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Invited review: Fermented milk as antihypertensive functional food

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ABSTRACT

Over the past decade, interest has been rising in fermented dairy foods that promote health and could prevent diseases such as hypertension. This biological effect has mainly been attributed to bioactive peptides encrypted within dairy proteins that can be released during fermentation with specific lactic acid bacteria or during gastrointestinal digestion. The most studied bioactive peptides derived from dairy proteins are antihypertensive peptides; however, a need exists to review the different studies dealing with the evaluation of antihypertensive fermented milk before a health claim may be associated with the product. Thus, the objective of this overview is to present available information related to the evaluation of fermented milk containing antihypertensive peptides by in vitro and in vivo studies, which are required before a fermented functional dairy product may be introduced to the market. It was finally concluded that although commercial fermented milks with antihypertensive effect exist, these are scarce and most are based on *Lactobacillus helveticus*. Thus, a great opportunity is available for the development of functional dairy products with new lactic acid bacteria that support heart health through blood pressure- and heart rate-lowering effects. Hence, the consumer may be willing to spend in foods with important functional benefits.

Key words: hypertension, heart rate, antihypertensive peptides, in vitro studies, in vivo studies

INTRODUCTION

Interest has been increasing in certain foods, known as functional foods, that besides traditional nutrition, promote health or reduce diseases (Flambard and Johansen, 2007). Among these foods are dairy products, as they contain several bioactive compounds such as calcium, medium-chain fatty acids, CLA, lactose, and peptides (Ebringer et al., 2008). Milk proteins are a good source of bioactive peptides (which are latent or encrypted within native protein and some regions of their primary structure contain peptide sequences, considered strategic zones, that may exert different biological effects (Flambard and Johansen, 2007). However, as they are latent or encrypted within the native protein, they need to be released by proteolysis during gastrointestinal digestion or food processing, such as milk fermentation with lactic acid bacteria (**LAB**; Torres-Llanez et al., 2005; González-Cordova et al., 2011). Peptide bioavailability after oral administration plays a major role because it is crucial that they remain bioactive during digestion and absorption so that peptides can reach target organs and tissues through blood circulation (Vermeirssen et al., 2004). Milk bioactive peptides consist of 2 to 20 AA residues that, besides being a valuable source of EAA, possess specific biological properties (Ricci-Cabello et al., 2012); some of these biological properties include mineral binding, antioxidant, antithrombotic, antimicrobial, opioid, and antihypertensive actions. These released peptides are present in fermented dairy products or are potential ingredients in health-promoting foods (Pihlanto et al., 2010; Ricci-Cabello et al., 2012).

Hypertension is a disease characterized by high values of blood pressure over the normal range (Chobanian, 2003). It is a chronic degenerative disease that affects more than 1 billion people throughout the world (WHO, 2011). Moreover, it is an important risk factor for developing other cardiovascular diseases, strokes, renal failure, cerebrovascular accidents, and many other complications (Bruce and Hanson, 2010). The renin-angiotensin system is the most important metabolic pathway in the control of blood pressure and vascular tone (Daien et al., 2012). 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 inhibition of ACE will cause a vasodilator response,

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which lowers blood pressure. Therefore, the pursuit for ACE-inhibitory substances, such as peptides, in food has been conducted so they could be used in the prevention and treatment of hypertension (Udenigwe and Mohan, 2014). Several ACE-inhibitory peptides have been identified in fermented milk and cheese (Pihlanto et al., 2010; Hernandez-Ledesma et al., 2011), although their actual antihypertensive mechanism is still unclear (Jäkälä and Vapaatalo, 2010). Although pharmacological therapies are the most widely used for hypertension treatment, they have long-term secondary side effects. Fermented milk was recommended as a nonpharmacological treatment for hypertension, mainly because they lack of undesirable side effects (Flambard and Johansen, 2007); however, the European Food Safety Authority (**EFSA**) considers that the evidence is insufficient.

To date, antihypertensive peptides derived from milk proteins are the most studied, although several reviews addressing their production, bioavailability, and incorporation to foods were published (Pihlanto and Korhonen, 2003; Korhonen and Pihlanto, 2006; Hernandez-Ledesma et al., 2011). However, a review of in vitro and in vivo studies dealing with the evaluation of antihypertensive peptides in fermented milk is needed. Thus, the objective of this overview is to present available information related to the evaluation of fermented milk containing antihypertensive peptides by in vitro and in vivo studies, which are required before a fermented functional dairy product may be introduced to the market.

IN VITRO STUDIES

The search for ACE-inhibitory peptides by in vitro studies is the most common strategy to select those fermented milks with antihypertensive potential. Generally, the Cushman and Cheung (1971) spectrophotometric method is the most broadly used technique for the evaluation of ACE-inhibitory activity. It uses hippuryl-His-Leu as a substrate, and it is hydrolyzed by ACE to hippuric acid and His-Leu. However, ACE inhibitors make sure this reaction does not take place. This activity may be expressed as percentage of ACE inhibition (**ACEI**) or as which is the minimum concentration of protein to inhibit 50% of the enzymatic activity ($\mathbf{IC_{50}}$; Hernández-Ledesma et al., 2011). Once, ACEI is tested and IC_{50} is determined in the water soluble fraction of fermented milk, peptides may be determined by HPLC and mass spectrometry.

Most studies consisted of the screening of different LAB for their capacity to exhibit high ACEI activity as a first step toward the development of antihypertensive fermented milk. Proteolysis by endogenous milk enzymes and enzymes from microbial cultures, such as LAB, have been shown to produce antihypertensive peptides during fermentations. The responsible bacterial enzymes are believed to be the cell wall proteinases, which break the protein to oligopeptides and use them for nutritional sources. The cell wall proteinase activity, the proteolytic system, and the activity of LAB are fundamental for the delivery of antihypertensive peptides (Flambard and Johansen, 2007). Thus, the specificity of the enzymes determines the sequence of the liberated peptides and is specific for each strain. Lactic acid bacteria in vitro screening for ACEI generally follows 2 approaches: the enzymatic characteristics of the bacterial cell wall proteinases and the properties of the strains to reduce ACE activity in vitro. The first approach was carried out on the *Lactobacillus helveticus* species, and it was reported that 3 specific cell wall proteinase patterns were correlated with ACEI in vitro (Flambard and Johansen, 2007).

Pihlanto et al. (2010) fermented milk with 25 different LAB and only 5 of them presented low IC_{50} in whey fractions: *Leuconostoc mesenteroides* 356 (0.44 mg/mL), *Leuconostoc mesenteroides* 358 (0.48 mg/mL), *Lactococcus lactis* spp. *lactis* ATCC 19435 (0.5 mg/mL), *Lactobacillus acidophilus* ATCC 4356 (0.42 mg/mL), and *Lactobacillus jensenii* ATCC 25258 (0.52 mg/mL). González-Córdova et al. (2011) fermented milk for 24 h with different *Lactobacillus* strains; *Lactobacillus reuteri* 14171, *Lactobacillus fermentum* ATCC 11976, and *Lactobacillus johnsonii* ATCC 33200 exhibited 42.04 to 83.36% ACEI activity, though *Lb. fermentum* presented the lowest IC_{50} (21 mg/mL) in whey fraction <3 kDa. In a preliminary screening of 20 wild strains of *Lc. lactis*, those isolated from artisanal dairy products presented the highest ACEI activity and the lowest IC_{50} (13–50 μg/mL) in whey fractions <3 kDa (Rodríguez-Figueroa et al., 2010). Furthermore, fermented milk fractions obtained through reversed-phase HPLC of 2 *Lc. lactis* strains showed low IC_{50} for strains NRRL B-50571 (0.034 μg/mL) and NRRL B-50572 (0.041 μg/mL; Rodríguez-Figueroa et al., 2012).

Another study evaluated yogurt ACEI activity containing additional probiotic strains during refrigerated storage (Donkor et al., 2007). Yogurt was prepared using either a sole yogurt culture including *Lactobacillus delbrueckii* spp. *bulgaricus* Lb1466 and *Streptococcus thermophilus* St1342, or with *Lb. acidophilus* L10, *Lactobacillus casei* L26, or *Bifidobacterium lactis* B94 besides the yogurt culture. All probiotic yogurts showed greater ACEI activity during the initial stage of storage (first 3 wk) versus the control; however, it decreased afterward and IC_{50} ranged from 27.79 to 103.30 μg/mL in whey fractions (Donkor et al., 2007). Thus, studying