Anti-hypertensive peptides released from milk proteins by probiotics

Maturitas
Review

Anti-hypertensive peptides released from milk proteins by probiotics

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ABSTRACT

The development and growth of agricultural products as well as the food industrialization have led to dramatic lifestyle changes, particularly in dietary patterns, which in turn has lead to increased occurrence of chronic diseases (i.e. cardiovascular, stroke and hypertension). In order to overcome these side effects, the food industry has developed functional milks. Milk products, particularly fermented milk containing probiotics are most popular. The advantages of utilizing probiotics is for gut health, reduced allergenicity, increased bio-accessibility of fats/proteins in foods, and lowering of blood pressure due to poly-amines and bioactive peptides. In addition, bioactive peptides have been shown to lower the risk of hypertension and cancer. Herein, we discuss the potential role of fermented milk as functional milk against hypertension. However, longer term research studies are necessary to evaluate the role of fermented milk drinks to support human health.

Keywords:

Anti-hypertensive peptides; Angiotensin converting enzyme; functional fermented milk; blood pressure; hypertension

1. Introduction

Nutrition concepts today are moving away from prevention to promotion of health and wellness, and due to increased education and awareness to consumers the link between diet and health has become a major focus. As such, this trend has created a demand for functional foods, or ‘foods that contain some health-promoting components beyond traditional nutrients’ [1]. The market for functional foods is large in the US, it was valued at US$21 billion (B) in 2006, with a 5 % annual growth till 2011 [2] and increased to 25 % and valued at US$43.27B in 2014 [3]. However, following the economic crisis in recent years, the market for functional foods has suffered mainly owing due to consumers turning to cheaper food options as well as changes being implicated in food regulations. In addition, in the European Union manufacturers are being pressured to provide scientific evidence for the health benefit claims of functional foods [3].

Foods can be modified to become ‘functional’ either by hydrolysis by digestive gastrointestinal enzymes or during food processing [4]. This effectively releases bioactive peptides from an inactive state in the protein molecule [4]. The role of bioactive peptides in promoting wellness has been attributed to their physiological and physiochemical benefits such as, anti-thrombotic, anti-hypertensive, anti-microbial, anti-oxidative and immunomodulatory [5]. To attain anti-hypertensive function, angiotensin converting enzyme inhibitory (ACE-I) peptides are required to be absorbed by the intestine in an active form prior to reaching the targeted organ. One of the challenges with oral ingestion however is their stability. As such, they need to survive degradation by gastrointestinal proteinases and peptidases before being absorbed into the system [5]. In fact, recent efforts have focused on transdermal delivery of anti-hypertensive peptides and drugs in order to bypass gastrointestinal degradation [6].

Bovine milk typically is composed of water (87.1 %), lactose (4.6 %), milk proteins (3.3 %) and fat (4 %) [7]. There are 2 types of milk proteins, 80 % casein and 20 % whey protein (WP) and are characterized based on their solubility at pH 4.6; casein is insoluble whereas WP is soluble [8]. WP is a mixture of bovine serum albumin (8 %), α-lactalbumin (25 %), β-lactoglobulin (65 %) and other minor constituents such as, immunoglobulins, protease peptones, lacto peroxidase, lysosome and lactoferrin [9]. WP usually comes in powder form either as whey protein concentrate (WPC; 85 % protein, low fat and cholesterol levels, high levels of bioactive compounds) or whey protein isolate (WPI; >90 % protein and low levels of fat, cholesterol and bioactive compounds) and is marketed as a dietary supplement with wide health claims [10]. In general, milk proteins are used as a source of bioactive peptides as they are considered economical and safe. The production of bioactive peptides from fermented milk products has been widely studied, and the effectiveness of bioactive peptides depend on its amino acid sequence [11, 12]. Herein, we present important studies on the anti-hypertensive properties of milk proteins in light of increasing scientific knowledge regarding ACE-I activity of these compounds.

2. Methodology

This review article presents current knowledge of the probiotics in particular lactic acid bacteria (LAB) in the fermentation of milk proteins to result in functional milk products. The LAB and the fermentation process produces bioactive peptides which have numerous health benefits. PubMed/Medline and Google Scholar were searched for relevant articles using the following key terms: LAB OR *Lactobacillus* AND bioactive peptides (this resulted in 161 papers), *Streptococcus* AND bioactive peptides (55 papers), *Bifidobacterium* AND bioactive peptides (24 papers), probiotics AND bioactive peptides (82 papers), probiotics AND health (5,794 papers, only screened titles from 2018 (451/5,794) and only chose those related to hypertension), probiotics AND hypertension (118 papers), sensory evaluation AND peptides AND milk (12 papers), whey/casein AND bioactive peptides (137 papers), bioactive peptides AND hypertension AND human AND milk (36 papers).

Publications in the last 10 years are mostly cited. Papers that were discarded are those that showed that probiotics resulted in bioactive peptides but were not relevant to anti-hypertensive properties; enzymatic and chemical synthesis of bioactive peptides; dairy food preservation; fruits and vegetables as source of bioactive compounds; non-dairy products; anti-bacterial properties of compounds; microorganisms and food contamination; heat shock proteins; bioactive peptides in functional meat products; gut microbiome; in silico characterization of bioactive peptides; conference abstracts and papers not in English were also excluded.

3. Fermentation of dairy products

Fermentation of dairy products can be dated as far back as 10,000BC and the fermentation process requires the use gram positive, acid-tolerant bacteria Lactobacillales or

LAB which produce lactic acid as a main end product of fermentation. LAB include, *Lactobacillus*, *Streptococcus* and *Bifidobacterium* ssp. and, are commonly referred to as probiotics (Table 1). Probiotics are defined as 'live microorganisms that when administered in adequate amounts confer health benefits to the host' [13, 14]. The genera of microorganisms, yeast and enzymes that are commonly used to release bioactive peptides from milk proteins are listed in Table 1 [15, 16]. Fermentation improves shelf life, taste and digestibility of milk [17]. Fermentation knowledge is essential in driving research forward into their role in health and how they can be used to overcome disease, such as, autoimmunity, allergies, diabetes and cancers [18]. The 'quality' of fermented dairy products (potency, efficacy, stability and immunomodulatory abilities) is determined by the equipment and methods used. One such approach is the use of the bioreactor which results in high quality fermented products with high yields (Figure 1). There are different types of bioreactors used such as, stirred tank reactors, tower fermenters, airlift fermenters and hollow fibre chambers; for research, small-scale bioreactors are used [18]. Bioreactors can be used to monitor and controlling pH by acid/base addition or CO_2/base addition, temperature regulation, sterile sampling capability and mixing such that the cultures remain in suspension. All this is achieved without damage to the micro-organisms. As such, the bioreactor process has been developed for dairy fermentation products including the production of yoghurt, cheese, butter milk, Kefir (Table 2) [19]. Although yoghurt containing *Lactobacillus acidophilus* or *Bifidobacterium* ssp. would generally be classified as 'fermented milk' [20], according to the Australian food standards code, the pH of yoghurt should be < 4.5 and therefore is prepared with *S. thermophilus* and *L. delbrueckii* ssp., *bulgaricus* or other LAB [21]. The fermentation processes results in changes in the structure of milk proteins, leading to the production of some amino acids and peptides from milk proteins with bioactive properties [17, 22-24].

4. Production of bioactive peptides

The protein content in food is a source of peptides, which when cleaved intact become active. Bioactive peptides are effective in promoting human health by decreasing the risk of chronic diseases, such as hypertension [25]. Bioactive peptides are produced during gastrointestinal enzymatic degradation of proteins or during food processing such as fermentation [26, 27]. In addition, bioactive peptides can be produced by heat, alkaline or acidic conditions, enzymes and microbial fermentation of proteins [27]. Enzymatic hydrolysis using trypsin, papain or subtilisin is used to produce low molecular weight bioactive peptides; subtilisin being more potent than papain and trypsin [28]. Likewise, subtilisin hydrolysis of rice bran proteins was more efficient in producing low molecular weight bioactive peptides than papain and pepsin [29]. In addition, ACE-I peptides are most commonly produced by trypsin. However, other enzymes and enzyme combinations of alkalase, pepsin, pancreatin and enzymes from bacterial and fungal sources, have been used to produce bioactive peptides [24].

Microbial fermentation involves the culture of some bacteria or yeast with proteins; bacteria and yeast secrete enzymes which break down proteins into bioactive peptides. The extent of hydrolysis is dependent on the strain used, type of protein and the time of fermentation. LAB such as *Lactobacillus helveticus* is usually used in milk processing to produce cheese. The release of amino acids by action of peptidases is an essential part of the LAB proteolytic system. Tri-peptides, IPP and VPP are generated from sour milk fermented with *Lactobacillus helveticus* CP790 and *Saccharomyces cerevisiae* (Ahtesh et al., 2018 submitted). Several studies have reported over ten peptides as part of *Lactobacillus helveticus* proteolytic system; (PepE, PepO, PepT, PepX, PepI, PepQ, PepR, PepD, PepV, PepC, PepN) [30, 31]. Another study identified seven oligo endo peptidases and eight di- and tri- peptidases in *Lactobacillus helveticus* strain CNRZ32 [32].

5. Functional food products

5.1. Health benefits of fermented milk products

The process of fermentation increases the concentration of enzymes and vitamin B, C, D and K content. LAB (*Lactobacillus, Streptococcus* or *Bifidobacterium*) are used to ferment milk products (i.e. yoghurt, buttermilk, kefir) which have been shown to confer health benefits beyond their nutritional qualities (Table 1, 2, Figure 1). The efficacy of some probiotics against diarrhoea has been reported [33], as well as providing intestinal microbial balance [34], reduces the risk of colon and breast cancer [35], reduces blood pressure [17, 22-24, 36], reduces lipid profiles [37], reduces allergies and food intolerances [38], inhibits the growth of pathogenic bacteria and is immunomodulatory [12, 39-42] (Figure 1). More recently, dysbiosis has been implicated to be involved in the pathogenesis of a number of disorders such as, Sjogren's syndrome [43], Parkinson's disease [44] and multiple sclerosis [45]. The use of probiotics and prebiotics could be used as therapeutic approaches against diseases. In fact, it was recently reported that consumption of probiotics had an overall positive relationship with lactose intolerance [46]. In order to determine the mechanism by which fermented milk consumption confers health benefits, serum metabolome was measured in 14 healthy males in a randomized crossover study following consumption of either non-fermented milk or yoghurt. It was noted that the yoghurt group had higher concentrations of 7 free amino acids, reduction of 5 bile acids and modulation of 4 indole derivatives compared to the non-fermented group suggesting novel health benefits of fermented milk products [47]. Fermented milk treatment in, *in vitro* semi-continuous anaerobic cultivation of the colon showed that butyrate producing bacteria were altered as well as some metabolites, including butyrate, ethanol and lactate [48]. In addition, *Lactobacillus plantarum* YW11 decreases nitric oxide, increases the content of short chain fatty acids, shows anti-oxidant activity and regulates microbiota activities in the intestine bringing new insights into the mechanisms conferred by probiotics [49]. Furthermore, in mice, consumption of probiotic yoghurt increased subcuticular folliculogenesis resulting in thick shiny fur [50]. Moreover, probiotics regulate cellular and humoral immune responses as well as inducing regulatory T cells and anti-inflammatory cytokines [39-42], beneficial for a number of inflammatory disorders. It is clear that fermented milk products confer an array of health benefits however, further randomized controlled studies are required to elucidate such benefits.

5.2. Regulation of blood pressure

Blood pressure is one of the leading risk factors for cardiovascular disease and this risk increases progressively above 120/80 mmHg. Hypertension is related to increase systolic and diastolic blood pressure and is defined as being above 140/90 mmHg. According to the Australian health survey 2011-2012, one third of the world's adult population were hypertensive in the year 2011-2012. Of these people > 75 % were overweight/obese and 42 % reporting to doing no exercise. Hypertension usually co-morbids with other risk factors, such as, metabolic syndrome, insulin resistance and high cholesterol levels (and chronic inflammation) [51-53]. High blood pressure in the short term affects the sympathetic nervous system whereas in the long term body fluid volume and the function of the kidneys are greatly affected [53, 54]. A key system that regulates body fluids and blood pressure is the hormone system, the renin-angiotensin system (RAS) (Figure 1) [55, 56]. Renin is secreted by the kidney when renal blood flow is low which converts angiotensinogen secreted by the liver into angiotensin I (AT1) which is then converted to ATII by the enzyme, angiotensin-converting enzyme (ACE) found in the lungs. ATII (a strong vasoconstrictor) stimulates the hormone aldosterone from the adrenal cortex and as a result increases blood pressure and water retention. The importance of the RAS in hypertension have been shown by the clinical benefits of ACE inhibitors (ACE-I), AT1 and ATII receptor blockers (ARBs) as well as renin inhibitors [57, 58]. Using molecular modelling, components of the RAS (especially ACE-I peptides) have been designed and used as anti-hypertensives [59]. More recently, alamandine, a vasoactive peptide of the RAS, was shown to reverse vascular dysfunction via the protein kinase A pathway [60]. Examples of ACE-I include benazepril hydrochloride, captopril, perindopril, enalapril maleate and of ARBs include candesartan, irbesartan, losartan, valsartan. Although ACE-I are used to treat hypertension, heart failure patients, to prevent kidney disease and improve survival following heart attacks and, ARBs are used to prevent diabetes and recurrence of atrial fibrillation, there are many side effects associated with these [61]. ACE-I side effects include, cough, skin rash, change in taste, angiodema, increased uric acid, elevated potassium levels, liver dysfunction, allergic reactions, low blood cell counts, pancreatitis and kidney failure. ARBs may cause dizziness, diarrhea, rash, orthostatic hypotension, indigestion, lung infections, kidney and liver failure, allergic reactions, decreased platelets and angioedema [61]. Due to the adverse effects of long term use of ACE-I and ARBs, much research has focused on the production of bioactive peptides from fermented milk products. Peptides are known for their high tissue affinities and thus, may be more slowly eliminated from tissues compared to synthetic drugs.

5.3. Anti-hypertensive bioactive peptides

Milk derived bioactive peptides with anti-hypertensive attributes are highly studied and a number of reviews regarding their production, bioavailability and inclusion into foods are published [26, 27]. Fermentation of WP with *Lactobacillus brevis* has higher ACE-I activity than whey protein fermented with *Lactobacillus -acidophilus, -casei, -helveticus, -lactis or -reuteri*. Thus, different microorganisms have different proteolytic systems and different ACE-I activities [62]. In addition, studies report the ability to hydrolyse α–lactalbumin, β-lactoglobulin and immunoglobulins from WPs in order to produce bioactive peptides [63].

5.3.1. *In vitro* ACE-I activity: *In vitro*, several peptides of varying amino acid lengths have been shown to inhibit ACE at □M concentrations [64]. Inhibition of ACE activity reduces the production of ANGII leading to lowered blood pressure. In addition, the bradykinin system involves ACE which causes blood vessels to dilate, consequently reducing blood pressure (Figure 1) [64, 65]. It is plausible that small molecular weight ACE-I peptides would move through the gut epithelium and into circulation to reach target sites and reduce blood pressure. As such, fermentation of milk proteins have been utilized to produce bioactive peptides with ACE-I activities. Bioactive peptides with ACE-I activities are aimed to block the conversion of ANGI to ANGII or inhibiting the degradation of bradykinin. In fact, *Lactobacillus helveticus* fermented whey protein produced the peptides AHKAL, APLRV, AQSAP and IPAVF with strong ACE-I. However, whey fermented with *Lactobacillus brevis* produced a different peptide AEKTK, but also with ACE-I activity [62]. Additionaly, using *Lactobacills casei* and kombucha cultures resulted in 21 new peptides (6-18 amino acids in length) of which LVESPPELNTVQ, VLESPPELN, WGYLAYGLD were confirmed to have ACE-I activity [66].

5.3.2. Anti-hypertensive activity in animal models: The WP fraction of yoghurt fermented with *Lactobacillus helveticus* has been found to contain the dipeptide YP with anti-hypertensive effects in spontaneous hypertensive rats (Figure 1) [67]. Furthermore, the peptides DPYKLRP and DY produced from lactoferrin and bamboo shoots respectively following fermentation with *Kluyveromyces marxianus* have ACE-I activity and reduce blood pressure in spontaneous hypertensive rats. The peptides FFVAPFPEVFGK, FFVAP and AVPYPQR derived from casein potentiate bradykinin in the contraction of the ileum of rats reducing blood pressure [68]. The renin inhibitory peptide IRLIIVLMPILMA hydrolyzed from red seaweed *Palmaria palmata* using the enzyme papain also reduces systolic blood pressure in spontaneous hypertensive rats [69]. Likewise, RVPSL peptide isolated from hen egg protein has been shown to be an ARB and has potential to be used in the treatment or prevention of hypertension [70].

5.3.3. Clinical studies: In humans, the peptides VPP and IPP released from casein following culture with *Lactobacillus helveticus* CP790 and *Saccharomyces cerevisiae* have been shown to reduce blood pressure [71]. These peptides were shown to be absorbed directly by the epithelium without being hydrolyzed by digestive enzymes reaching the target sites for anti-hypertensive effects [72, 73]. In a 12 weeks double-blind parallel intervention study of 89 hypertensive subjects who consumed either VPP/IPP peptides in milk compared to placebo milk significantly reduced arterial stiffness (augmentation index) [74]. An addition, 94 hypertensive patients in a randomized double blinded placebo-controlled study who consumed

Lactobacillus helveticus fermented milk (with a high concentration of VPP/IPP peptides), twice a day for 10 weeks, lowered blood pressure compared to control product [75]. In a longer study over 21 weeks similar anti-hypertensive effects were noted [76]. Likewise, in hypertensive humans, who consumed fermented milk or a spread containing the ACE-I peptides, VPP and IPP, daily for 10-12 weeks showed reduced blood pressure [77]. IPP and VPP interact with subsides S_1, S_1' and S_2' at the active site of ACE; thus ACE prefers hydrophobic amino acid residues in the three C-terminal positions. As a consequence of the anti-hypertensive abilities of the bioactive peptides, several fermented milk products have been developed and are consumed for the management of hypertension (Figure 1), although, the European food and safety authority has not approved such approach to be used alone as a treatment and management of hypertension. Further human intervention studies are therefore crucial to establish the anti-hypertensive and health benefits of milk derived bioactive peptides.

6. Sensory evaluation of milk products

Evaluation of the sensory characteristics is defined as a scientific judgment of food quality using senses, such as smell, taste, touch and sight [17]. It is divided into 2 categories, (i) objective testing which uses laboratory equipment with no involvement of senses and (ii) subjective testing involving a group of panellists which includes the senses (sight, smell, taste, flavor, touch, sound). Both objective and subjective tests are required in sensory evaluation [17]. As such the Hendonic scale is commonly used by panellists to evaluate liking of food products, such as, a bioactive milk product. After food production and before reaching the marketplace, new food products go through many tests to accurately judge how well people will accept them [17, 78]. Manufacturing companies are required to evaluate the new product by particular food gastronomy and respond to the following: (i) will people like the product? (ii) will they buy the product? what price? (iii) how can the product be successfully marketed to people? and (iv) will they prefer the product over others? Useful information can be obtained by posing specific questions to panels about age, sex, religion, geographic nationality, location, employment and education. Lastly, marketing and quality control are additional applications of sensory testing [78]. Sensory evaluation is a valuable tool, especially for commercial companies, as the outcomes are fast, practical and simple [79].

7. Conclusion

Several species of probiotics strains are potential contributors to the regulation of high blood pressure, lipid metabolism, diabetes and are immunomodulatory, due to the effectiveness of the bioactive peptides produced during fermentation. We described anti-hypertensive peptides released from fermented milk products eventhough there are still a large number of peptides released from milk protien hydrolysis still to be evaluated. There is a need to determine the mechanisms of bioactive peptides and their metabolites and their gene changes on cells using RNAsequencing in order to better understand the mechanistic effects of peptides at the molecular level. This information will aid in the development of more effective therapeutic strategies and achieve better outcomes.

Conflict of interest

The authors declare no conflicts of interest

Author contributions

FA wrote the review article under the guidance of VA and LS. FA, LS and VA edited and reviewed the article.

Ethical approval

No ethics was required for this article

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Table 1
The genera of microorganisms, yeast and enzymes that are commonly used to release bioactive peptides from milk proteins.

Microorganisms	**Yeast**	**Enzymes and others**
L. acidophilus	*Candida krusei*	Alcalase®
L. casei	*Galactomyces geotrichum*	*Bacillus cereus*
L. delbrueckii ssp. Bulgaricus	*Geotrichum candidum*	*Bacillus clausii*
L. farciminis	*Kluyveromyces marxianus*	*Bacillus oligonitrophilis*
L. gasseri	*Pichia subpelliculosa*	*Clostridium butyricum*
L. helveticus	*Saccharomyces boulardii*	*Enterococcus faecium*
L. johnsonii	*Saccharomyces cerevisiae*	Flavourzyme®
L. paracasei	*Saccharomyces kefir*	*Propionibacterium*
freudenreichii	*Schizosaccharomyces pombe*	
L. rhamnosus		
L. salivarius		
Lactococcus lactic ssp. cremoris		
Lactococcus lactis		
B. acolescentis		
B. animalis		
B. bifidum		
B. breve		
B. infantis		
B. lactis		
B. longum		
B. thermophilum		

Bifidobacterium (B), Lactobacillus (L)

Table 2
Fermented products, microorganisms used and health benefits

Dairy product	**Milk used**	**Microorganism used**
Yoghurt	Cow	*S. thermophilus* *L. bulgaricus, L. acidophilus*
Lassi	Buffalo, Cow	*L. bulgaricus*
Kefir	Sheep, cow, goat	*Saccharomyces, S. lactis, Kefir*
Cheese	Cow, goat, sheep, buffalo	*L. lactis, S. therpmophilus* *L. delbueckii* subsp. *bulgaricus* *Penicillum roqueforti, etc*
Curd	Cow, buffalo	*L. lactis, S. thermophilus* *L. delbueckii* subsp. *bulgaricus* *S. cremoris*
Acidophilus milk	Cow	*L. acidophilus*
Bulgarian butter milk	Cow	*L. delbueckii* subsp. *bulgaricus*
Fermented products	**Constituents**	**Health benefits**
Kefir (milk)	Vitamin B12, K2, calcium magnesium, biotin, folate	Immunomodulator, improves - irritable bowel syndrome, allergies, digestion and kills candida
Yoghurt (milk)	Vitamin B2, B12, calcium, potassium, magnesium, protein,	Immunomodulator, improves cardiovascular system, increases triglycerides
Kombucha (black tea and sugar)	Vitamin B, enzymes acetic acid, lactic acid	Improves digestion, energy, prevents cancer, immunomodulator
Pickles (i.e. cucumber)	Vitamin A, K2, fibre anti-oxidants, calcium	Improves heart and bone health
Sauerkraut (cabbage)	Vitamin A, B, C, K, iron manganese, magnesium,	Improves digestion, circulation, reduces cholesterol and inflammation
Tempeh (soybean)	Vitamin B2, 3, 5, 6, high protein	Reduces cholesterol, increases bone density, muscle recovery, reduces menopausal symptoms
Miso (barley, soybean or brown rice)	Vitamin K2, omega-3, fibre, zinc, protein,	Anti-ageing, immunomodulatory, reduces cancer risk, improves bone health and nervous system
Natto (soybean)	Vitamin K2, calcium enzyme nattokinase,	Immunomodulatory, improves cardiovascular system, bone and digestion, anti-cancer

Bifidobacterium (B), Lactobacillus (L), Streptococcus (S)

Figures

Fig. 1. Schematic representation of the bioreactor to produce fermented dairy products from milk and lactic acid bacteria (LAB). Fermented dairy products have an array of health benefits including that against hypertension. In addition, milk proteins (whey and casein) when mixed with LAB at the appropriate pH and time, release bioactive peptides from protein which have angiotensin converting enzyme (ACE)-inhibitory activity, angiotensin receptor blocking (ARB) activity or bradykinin protective activity.