Fermented milk derives bioactive peptides with antihypertensive effects

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Abstract

Functional foods present great promise for future developments in human nutrition. In the fermented dairy products, milk proteins serve as an important source of a range of bioactive peptides encrypted within the sequence of the native proteins and thus be released by proteolysis. From the bioactive peptides detected in dairy products, is inhibitors of angiotensin I-converting enzyme (ACE), which has a central role in the regulation of blood pressure in mammals. Therefore, the objective of this study is to review the possible mechanisms of milk protein proteolysis during fermentation and their relation to function bioactive peptides with antihypertensive effects.

Introduction

Chronic hypertension plays a key role in the development of cardiovascular diseases i.e. arteriosclerosis, stroke and myocardial infarction which lead to renal disease in the end-stage [1]. The high cost and side effects associated with hypertension drugs have encouraged the scientific community to look for alternatives [2]. Lactic acid bacteria are fastidious microorganisms with regard to nutrition requirements [10]. They have limited biosynthetic ability hence the requirement for an exogenous source of amino acids (such as isoleucine, leucine, valine, histidine and methionine) or peptides for optimum growth [11,12]. Since milk is deficient in such low-molecular components the growth of the starter bacteria depends on their proteolytic systems to hydrolyze caseins [13]. The amino acids released by the bacteria and accumulated in the milk affect the nutritional potential and biological value of the fermented product. Amino acids may not be directly contributory to the flavour and aroma of fermented milk. However, they act as precursors for a number of reactions that produce carbonyl compounds [14]. The spectrum and level of free amino acids in fermented milk depend on several variables such as type of milk, composition of the starter, method of preparation and storage conditions. Caseins are the main source of amino acids ensuring 98% of the growth [15]. The contribution of caseins to the provision of essential amino acids depends on the type of proteinase [15]. Protease is capable of initiating the degradation of casein to oligopeptides which are transported into the bacteria and afterwards degraded through a complex sequence of intracellular peptidases [15]. The amino acid necessity and production activity in mixed cultures can be modified using selected strains of lactobacilli [16] capable of intracellular splitting of oligopeptides or of attacking peptides and proteins in the nutrient medium by means of the proteolytic enzyme systems synthesized [16].

For example, in the mixed yogurt culture L. bulgaricus has higher proteolytic activity than S. thermophilus and thus the free amino acids produced by L. bulgaricus are also used by S. thermophilus [6,17]. The total amino acid content in yogurt reflects the balance between proteolysis and assimilation by bacteria [17]. The pathway of peptide hydrolysis in yogurt bacteria ensures the release of amino acids respectively and the growth relation between S. thermophilus and L. bulgaricus [6,18]. Proteolysis in fermented milk is mainly related to

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yogurt cultures which explain the high level of proteolysis in fresh biokefir after storage compared to other fermented milk [17]. The pathway of casein catabolism through yogurt organisms can be altered via endopeptidase activity as described for strains of *S. thermophilus* and *Lactococcus lactis* ssp. *lactis*, and aminopeptidase as described for *L. bulgaricus* and *Lactobacillus helveticus* [17].

**Proteolytic agents in fermented milk**

During milk fermentation proteolysis in milk is catalysed by enzymes from:

1. Coagulant (e.g. chymosin, pepsin, microbial or plant acid proteinases).
2. Milk (plasmin and perhaps cathepsin D and other somatic cell proteinases).
3. The starter or non-starter culture.
4. Secondary cultures (e.g. *P. camemberti*, *P. roqueforti*, *Propionibacterium* sp., *B. linens* and other coryneforms).
5. Exogenous proteinases or peptidases, or both, which are produced during milk fermentation.

The initial hydrolysis of caseins during milk fermentation is occurred by the coagulant and to a minor range by plasmin which caused the creation of large- (water-insoluble) and intermediate-sized (water-soluble) peptides that are released afterward by the coagulant and enzymes from the starter and non-starter microflora of the milk [11,12]. The extracellular cell envelope-associated proteinase of *Lactococcus* (lactocepin, endopeptidase lactocepin) contributes to the formation of small peptides in milk. This occurred possibly by the hydrolysis of larger peptides produced from αs1-casein by chymosin or from β-casein by plasmin [19,20]. The peptides (which are intracellular) released from lysed cells are responsible for the breakdown of short peptides and the liberation of free amino acids [17,21]. The resulted products of proteolysis are free amino acids. Thus, their quantity in milk at any phase of fermentation is the net result of the released amino acids from casein, their degradation to catabolic products and maybe some synthesis by the milk microflora [14]. Therefore proteolysis can vary significantly between variety e.g. coagulants which are completely denatured by fermentation temperature used in milk manufacture.

**Functionality of bioactive peptides**

The physiologically active components of proteins in the food are being widely recognized. The physiological action of natural proteins present in raw food materials can exert directly or indirectly upon enzymatic hydrolysis in vitro or *in vivo*. Dietary proteins deliver a good source of naturally active peptides [22]. These peptides are inactive within the structure of the native protein but can be liberated via hydrolysis with digestive enzymes and/or proteolytic activity of microorganisms or plants. It is currently well-known that biologically active peptides are generating from food proteins during fermentation with LAB and gastrointestinal digestion. The production and properties of bioactive peptides have been reported in several studies [12,21,23,24].

Bioactive peptides have been defined as particular protein fragments that have physiological effect on human body and may eventually influence health [25]. Oral administration of bioactive peptides has significant effect on functions of human body systems such as cardiovascular, digestive, immune and nervous systems depending on the amino acid sequence of these peptides. Therefore, the potential of different dietary peptide sequences to stimulate human health by lowering the risk of chronic diseases or improving immune system has been widely studied [12,21,24]. Several known peptide sequences showed therapeutic properties such as anti-microbial, anti-oxidative, anti-thrombotic, anti-hypertensive and immunomodulatory activities [23]. These activities are relied on the amino acid composition and sequence of these peptides. The length of bioactive peptides is generally 2-20 amino acid residues and some peptides can exert multi-functional properties [26]. Nowadays, the most important source of bioactive peptides is milk proteins because these peptides have been identified and isolated from milk protein hydrolysates and fermented dairy products [12,15,21,24].

**Hypertension**

Hypertension is the most common cardiovascular diseases. It is a universal problem of epidemic proportions, that affects 10%-20% in the adult population and 40%-50% in people aged 50 or older [27]. It is one of the serious chronic health problems associated with several diseases such as arteriosclerosis, stroke, myocardial infarction and end-stage renal disease. So, the role of the rennin–angiotensin system (RAS) in cardiovascular physiology is well studied and exploited pharmacologically.

The angiotensin converting enzyme (ACE), a component of RAS catalyzes the formation of the strong pressor agent angiotensin II from angiotensin I help to control high blood pressure [28,29]. ACE inhibitors are competitive substrates for ACE. The primary structural control this inhibitory response is the C-terminal tripeptides sequence. These peptides may interact with subsites s₁, s₁’ and s₂ at the active site of ACE. Substrates and inhibitors containing hydrophobic amino acid residues in the three C-terminal positions are preferable for ACE [30]. For example, aliphatic, basic and aromatic residues are binding in the penultimate positions, whereas aromatic, proline and aliphatic residues are binding in the ultimate positions. The positive charge of arginine or the ε-amino group of lysine at the C-terminus has been shown to play role of several ACE-peptides [31]. Several ACE inhibitors such as captopril, enalapril, lisinopril and temocapril are known for the management of hypertension. All of these drugs produced side effects thus, justifying the search for natural ACE inhibitors for safe and economical use [29,32].

**Milk-protein-derived peptides with antihypertensive effects**

Recently the ingesting of fermented milk has increased because of the fact that this dairy product fulfills several of human nutritional requirements. It is a ready to eat food moderately low in fat and fulfill the requirements of human nutrition. Recently, the use of functional foods has been increased because of increasing consciousness among people of the linkage between food and health [23]. Fermented milk considered being functional food particularly when it contains probiotic bacteria. It is also offer additional benefits related to the bioactive peptides that are generated during manufacturing and storage. Presently, an excessive attention has been focused on bioactive-peptides that can reduce the blood pressure in hypertensive people [23].

The action of these peptides is relying on the inhibition of angiotensin-1 converting enzyme (ACE, E.C. 3.4.15.1). However, the activity of these peptides could include various complex mechanisms that may increase the therapeutic properties of fermented milk followed by further benefits for consumer health [8,33,34]. ACE is an enzyme that has an important role in the rennin-angiotensin system
by controls the arterial blood pressure and the balance of water and salt in the body. An elevation in blood pressure is occurred when the enzyme catalyzes the hydrolysis of angiotensin I to angiotensin II that act as strong agent (vasoconstrictor) with the aid of vasodilative action resulted of the degradation of bradykinin [29]. The proteolytic activity of LAB during milk fermentation and/or the action of pure proteinases on milk proteins led to produce numerous of peptides with anti-hypertensive properties [7,9,17]. Several peptides have been shown anti-hypertensive action on spontaneously hypertensive rats and on small groups of human volunteers [23,35]. In addition, several ACE inhibitory peptides have been isolated from the enzymatic hydrolysis of fermentation milk with lactic acid bacteria [1,12,17] or chemical synthesis of peptides according to milk protein sequences [2].

The degradation of milk proteins with proteinases from L. helveticus produced peptides with ACE-inhibiting activity had a significant antihypertensive effect in spontaneously hypertensive rats [36]. The same effect was observed with fermented milk containing L. helveticus [37]. Two tripeptides valyl-prolyl-proline (Val-Pro-Pro; VPP) and isoleucyl-prolyl-proline (Ile-Pro-Pro; IPP) were identified as the active peptides which were responsible for this effect [37]. A liquid chromatography–mass spectroscopy (LC-MS) method with Ala-Pro-Pro as an internal standard was used for the quantitative determination of these two peptides in casein hydrolysates [38]. In several short- and long-term human studies where VPP and IPP containing fermented milk products were ingested a blood-pressure lowering effect was observed [36,39,40].

**Production of fermented dairy products with ACE inhibitory peptides**

In dairy products, the production of ACE inhibitory and anti-hypertensive peptides in situ aroused a lot of interest from scientists since this provides further therapeutic properties to fermented dairy products. During milk fermentation an excessive amount of peptides are liberated from milk proteins as a result of the action of plasmin (indigenous milk enzyme) and proteolytic activity of starter and non-starter LAB [41,42]. Ferment milk with highly proteolytic species of LAB is widely used to increase the amount of bioactive peptides [41,42]. The formation of peptides such as bitter peptides but yet to provide a high proteolysis of bioactive peptides such as ACE-inhibitory peptides. Since the concentration of ACE-inhibitory peptides appears to rely on a balance between their formation and degradation into inactive peptides and amino acids subject to storage periods and conditions [41,42].

Manipulation of bacterial fermentation of milk play crucial role in increasing anti-ACE-1 activity. The enhance anti-ACE-1 activities associated with herbal extracts or type of milk used in fermented milk has been studied [43-45]. This may imply unique properties of herbal extracts and milk interactions towards preferential formation of bioactive peptides, some of which may be have ACE-inhibitory activity. In addition, fish collagen has showed significant effect on increased anti-ACE-1 activities [46,47]. Further studies are needed to isolate and identify the bioactive peptide with anti-ACE-1 activities from fermented milk in the presence of stimulate bacterial growth materials such as medicinal plant and fish collagen.

**References**

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