Journal of Biology and Today's World
ISSN 2322-3308
http://www.journalbio.com

Received: 25 August 2014 • Accepted: 23 November 2014

Research

doi:10.15412/J.JBTW.01040105

Mixtures of soy- and cow's milk as potential probiotic food carriers

Zahra Hassanzadeh-Rostami[1], Seyed Mohammad Mazloomi[2*], Samane Rahmdel[3], Asma Kazemi[4]

[1] Department of Clinical Nutrition, Student Research Center, School of Nutrition and Food Sciences, Shiraz University of Medical Sciences, Shiraz, Iran

[2] Department of Food Hygiene and Quality control, Nutrition and Food Sciences Research Center, School of Nutrition and Food Sciences, Shiraz University of Medical Sciences, Shiraz, Iran

[3] Department of Food Hygiene and Public Health, School of Veterinary Medicine, Shiraz University, Shiraz, Iran

[4] Department of Clinical Nutrition, School of Nutritional Sciences and Dietetics, Tehran University of Medical Sciences, Tehran, Iran

*correspondence should be addressed to Seyed Mohammad Mazloomi, Department of Food Hygiene and Quality control, Nutrition and Food Sciences Research Center, School of Nutrition and Food Sciences, Shiraz University of Medical Sciences, Shiraz, Iran; Tell: +987137251001; Fax: +987137260225; Email: mazloomi@sums.ac.ir.

ABSTRACT

The current global burden of chronic diseases necessitates the introduction of new healthy food, including probiotic ones. Thus, the aim of the present study is to evaluate the suitability of different mixtures of soy and cow's milk as probiotic food carriers. Soy milk prepared from soybeans was combined with cow's milk at the concentrations level of 0, 20, 40 and 60% (v/v). *Lactobacillus acidophilus* La-5, *Lactobacillus casei* and yogurt starter (*Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus*) were used in a single culture for fermentation of different mixtures. Viable cell count, pH and titratable acidity of samples were determined on the first day after fermentation. The viable cell counts of all samples were over recommended levels for probiotic action (10^6 cfu mL^{-1}). While *L. acidophilus* exhibited the highest capacity to grow in the presence of soy milk, the lowest bacterial population was found in the mixture containing 60% soy milk and was fermented with yogurt starter (*P*>0.005). Samples inoculated with yogurt starter showed the lowest pH and the highest titratable acidity values; however, ones into which *L. acidophilus* was injected had the highest pH and the lowest titratable acidity values. The present study showed that mixtures of soy- and cow's milk could serve as vessel for probiotics. However, more data are needed before industrial production. In order to achieve short fermentation time and pleasant fermented products, strain selection for ability to ferment mixture rapidly along with maintaining the significant probiotic yields should be taken in to consideration.

Key words: soymilk, cow's milk, Lactobacillus acidophilus, Lactobacillus casei, yogurt starter, fermentation

1. INTRODUCTION

Chronic diseases such as gastrointestinal disorders, cardiovascular disease, hypertension, cancer and osteoporosis have increased over the recent decades(1, 2). These serious health problems are usually attributed to improper nutrition arising from stress and busy lifestyle of humans. Consumption of food that are enriched with physiologically active components such as probiotics is considered to be a promising solution for prevention or elimination of these diseases (2). Probiotics are live microorganisms that are beneficial for the health of consumers when it is ingested in sufficiently high levels and mainly belong to the genera *Lactobacillus* and/or *Bifidobacterium* (3). These microorganisms have several health promoting effects including activation of immune system, inhibition of pathogens, prevention of upper intestinal tract disorders and colon cancer as well as improvement of lactose utilization, reduction of serum cholesterol level and also alleviation of hypertension (4-6). Even though the most concentrated sources of calcium are dairy products (7) however, they still are the most typical carriers of probiotic bacteria, lactose intolerance, cholesterol content and also allergic potency of milk-proteins that are some inconveniences related to their consumption. Hence, there is an increasing demand for non-dairy probiotic products lacking the dairy allergens (8, 9). Soy milk, the water extract of soybean, offers a promising performance as a carrier of probiotics (9). Furthermore, it is enriched in nutritive elements like proteins, unsaturated fatty acids, lecithins, isoflavones, mineral substances, free amino acids and polypeptides (10), while containing only a small amount of saturated fatty acid and it lacks cholesterol or lactose (6, 11). From a different point of view, fermentation of soymilk with probiotics could be considered a way to diminish the problems of beany flavor and flatulence that are attributed to the oligosaccharide constituents in soybean (11). However, in comparison to cow's milk, soy milk and other non-dairy milks have lower levels of calcium and are now often fortified with the same amount of calcium found in cow's milk (7). Thus, the incorporation of probiotics into the mixture of cow's milk and soy milk seems to offer additional health-promoting features. In the present study, the suitability of different mixtures of cow's milk and soy milk as substrates for the culture of *Lactobacillus acidophilus*, *Lactobacillus casei* and yogurt starter was evaluated.

2. MATERIALS AND METHODS

2.1. Materials

Soybeans (*Glycine max*) were purchased from a local store in Shiraz, Iran. All used chemicals and cultures were supplied by Merck Co. (Darmstadt, Germany).

2.2. Starter organisms and inoculums preparation

Lyophilized *Lactobacillus acidophilus* La-5, *Lactobacillus casei* and yogurt starter (*Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus*) were obtained from Christian Hansen (Hørsholm, Denmark). The inoculums was prepared with inoculating sterilized cow's milk with 0.01% of each lyophilized organism and then incubated at 37 °C until a pH of 4.50 was reached.

2.3. Preparation of fermented product

Soybeans were initially soaked in distilled water for 12-14 h at 5 ˚C. After rinsing them, they were grinded with water at a ratio of 1:5 (w/v) using a commercial blender unit. Afterwards, the resultant slurry was filtered through double-layered cheesecloth to filter out insoluble residues. In order to prepare different fermentation mediums, homogenized and standardized low fat cow's milk (1.5%) was mixed with the produced soy milk as following: a) 100% cow's milk; b) 80% cow's milk inoculated with 20% soy milk (v/v); c) 60% cow's milk inoculated with 40% soy milk (v/v); and d) 40% cow's milk inoculated with 60% soy milk (v/v). These mixtures were sterilized by autoclaving at 121°C for 15 min, followed by cooling down to 40°C. Each of the above mentioned fermentation medium was divided into three equal portions which were then inoculated with *L. acidophilus* La-5 or *L. casei* or yogurt starter inoculums at a concentration of 3% v/v. The preparations were subsequently incubated at 37 °C and then stored at 4 °C. Bacterial count, pH and titratable acidity of all samples were determined on the first day after fermentation. The entire experiment was replicated five times.

2.4. Enumeration procedure

For each sample, the viable counts of starter cultures were enumerated by a pour-plate method. In this regard, serial ten-fold dilutions were prepared in a solution of 0.9% NaCl (w/v) and suitable dilutions were plated onto de Man, Rogosa and Sharpe agar (MRS agar) plates. The set agars were incubated anaerobically at 40 °C for 72 h. Colony counts were calculated in $\log_{10}$ cfu mL^{-1}.

2.5. pH and titratable acidity

The pH was measured by using a Metrohm Model 827 pH meter (Metrohm Ltd., Herisau, Switzerland) calibrated with commercial pH 4.00 and 7.00 buffer solutions. Titratable acidity (TA) was determined according to AOAC (1997) (12). The results were reported as g lactic acid/ 100 mL.

2.6. Statistical analysis

Data were statistically analyzed using SPSS 16.0 (SPSS, Inc., Chicago, IL, USA). Group comparison was performed by means of the Kruskal-Wallis and Mann-Whitney U tests. The 0.05 level of statistical tests was considered as a significant point.

3. RESULTS AND DISCUSSION

3.1. Cell growth

As shown in Table 1, the viable counts of bacteria were not significantly affected by the probiotic strain, as well as the mixture type and for all of the starter cultures, *L. acidophilus* La-5, *L. casei* and yogurt starter, were at the range of 7.63-8.26 $\log_{10}$ cfu mL^{-1} in different fermented products.

Table 1. The viable counts ($\log_{10}$ cfu mL^{-1}) of *L. acidophilus*, *L. casei* or yogurt starter (*L. delbrueckii* ssp. *bulgaricus* and *S. thermophilus*) in 4 mixtures of milk and soy milk on the first day after fermentation

Treatments	***Starter culture***			***P value****
	L. acidophilus	***L. casei***	**Yogurt starter**	

Milk	7.63±0.86	8.25±0.36	8.17±0.58	0.20
Milk- 20% soymilk	8.10±1.11	8.26±0.47	8.17±1.11	0.95
Milk- 40% soymilk	8.26±0.52	7.70±0.83	8.15±0.85	0.37
Milk- 60% soymilk	8.13±0.56	7.81±0.80	7.63±0.95	0.50
P* value	0.58	0.31	0.65	

Values are given as mean ± standard deviation of five replicates. * *P*-values were resulted from Kruskal-Wallis Test.

Of the three starter cultures used in this study, *L. acidophilus* La-5 showed the greatest ability to grow in the presence of soy milk. However, the highest population of *L. casei* was observed in the cow's milk (0% of soy milk) and also in the cow's milk containing 20% (v/v) soy milk after fermentation. Although, the cow's milk fortified with 60% (v/v) soy milk had the lowest concentration of yogurt starter at time of the test, soy milk at the levels of 20 and 40% (v/v) did not have an adverse effect on the viability of this starter.

3.2. pH and titratable acidity

As the results show, the TA values were not significantly different for samples of one mixture type inoculated with different starter cultures however, a lower acid production was found in mixtures fermented with *L. acidophilus* La-5. Fermentation with mixed cultures of *L. delbrueckii* ssp. *bulgaricus* and *S. thermophilus* (yogurt starter) gave TA values over 0.70% in the cow's milk and also in the samples containing 20 and 40% (v/v) soy milk. Similarly, titratable acidity of about 0.70% was seen in the majority of mixtures fermented by *L. casei*. Regardless of the starter type, the cow's milk fortified with 60% (v/v) soy milk had the lowest titratable acidity values (Table 2).

Table 2. The titratable acidity values (g lactic acid/ 100 mL) of 4 mixtures of milk and soy milk fermented by *L. acidophilus*, *L. casei* or yogurt starter (*L. delbrueckii* ssp. *bulgaricus* and *S. thermophilus*) on the first day after fermentation

Treatments	***Starter culture***			***P value****
	L. acidophilus	***L. casei***	**Yogurt starter**	
Milk	0.53±0.23	0.71±0.08^a	0.82±0.12^a	0.07
Milk- 20% soymilk	0.56±0.26	0.66±0.07^a	0.77±0.05^a	0.07
Milk- 40% soymilk	0.54±0.21	0.74±0.27^a	0.71±0.08^a	0.42
Milk- 60% soymilk	0.42±0.20	0.44±0.05^b	0.51±0.04^b	0.31
P* value	0.45	0.01	0.006	

Values are given as mean ± standard deviation of five replicates. Numbers in the same column with different superscript letters (a, b) are significantly different ($P< 0.05$) according to Mann-Whitney U test. * *P*-values were resulted from Kruskal-Wallis Test.

Different mixtures containing the same starter did not exhibit significant differences among their respective pH values. However, as shown in Table 3, *L. delbrueckii* ssp. *bulgaricus* in conjunction with *S. thermophilus* as yogurt starter cultures resulted in pH values below 4.5 in all experimental mixtures which were significantly lower than those of the other treatments.

Table 3. The pH values of 4 mixtures of milk and soy milk fermented by *L. acidophilus*, *L. casei* or yogurt starter (*L. delbrueckii* ssp. *bulgaricus* and *S. thermophilus*) on the first day after fermentation

Treatments	***Starter culture***	***P value****

	L. acidophilus	*L. casei*	Yogurt starter	
Milk	5.10±0.97	4.63±0.28	4.37±0.21	0.06
Milk- 20% soymilk	4.95±0.94[a]	4.49±0.14[a]	4.22±0.04[b]	0.01
Milk- 40% soymilk	4.89±0.84[a]	4.45±0.10[a]	4.22±0.03[b]	0.01
Milk- 60% soymilk	4.87±0.88[ab]	4.42±0.10[a]	4.22±0.04[b]	0.04
P* value	0.33	0.37	0.06	

Values are given as mean ± standard deviation of five replicates. Numbers in the same row with different superscript letters (a, b) are significantly different ($P< 0.05$) according to Mann-Whitney U test. * *P*-values were resulted from Kruskal-Wallis Test.

The highest pH values were found in preparations fermented with *L. acidophilus* La-5, while in the samples inoculated with *L. casei*, the mean pH values ranged from 4.42 to 4.63. Because of health benefits of probiotics, there has been a considerable interest in combining them with different food. However, the minimum concentration of probiotic intake through cultured milk or soymilk to achieve beneficial effects on the host is suggested to be 10^6 cfu mL^{-1} (13). In the present study, the viable counts of *L. acidophilus* La-5, *L. casei* and yogurt starter (*L. delbrueckii* ssp. *bulgaricus* and *S. thermophilus*) in all the fermented samples were above the recommended level for probiotic effect after 5 h of fermentation. Of the three starter cultures, *L. acidophilus* La-5 grew well in samples fortified with soymilk that can be attributed to its ability to ferment sucrose, which is the main sugar in soy milk (14). On the other hand, the low free amino acids content of cow's milk can limit the growth of nonproteolytic bacteria (15). However, the yogurt starter reached its maximum numbers in cow's milk presumably due to the ability of *L. delbrueckii* subsp. *bulgaricus* to break down cow's milk proteins supporting the growth of nonproteolytic strain, *S. thermophilus* (15). Although *L. delbrueckii* subsp. *bulgaricus* cannot ferment sucrose and other soy carbohydrates, *S. thermophilus* is able to consume sucrose (4). Thus, samples containing 20 and 40% soy milk had a more suitable environment for the growth of yogurt starter. In the study conducted by Wang, Yu, et al. (14), among lactic acid bacteria inoculated into soymilk, *L. acidophilus* and *L. bulgaricus* showed the highest and the lowest viable counts after 48 h of fermentation, respectively; on the other hand, *S. thermophilus* grew well in soy milk and its maximum population was reached after 24 h. Farnworth et al. (15) indicated that *S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus* grew well as a mixed culture in both cow's milk and soy beverage. In another study, when soy milk was inoculated with *L. acidophilus* (LAFTI® L10 and La4962), *L. casei* (LAFTI® L26 and Lc279), *L. delbrueckii* ssp. *bulgaricus* Lb1466 and *S. thermophilus* St1342 individually, all strains reached above recommended level of 10^6 cfu mL^{-1} during 48 h of fermentation at 42 °C; however, *L. acidophilus* L10 and *L. delbrueckii* ssp. *bulgaricus* Lb1466 exhibited slow growth in comparison to the other organisms throughout the incubation (4). Soy milk as an applicable delivery medium for *L. acidophilus* L10 as well as *L. casei* L26 was also shown by Donkor and Shah (16). Another research (14) reported that after 6 h of fermentation, growth rates of *L. casei* Zhang in both the soy milk and bovine milk were similar. However, from a commercial perspective, not only they grow well in soy milk, it is also important that bacterial fermentation to the desired pH of 4.5 could well occur within the short period of incubation (17), because pH can affect texture, physical stability, flavor and aroma of the soy yogurt (18). In the present study, the lowest pH and the highest TA values were found in mixtures fermented with yogurt starter followed by ones fermented with *L. casei*. As reported by Farnworth et al. (15), the constructive interrelationship occurring between *S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus* in milk fermentation seems to also occur in the soy milk. Donkor et al. (4) reported that twelve hours is required to reach a pH of 4.12 in the soy milk fermented with *L. casei* L26; while the pH of soy milk samples fermented with the other strains including *L. acidophilus* declined to below 4.5 after a prolonged incubation (48 h). In this study, the pH of samples inoculated with *L. acidophilus* was highest after 5 h of fermentation and also Fermentation by *L. acidophilus* gave the lowest amount of TA nonetheless, it flourished well. Previous studies have also reported low production of organic acids by lactic acid bacteria growing well in soy milk (4). On the other hand, the mixture containing 60% (v/v) soy milk exhibited the lowest TA level for each starter, even though it had a pH value similar to that of the other mixtures. This observed phenomenon was in agreement with the reports of Farnworth et al. (15), Wang, Guo, et al. (19) and Champange et al. (17) and could attribute to the lower buffering ability of soy milk as compared to that of cow's milk. In order to create shorter fermentation time as well as a significant probiotic yields and at the same time generating a product with pleasant flavor, it seems that a suitable way would be to combine probiotic strains with a yogurt strain (17).

4. CONCLUSION

The present study shows that soy milk in combination with cow's milk could be a suitable substrate for the culture of *Lactobacillus acidophilus*, *Lactobacillus casei* and yogurt starter. Such functional food could be a desirable choice of food for consumers, particularly those who are lactose intolerant. However, the organoleptic acceptability of such products should be evaluated and further research is required, to screen starters that showed great potential for application in the development of soymilk-based products.

ACKNOWLEDGMENT

This study was entirely financed by Shiraz University of Medical Sciences. And it was edited by the RCC center of Shiraz University of Medical Sciences.

AUTHORS CONTRIBUTION

This work was carried out in collaboration among all authors.

CONFLICT OF INTEREST

The authors declared no potential conflicts of interests with respect to the authorship and/or publication of this article.

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