et al. (33) isolated 231 microorganisms from raw cow milk samples and then assayed the ACE inhibitory activity of the resultent fermented milk produced with be isolated microorganisms in SHR. Four \textit{Enterococcus faecalis} strains were selected as producers of fermented milk with potent ACE inhibitory activity. Highly significant decreases in the systolic blood pressure and in the diastolic blood pressure were observed when the fermented milk was administered to SHR. So the study suggested that raw cows’ milk is an excellent source of lactic acid bacteria to produce fermented milk with ACE inhibitory activity furthermore four selected strains of \textit{E. faecalis} were able to produce pasteurized fermented milk with potent ACE inhibitory and antihypertensive activity. Several bioactive peptides have been found in cheese and may due to the intense proteolysis during cheese ripening (8). Cheese contains phosphopeptides as natural constituents, and secondary proteolysis during cheese ripening leads to the formation of other bioactive peptides.
such as those with ACE inhibitory activity (1). Different types of cheeses have recently been used as source of antihypertensive peptides. Smacchi and Gobbetti (34) identified water-soluble peptides from Italian cheeses, Mozzarella, Italiano, Crescenza and Gorgonzola. Most of these peptides were found to be effective in reducing the activity of the Pseudomonas fluorescens ATCC 948 endopeptidase and the ACE. It was also determined that compared to the conventional lactic acid bacteria (such as L. casei subsp. casei) seemed to be less sensitive to the inhibition. Bioactive peptides having ACE inhibitory activity was also obtained from new developing cheese types which have probiotic bacteria as starter culture. Ryhänen et al. (36) developed a new type of ripened and low-fat cheese with bioactive peptides. The cheese was produced by using cheese starter cultures in combination with Lactobacillus acidophilus and bifidobacteria. In the analyses, the ripened cheese was found contain bioactive peptides with potential antihypertensive effects and so it was suggested that new cheese may have a beneficial impact on health. The inhibitory activity to ACE and decrease in the systolic blood pressure in SHR were measured before and after oral administration of each peptide sample obtained from seven kinds of ripened cheeses. The strongest depressive effect in the systolic blood pressure (-24.7 mm Hg) and intensive inhibitory effects and so it was suggested that new cheese may have a beneficial impact on health. The inhibitory activity to ACE and decrease in the systolic blood pressure in SHR were measured before and after oral administration of each peptide sample obtained from seven kinds of ripened cheeses. The strongest depressive effect in the systolic blood pressure (-24.7 mm Hg) and intensive inhibitory effects and so it was suggested that new cheese may have a beneficial impact on health. The inhibitory activity to ACE and decrease in the systolic blood pressure in SHR were measured before and after oral administration of each peptide sample obtained from seven kinds of ripened cheeses. The strongest depressive effect in the systolic blood pressure (-24.7 mm Hg) and intensive inhibitory effects and so it was suggested that new cheese may have a beneficial impact on health. The inhibitory activity to ACE and decrease in the systolic blood pressure in SHR were measured before and after oral administration of each peptide sample obtained from seven kinds of ripened cheeses. The strongest depressive effect in the systolic blood pressure (-24.7 mm Hg) and intensive inhibitory effects and so it was suggested that new cheese may have a beneficial impact on health. The inhibitory activity to ACE and decrease in the systolic blood pressure in SHR were measured before and after oral administration of each peptide sample obtained from seven kinds of ripened cheeses. The strongest depressive effect in the systolic blood pressure (-24.7 mm Hg) and intensive inhibitory effects and so it was suggested that new cheese may have a beneficial impact on health.

CONCLUSION

Recently it can been seen that ACE inhibitory peptides are the most favourite bioactive peptides for application to foodstuffs formulated to provide specific health benefits. This review has focused on production and releasing of ACE inhibitory peptides from milk proteins and their occurrence in dairy products. It has been demonstrated that peptides contained in milk, cheeses and fermented milks may regulate particular bodily processes and one suggested as a daily aid to maintain good health. If not released under physiological conditions in vivo, bioactive peptides could be produced commercially and used as nutraceuticals in disease prevention and treatment. For this purpose there is a need to develop novel technologies by means of which active peptide fractions can be produced and enriched. Furthermore, it is important to study the technological properties of the active peptide fractions and to develop new foods which contain these peptides and retain their activity for a certain period. Microbial fermentation seems to be a potential natural technology which can be applied to the production of bioactive peptides from milk proteins. This potential is already well-demonstrated by the presence of bioactive peptides in fermented milks and cheese varieties. For some of the ACE inhibitory peptides, significant antihypertensive effects have been demonstrated in rats and in vitro. The widespread use of these peptides in functional foods / nutraceuticals requires sufficient data based on human studies to demonstrate their long-term efficacy and safety. Furthermore as the mechanism of antihypertensive effect is not clear in vivo, molecular studies are needed to assess the mechanisms by which the bioactive peptides exert their activities. Finally, ACE inhibitory peptides obtained from milk proteins can be used as pharmaceutical and commercial nutraceutical. Also, consumption of dairy products having ACE inhibitory activity should not be forgotten in prevention of diseases.

REFERENCE AND NOTES

21. B. Hernández-Ledesma et al., "Application of high-performance liquid chromatography-lantern mass spectrometry to the identification of biologically active peptides"


34. E. Smacchi, M. Gobbetti, "Peptides from several Italian cheeses inhibitory to proteolytic enzymes of lactic acid bacteria, Pseudomonas fluorescens ATCC 948 and to the angiotensin I-converting enzyme", Enzyme and Microbial Technology, 22, pp. 687-694 (1998)


