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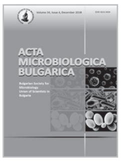

Review

Soymilk and Lactic Acid Fermentation

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Abstract

Modern consumers are directing their attention to functional foods that have both nutritional value and enhanced health benefits. This has led to an increased interest in non-dairy fermented soy products. They can become part of the growing market for functional foods, or carriers of probiotics for a specific category of consumers. A big challenge is to transform the beneficial, but non-digestible ingredients into a bioavailable form, transforming them into novel nutritional and efficient products.

The aim of the present review is to summarize the role of lactic acid bacteria (LAB) in the transformation of isoflavones, non-digestible oligosaccharides and insoluble proteins of soymilk. During fermentation, LAB of various genera, often recognized as probiotic, can play a significant role in improving the nutritional/favorable profile of soymilk and soy yogurt-like products. In addition, the role of LAB strains in the reduction of negative gastrointestinal effects typical of unfermented soybean milk is discussed. This beneficial role is proven to be strain-specific. Characterization of the role of lactobacilli in soymilk fermentation is a prerequisite for successful selection of strains with metabolic activities appropriate for the development of new types of functional foods with enriched properties, desirable to both people with health problems and the increasing vegan population.

Keywords: soy, soymilk, lactic acid bacteria, fermentation, *Lactobacillus*

Резюме

Съвременните потребители насочват вниманието си към функционални храни, които имат както хранителна стойност, така и допълнителни здравни ползи. Това води до повишен интерес към ферментационно-получени немлечни продукти и основно соеви. Те могат да бъдат част от нарастващия пазар на функционални храни или носител на пробиотици за конкретна категория потребители. Голямо предизвикателство е да се трансформират техните полезни, но несмилаеми съставки в бионалична форма, превръщайки ги в търсени и полезни храни.

Целта на настоящия преглед е да се обобщи ролята на млечнокиселите бактерии (МКБ) в трансформацията на изофлавони, несмилаеми олигозахариди и трудно-разградими протеини от соево мляко. По време на ферментацията, МКБ от различни видове, признати като пробиотици, могат да играят важна роля за подобряване на хранителния профил на соево мляко и за получаването на йогурт от соя. В допълнение е дискутирана тяхната роля за намаляване на негативните ефекти в стомашно-чревния тракт при консумация на соево мляко. Доказана е щамово специфична метаболитна активност при представители на различни лактобацилни видове. Комплексната характеристика на тяхната роля при ферментацията на соево мляко е предпоставка за разработване на нов тип функционални храни, с обогатени свойства, желани както от хора със здравословни проблеми, така и от нарастваща веганска популация.

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Functional foods, probiotics, prebiotics

In recent years, as research has proved the link between diet and health, consumers have been directing their attention to the so-called "functional foods". Those are foods which promote optimal health beyond basic nutrition. Functional foods and ingredients, such as probiotics, prebiotics, dietary fibers, soy and/or other derivatives, could be effective in enhancing health and, in addition to disease prevention, could be even useful in disease treatment (Tufarelli and Laudadio, 2016).

Among them, fermented products are widely accepted as highly nutritional and beneficial foods. Fermentation improves the bioavailability and nutrient profile. Fermented products are a rich source of live microorganisms some of which possess potential probiotic properties (Michalak and Chojnacka, 2016). According to FAO/WHO (2002) definition, probiotics (*pro*=for and *bios*=life) are "live microorganisms which, when administered in adequate amounts, provide a health benefit to the host" (Hill *et al*., 2014). Commonly accepted probiotics are lactobacilli, bifidobacteria and some non–pathogenic yeast species. Many studies report that probiotics improve a number of digestive issues (Aidy *et al*., 2015), homeostasis (Backhed *et al*., 2012) and the barrier functions of the gut epithelium (Marzorati *et al*., 2014), the immune (Levy *et a*l., 2016), cardio-vascular (Thushara *et al*., 2016) and central nervous system (Hsiao *et al*., 2013), sugar metabolism (Marzorati *et al*., 2014), the metabolic syndrome (Cani and Van Hul, 2015). In order to support a health claim, each probiotic product is required to enclose the minimal daily ingested dose needed for specific health benefit(s) (FAO/WHO, 2002). Some food products are known as "synbiotics", due to high content of both prebiotics and probiotics (Homayouni *et al*., 2007). Since legumes and cereals are a naturally rich source of non-digestible oligosaccharide fibers, fermented products could be considered natural pre-synbiotics. The reason is their high prebiotics content and the resulting health benefits on the bifidogenic gut microbiota as well as the possible combination with probiotic microorganisms.

Prebiotics (or fibers) are non-digestible in the upper part of the gut substances, such as – non-digestible carbohydrates, which are utilized by beneficial indigenous bacteria in the intestine and promote their growth (FAO/WHO 2002). Food matrix suitability for probiotic delivery is being actively studied, but still, not enough information is available (Sanders and Marco, 2010).

Nutritional challenges and soymilk

Fermented milk products have long been recognized as widely consumed and functional foods which are able to deliver a daily dose of probiotic bacteria. However, part of the population is unable to consume milk due to growing lactose intolerance and allergy to casein and other milk proteins, as well as the problem with high cholesterol. These problems, along with a growing interest in a vegetarian and vegan lifestyle, contribute to the emergence of new tendencies on the market. Consumers are looking for milk alternatives as a suitable source of nutrients and probiotics. Non-dairy fermented probiotic products of soy, cereal, vegetable or fruit origin are gaining popularity (Prado *et al*., 2008). In addition, their naturally high prebiotic content makes them ideal "synbiotics". Moreover, some of them have been traditionally used for centuries around the world.

Novel functional food development is a challenging and expensive endeavor. Appropriate selections of a suitable food matrix, technological and processing techniques are important determinants of probiotic viability. In addition, probiotics need to retain a high viable number during long-term storage (Endo *et al*., 2014). Thus, appropriate selection combination of delivery substrate and starters/adjuncts strains is also required for appropriate growth, productivity and stability during processing and storage (Ranadheera *et al*., 2010). Appealing sensory characteristics and enhanced nutritional value are also essential factors for the success of novel products (Rouhi *et al*., 2013).

The aim of this review is to summarize published data on the role of lactic acid bacteria (LAB) in soy fermentation processes and their contribution to the production of new types of soymilk-based yogurt-like products with enhanced nutritive properties. We discuss the potential of probiotics to increase the bioavailability of important nutrients or active compounds during soymilk fermentation and the role of LAB in contributing to new types of non-dairy functional foods. The inclusion of probiotics that can carry out such tasks is an efficacious way to improve the quality of products and an inexpensive and natural way to add functionality to soy products.

Soybean as nutrients source

Soybean (*Glycine max*), a crop belonging to the legume family, is native to East Asia, but presently – well-recognized throughout the world. In the Asian region it is consumed as soy flour, milk, fermented products such as tempeh and miso, tofu, soy yogurt. Soybeans are generally composed of ~35–40% protein, ~20% lipids, ~9% dietary fiber, and ~8.5% moisture based on the dry weight of raw seeds (He *et a*l., 2013).

According to FDA health claims, soy contains high amounts of protein, fiber, iron and essential fatty acids, but is low in fat (FDA, 1999). It has a low amount of saturated fat, and is therefore suitable for the prevention of cardiovascular diseases. The high protein content makes soy a suitable nutrient containing essential amino acids. Soy-based foods have been discussed as an approach to improve – the protein-calorie malnutrition problem and to effectively replace animal proteins in one's diet (Montgomery, 2003).

Several beneficial properties of soy products have been suggested: anti-microbial (Singh *et al*., 2015) and anti-oxidative activities (Martinez-Villauenga *et al*., 2012), anti-hypertensive (Tsai *et al*., 2006), anti-hypercholesterolemic (Kim *et al*., 2014), anti-obesity (Lee *et al*., 2013) and anti-cancer (Lai *et al*., 2013) effects. However, together with the beneficial properties, soybean/soy products are included in the list of the top 10 most allergenic foods.

Soymilk fermentation processes and bio-active compounds

Soymilk is obtained from soy beans that have undergone a processing procedure to obtain soybean water extract from crushed soybeans (Fig. 1). It is an economically suitable substitute for dairy products, whose protein content is similar to cow's milk however, it is free of lactose. Soymilk contains around 3.5% protein, 2.9 % carbohydrates, 2% fat and 0.5% ash. Besides its high protein content, soymilk also contains isoflavones, flavonoids, minerals, vitamins, saponins, etc. The processes that enhance the nutritive properties and improve the bioavailability of different components of soybean products are especially valuable. In addition to processing techniques, the content of soy oligosaccharides in milk, particularly raffinose and stachyose, could be decreased by fermentation. These oligosaccharides found in soybeans have invaluable prebiotic properties. Non-digestible oligosaccharides, which act as prebiotics, promote the growth of colon probiotic bacteria (Benno *et al*., 1987). However, they are also a reason for gastric distress, bloating and flatulence. Hydrolyzation of oligosaccharides to simpler sugars during fermentation reduces negative side effects (Adaeymo and Onilude, 2013).

Another anti-nutritional factor in the soy substrate is the high amount of insoluble proteins such as glycinin and beta-conglycinin. Their content in soy accounts for 65-80% of the total protein content (Wynstra *et al*., 1986). Soy proteins may act as allergens with children under the age of 3 being most susceptible (Wynstra *et al*. 1986). Due to an increase in soy products consumption around the world, science and industry have been looking for various processing methods to reduce soy protein allergenic properties (Shriver and Yang, 2011). Microbial and plant proteases are being employed to disrupt allergenic proteins. Fermentation is also able to hydrolyze proteins to simpler forms and thus reduces allergenic properties (Singh *et al*., 2014). In addition, soy protein hydrolysates are more effectively absorbed in the intestines than non-hydrolyzed proteins (Ziegler *et al*., 1998) and some of them may act as precursors to bioactive peptides (Singh *et al*., 2014).

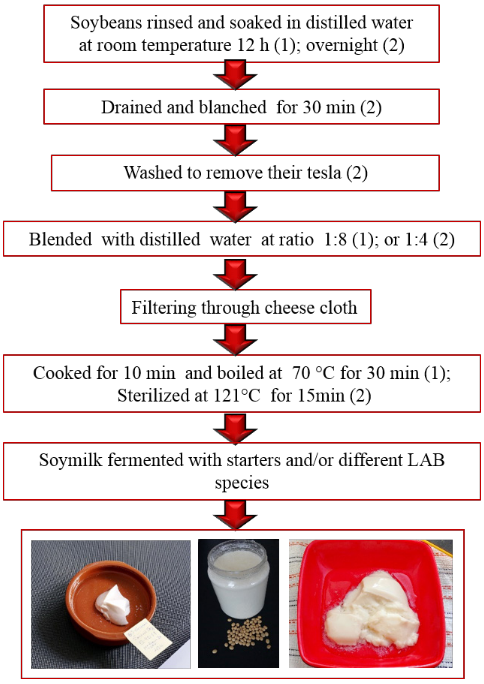

Fig. 1. Diagram flow of soymilk preparation and soy yogurt-like products. Adapted from (1) Sumarna (2010) and (2) Hati *et al.* (2014), with illustrations from personal archive of Dragnev K.

Lactic acid bacteria and soya fermentation

Dairy products have been proven to be lactic microbiota and probiotic carrier food. Lactic acid bacteria (LAB) have been extensively studied for various applications. Besides milk, they can utilize various substrates of plant origin, such as legumes, cereals, vegetables, etc. Soymilk could provide a cheaper and efficient probiotic delivery matrix alternative, especially in soybean producing countries, such as in Asia and South America (Tou *et al.*, 2007).

Beneficial effects of lactic acid bacteria during soy fermentation

Plant substrates have specific health properties and provide unlimited freedom in creating new products for different health conditions. During fermentation, along with a specific taste and flavor, bioactive compounds are also generated and enhance the bio-functionality of the products. These processes by different lactic acid bacteria, yeasts or some micromycetes, contribute to the functionality and benefits of fermented raw materials.

In the soybean substrate, fermentation by lactic acid bacteria increases soymilk functional aspects. Most important are the hydrolysis of soy proteins, production of bioactive peptides, conversion of isoflavones along with a decrease in non-digestible oligosaccharides, such as raffinose and stachyose (LeBlanc *et al.*, 2004). These oligosaccharides cause flatulence, digestive issues and a beany flavor, which are responsible for the limited consumption of soymilk presently.

LAB and isoflavones conversation

Soymilk is very rich in phenolic phytochemicals - isoflavones. They possess an oestrogen-like structure. Soy isoflavones have been considered for their potential in preventing major age-related and degenerative diseases (Wang *et al.* 2013), such as cancer (Jiang *et al.*, 2016), cardiovascular (Yan *et al.*, 2017), osteoporosis (Zheng *et al.*, 2016). They could also have a positive effect in relieving menopausal symptoms (Okabe *et al.*, 2011).

Soy isoflavones exist in two basic forms: glucosides (primary daizidin and genistin) and aglycones (primary daidzein and genistein). Aglycones are low molecular weight compounds, which are more easily absorbed in the intestine and thus are considered to possess greater bio-availability. The content of bioflavonoids in fresh milk is about 80-93% glucosides and 7-15% aglycones (Ding and Shah, 2010). Isoflavone content is also dependent on soybean and the processing techniques used. Germination, heat treatment, enzymatic hydrolysis and cooking of soybeans increase aglycone content through glucoside hydrolysis (Zhou and Erdman, 1997). Fermentation of soymilk by different LAB increases the bioavailability of isoflavones by hydrolyzing glucosides to aglycones. Natural conversion due to intestinal microflora also occurs in the lower small intestine, but there is a large variability in individual conversion capacity (Chun *et al.*, 2008). The increase in the aglycone content is closely related with the intensification of estrogenic properties and antioxidant activity of fermented soy products.

Several LAB are capable of increasing aglycone content in soymilk (Table 1). Chien *et al.*, (2006) tested soymilks (fermented individually or in combination) fermented with strains of *Streptococcus thermophilus* BCRC 14085, *Lactobacillus acidophilus* BCRC 14079 and bifidobacteria (*Bifidobacterium infantis* BCRC 14633, *B. longum* B6). Due to the highest ß-glucosidase activity of *S. thermophilus*, the aglycone contents of soymilk increased (Chien *et al.*, 2006). Specifically, bacteria with high beta-glucosidase activity are responsible for conversion of isoflavones. Beta-glucosidase is a microbial enzyme which is able to carry out hydrolysis of glucosides to the more bioavailable aglycones. Various LAB, as a single strain or in combination with other microorganisms, have been found to possess high beta-glucosidase activity in soymilk (Table 1). A strain-specific beta-glucosidase activity of LAB strains in soymilk was also reported. *L. rhamnosus* C6 revealed the highest beta-glucosidase activity and increased levels of aglycones during 12 h fermentation. In addition, conversion of isoflavones and the growth of probiotic bacteria are correlated (Hati *et al.*, 2014).

Generally, several probiotic strains revealed well-supported growth and viability in soymilk. Fermentation of soymilk for 12 hours with *L. paraplantarum* KM, *Weissella* sp. 33, or *L. paraplantarum* KM co-cultured with *Enterococcus faecium* 35, resulted in successful conversation of 92-100% of daidzin and 98-100% of genistin to corresponding aglycones (Chun *et al.*, 2008). This important characteristic is rather strain-specific than species-specific. Significant variations of reported conversation ratio of glucosides, during the fermentation of soybean substrates (soymilk, soy proteins) were also pointed. Different authors observed high variability in the beneficial beta-glucosidase capacity of LAB. Aglycones in soymilk were found to increase up to 90%-100% after fermentation with *L. rhamnosus CRL98*1 (Marazza *et al.*, 2009), and up to 71% with *L. casei* L26 (Donkor and Shah, 2008). Some of these strains are deemed suitable for preparation of fermented soy foods such as soy yogurt, soy beverage, soy cheese, and soy dahi. A functional soy beverage with *L. rhamnosus* CRL981 possessed higher antioxidant activity (71.2 ± 4.0%) in comparison to unfermented milk. The strain CRL981 was selected for its highest beta-glucosidase activity in soymilk among 63 LAB strains (Marazza *et al.*, 2009).

Table 1. Lactic acid bacteria and conversation of soy proteins, oligosaccharides and isoflavones

Function	Lactic acid bacteria responsible	Reference
Protein hydrolysis	*L. rhamnosus* C6	Suborta *et al.*, 2013
	S. thermophilus MD2 and L. rhamnosus NS6	Hati *et al.*, 2017
	L. bulgaricus Lb1466	Donkor *et al.*, 2007
	L. acidophilus LAFTI® L10, *B. lactis* LAFTI® B94, and *L. paracasei* LAFTI® L26 and starter culture (*L. bulgaricus* Lb1466, *S. thermophilus* St1342)	Donkor *et al.*, 2005
Oligosaccharides hyrdolysis	*L. plantarum* (SMN 25), *L.* pentosus (SMN 01 and FNCC 235)	Sumarna,2008
	B. lactis B94*, S. thermophilus* St1342, *L. acidophilus* La4962	Donkor *et al.*, 2007
	L. fermentum K4 *L. fermentum* K3	Mishra *et al.*, 2017
	L. rhamnosus C6 and *L. casei* NCDC17	Hati *et al.*, 2014
	L. plantarum MTCC 542	Rooparshi and Varadaraj, 2014
	L. bulgaricus and S. thermophilus	Omogbai *et al.*, 2005
	L. rhamnosus LR C8	Singhv and Vij 2018
Isoflavones conversion	*S. thermophilus* BCRC 14085	Chien *et al*, 2006
	L. plantarum SMN 025, *L. rhamnosus* FNCC 098	Sumarna, 2010
	L. acidophilus 4461	Otieno *et al.*, 2006
	L. helveticus with *Saccharomyces boulardii*	Rekha and Vijayalakshmi, 2008
	L. rhamnosus C6	Hati *et al.*, 2014
	L. paraplantarum KM, *Weissella* sp. 33, *L. paraplantarum KM* co-cultured with *Enterococcus faecium* 35 .	Chun *et al.*, 2008
	L. plantarum S48 and P1201	Lee *et al.*, 2017
	S. thermophiles BCRC 14085	Chien *et al.*, 2006

According to Marazza *et al.* (2009), *L. rhamnosus* strains generally reveal higher beta-glucosidase specific activity, compared with that of other lactobacilli.

In a study by Fakri *et al.* (2016), 12 *Lactobacillus* and *Pediococcus* strains were used to ferment commercial soymilk for 48 h. Reduction of glucoside content by 64.2% - 95.9% and a 2.1–6.5-fold increase in aglycone content (mainly daizdzein) was observed in comparison to control unfermented milk. Increased overall – strain-specific antioxidant activity during the fermentation was also reported, with no single strains possessing both activities (Fakri *et al.*, 2016). A similar effect on the antioxidant activity and aglycone content of soy powdered milk and soy powdered yogurt was observed with *L. plantarum* strains S48 and P1201 (Lee *et al.*, 2018).

Overall, different LAB can contribute to bioactive potential and antioxidant properties of processed soybeans. They are appropriate starters or adjuncts for new functional soy products, enriched with aglycone forms of isoflavones-containing foods.

LAB and oligosaccharide conversion

Lactic acid bacteria play an important role in the conversion of oligosaccharides (stachyose, raffinose and sucrose) during the fermentation of soy and other non-dairy products. Production of specific enzymes in soymilk and media supplemented with complex oligosaccharides and polysaccharides have been determined for various LAB taxons (Iazzeh *et al*., 2009).

The presence of α and β-galactosidase has been reported for *Bifidobacteria* genera and for some strains belonging to different species from the genus *Lactobacillus, Leuconostoc* and *Pediococcus (*Garro *et al.,* 1994). Lactobacilli producing the enzyme α-galactosidase are able to utilize complex oligosaccharides as a major growth substrate, thus the elimination of non-digestible oligosaccharides could be achieved in soy-based products (LeBlanc *et al*., 2004).

Several other non-digestible oligosaccharides, such as stachyose, verbascose, galactinol that are contained in soy could be hydrolyzed by α-galactosidase. Alpha-galactosidase is suitable for oligosaccharide digestion in legume products (Townsend and Pitchford, 2012).

According to Rodriguez *et al*. (2009), *L. plantarum* is the most commonly used commercial starter in plant substrates fermentation, due to the presence of α-galactosidases. Wang *et al*. (2016) reported an expressively higher oligosaccharide reduction (84 – 99%) by an *L. plantarum P8* strain in soybean meal. A strain-specific capacity to metabolize raffinose and stachyose was demonstrated during soy fermentation by 7 *Lactobacillus* strains. Each of them was able to reach a desired probiotic level of viability (10^8 CFU/ml), due to the capacity to metabolize soy oligosaccharides (Sumarna, 2008). The most active strains, *L. plantarum* (SMN 25), *L.* pentosus (SMN 01 and FNCC 235) reduced raffinose (by 81.5%, 73.0%, 67.0%) and stachyose (by 78.0, 72.5, 66.0%), respectively, in soymilk during fermentation at 41°C. In comparison, Donkor *et al*. (2007) determined raffinose reduction (by 77.4%, 64.5% and 55.9%) for *B. lactis* B94, *S. thermophilus* St1342 and *L. acidophilus* La4962 strains, respectively.

Several studies determined *L. rhamnosus* as effective α-galactosidase producer in soymilk. *L. rhamnosus* K4 and *L. fermentum* K3 were selected out of 49 lactobacilli, belonging to different species, due to their high α-galactosidase activity in soymilk (Mishra *et al*., 2017). On the other hand, two phylogenetically closely related strains, *L. rhamnosus* C6 and *L. casei* NCDC17, were found to exhibit the highest α-galactosidase activity in soymilk fortified with 1.5% whey protein (Hati *et al*., 2014). The level of α-galactosidase production, like other metabolic activities, is also strain-specific. Alpha-D-galactosidase of *L. plantarum* MTCC 542 reduced raffinose and stachyose content by almost 99% in soy-based probiotic curd product and reached high cell density content of 9.4 $\log_{10}$ CFU/g (Rooparshi and Varadaraj, 2014). In another study, stachyose content was reduced up to 90% by strain *L. rhamnosus* LR C8, while 6 strains of *L. plantarum*, 4 strains of *L. rhamnosus* (RC25, LR C28, LRC34) and *L. helveticus* (NCDC 288) strains reduced stachyose content less than 37% (Singh and Vij, 2018). In the same study highest a-galactosidase activity for all tested LAB was estimated after incubation for 24 h in soymilk. The result was fast coagulated (6 -12 h), a yogurt-like and strain-specific product of fermentation. Such specificity likely is determined by period and conditions of cultivation, and also by growth and metabolic differences of strains, during the LAB acclimatization and fermentation of the specific substrates (Singh and Vij, 2018). Commonly used yogurt starter bacteria – *L. bulgaricus and S. thermophilus* were able to reduce stachyose soymilk content by 31.5% after 8 h of fermentation (Omogbai *et al*., 2005). Starter culture *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* (IM 025) were capable to work and to grow together with other probiotic bacteria – such as *L. johnsonii* La-1, *L. rhamnosus* GG, *Bifidobacterium sp.*, producing soy beverage and cow milk yogurts. Subsequent analysis revealed different sugar utilization by probiotic bacteria, depending on the substrate - cow milk or soy beverage (Farnworth *et al*., 2007).

Soy fermentation and proteins hydrolysis

Lactic acid bacteria are able to degrade different proteins to simpler form - peptides (di-, tri-, and oligopeptides) with the help of bacterial extracellular proteinase and peptidases (Sanjukta and Rai, 2016). Some of them use soy proteins as a nitrogen source. Bioactive peptides are produced from larger soybean proteins by microbial fermentation, food processing, and gastrointestinal digestion and/or enzymatic hydrolysis (Chatterjee *et al.,* 2018). Peptides are also formed from precursor simple forms (Singh *et al*., 2014). A release of peptides with functional properties in soymilk was revealed for various LAB strains. They are associated with multiple metabolic effects, both beneficial and undesirable (Singh *et al*., 2014). During fermentation, the allergenic properties of proteins/long chain peptides are decreased due to their degradation. Soy bioactive peptides have been characterized for their health properties, such as antioxidant (Liu, Chen and Lin, 2005), antihypertensive (Tsai *et al*., 2006; Martinez-Villauenga *et al*., 2012), antimicrobial (Singh *et al*., 2015), anti-tumor (Hernandez-Ledesma *et al*., 2009) activities.

The major soy proteins are glycinin and β-conglycinin. Beta-conglycinin is a major soy protein with extensively studied benefits. Enrichment of diet with this protein possesses significant biological effects on lipid metabolism and has been shown to inhibit *in vivo* atherosclerosis development *in mice* (Adams *et al.,* 2004).

Soy proteins are degraded differently due to their structural variations. According to Aguirre *et al*. (2014), glycinin, which has acidic and basic fractions, is susceptible to better hyrolyzation of the acidic fraction by most LAB strains. Regarding β-conglycinin, which consists of 3 subunits: α subunit, α' and beta subunits, the best hydrolyzation is observed for α subunit, due to its structure and easy accessibility by bacterial proteinases.

Proteolytic activity has been observed in soymilk, and for various LAB proteolytic activity in the plant substrate was shown. Hati *et al*. (2013) studied *L. rhamnosus strains* NCDC19, NCDC24, C2, C6 and *L. casei* NCDC17, *L. casei* NCDC297 for their proteolytic activity in soymilk supplemented with whey protein. *L. rhamnosus* C6 was selected for its highest proteolytic activity, as well as high antioxidant and polyphenol content reduction. In another study, a high proteolysis rate was observed for 5 *Lactobacillus* strains (1 *L. plantarum* and 4 *L. rhamnosus* strains) compared to control proteolytic *L. helveticus* strain NCDC 288 (Singh and Vij, 2018).

Comparing milk and soymilk proteolytic activity, Hati *et al*. (2018) noted high proteolytic activity for *L. rhamnosus* NS6 and *S. thermophilus* MD2 in both skim and soymilk. In addition, *S. thermophilus* MD2 exhibited the highest ACE-inhibitory activity and high antimicrobial activity. However, better performance of the tested LAB was achieved in milk (Hati *et al*., 2018). Cultures used as starters in milk, such as *S. thermophilus, L. delbrueckii* subsp. *bulgaricus,* are also able to keep their high proteolytic activity in both milk and soymilk, even though the soy beverage generally does not support high growth of *L. bulgaricus* (Murti *et al*., 1993). Lower growth of *L. bulgaricus* is also reported by Wang *et al*, 1974, due to its inability to ferment sucrose and other soy carbohydrates. On the other hand, *S. thermophilus* is able to utilize sucrose and good growth is observed in soymilk (Murti *et al*., 1993). The process initiated in soymilk is similar to milk fermentation, i.e. streptococci initiate fermentation and lactobacilli acidify the substrate in the process (Farnworth *et al*., 2007). In a study by Donkor *et al*. (2007), *L. bulgaricus* Lb1466 showed the highest proteolytic activity in soymilk, in comparison to *L. acidophilus* (LAFTI L10 and La4962), *L. casei* (LAFTI L26 and Lc279), *Bifidobacterium* (*lactis* LAFTI B94 and *longum* Bl536). The poor proteolytic activity is a reason for restrained growth in milk/soymilk (Klaver *et al*., 1993). *L. bulgaricus* Lb1466 is able to keep its high proteolytic activity in both milk and soymilk. In addition to being strain-dependent, the study revealed time-dependent proteolytic activity. Only a slight increase in liberated amino acids and peptides was observed in the first 24 hours, which, however, increased significantly between 24-48 h. A correlation was observed between proteolytic activity and growth, due to the ability of some strains to utilize both oligosaccharides and proteins in soymilk.

Proteolytic activity is of upmost significance for growth in the substrate as well as ability for soy oligosaccharides (stachyose, raffinose, sucrose) utilization (Nielsen *et al.,* 2001). In addition, the metabolic activity is often strain-dependent (Aguirre *et al*., 2014). LAB's proteolytic activity in soymilk, as well as in soy-based products, revealed modulation according to the culture medium/substrate used for LAB growth and corresponding repression or induction capacity (Donkor *et al*., 2005). Texture and product flavor formation are also dependent on appropriate strains with specific proteolytic activity (Pescuma *et al*., 2010). In comparison to milk, the proteolytic system of LAB in soybean/soymilk is still poorly studied and more information is needed (Shihata and Shah, 2000).

In soy yogurt, co-fermentation of standard dairy starter cultures (*L. delbrueckii* ssp. *Bulgaricus* Lb1466 and *S. thermophilus* St1342) along with probiotics such as *L. acidophilus* LAFTI L10, *Bifidobacterium lactis* LAFTI B94, and *L. paracasei* LAFTI L26), resulted in significantly higher proteolytic activity in comparison to starter culture only fermented control yogurt. Both starter culture and probiotic cultures grew better together due to the higher level of essential growth factors, released by the strains. The increased proteolytic activity of probiotic organisms improved *L. delbruekii* ssp. *bulgaricus* Lb1466 survival. This also resulted in a product with higher ACE inhibitory activity compared to the control, which is partly a result of the higher proteolytic activity of probiotics (Donkor *et al*., 2005).

In addition to the invaluable properties of LAB fermentation soymilk products, LAB in soymilk have been studied for additional activities, such as reduction of polyphenol content in soymilk (Subrota *et al*., 2013), vitamin B production (Gu *et al*., 2015) and also for production of GABA-enhanced soymilk products (Park and Oh, 2007).

Conclusion

Different LAB strains which are able to grow in soymilk and to promote bioavailability of soy nutrients should be considered promising candidates for further scientific and industrial evaluation. Characterization of their strain-specific metabolic activities and technological relevance is a way for development of new non-dairy functional products for consumers with specific demands.

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