Effect of adding soymilk on physicochemical, microbial, and sensory characteristics of probiotic fermented milk containing *Lactobacillus acidophilus*

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(Received 9 Jun 2013; revised version 14 Dec 2013; accepted 15 Dec 2013)

Summary

The aim of the present study was to investigate the effect of addition of soymilk on physicochemical, microbial, and sensory characteristics of milk fermented with *Lactobacillus acidophilus*. Soybeans were blended 1:5 w/v with distilled water. The prepared soymilk was added to milk in combinations of 20%, 40%, and 60%. Milk was used as the control. All the samples were sterilized and fermented with *Lactobacillus acidophilus* LA-5 as probiotics. Then, they were kept at 5ºC for 14 days. Microbial count, titratable acidity, pH, syneresis, color parameters and sensory evaluation were performed during the storage time. Results showed that all the samples possessed minimum effective dose of LA-5 on day 14, although a significant decrease in LA-5 was observed in the sample with 60% soymilk. In each experimental day, there was a noticeable decrease in the pH of the samples. Addition of soymilk to milk also significantly increased syneresis, particularly in samples with 60% soymilk. Sensory evaluations showed that scores of texture, mouth sense, aroma, and flavor of the samples with 40% and 60% soymilk were significantly lower than other samples. With respect to color, “L” value decreased significantly in the fermented product with 60% soymilk and the decrease was more pronounced with longer storage times. In conclusion, addition of 20% soymilk did not substantially alter physicochemical and sensory characteristics of milk while providing an appropriate growth culture for LA-5. The mixture of milk-20% soymilk can be introduced as a good probiotic product with lower lactose content and additional nutritional benefits.

Key words: Probiotics, Milk, Soymilk, *Lactobacillus acidophilus*

Introduction

Probiotics are live non-pathogenic bacteria with the potential of colony formation in the gastrointestinal tract of humans (Williams, 2010). Probiotics have many health benefits including treatment of intestinal disorders, such as diarrhea (Guandalini, 2011) and inflammatory bowel disease (Meijer and Dieleman, 2011), enhancement of host immunity against intestinal infections (Lomax and Calder, 2009), and prevention of colorectal cancer (Azcárate-Peril *et al*., 2011). Lactic acid bacteria (i.e. *Lactobacillus* and *Bifidobacterium* strains) are the most widely used probiotics (Williams, 2010).

To convey probiotics to human gut, a delivery or carrier system is needed to carry probiotics to the intestine and protect them against gastric acid (Sanders and Marco, 2010). Dairy products are the most common delivery systems for probiotics. Milk has been reported to increase the viability of acid-sensitive *Lactobacillus* and *Bifidobacterium* strains during simulated passage from the stomach (Sanders and Marco, 2010).

Milk is one of the most valuable foods with eminent quality for proteins, minerals, and vitamins. However, its high quantities of casein and lactose make it allergenic and intolerable by some individuals. Soymilk is a good substitute for milk for such individuals because it contains no casein and lactose (Fiocchi *et al*., 2003). Furthermore, because of low content of saturated fats, high amounts of polyunsaturated fats, absence of cholesterol and presence of plant sterols, soymilk, unlike milk possesses anticholesterolemic and antiatherogenic properties (Sacks *et al*., 2006; Gardner *et al*., 2007). Soy also possesses isoflavones, which are phytoestrogens and may be advantageous in cases of low estrogen and prevention of cardiovascular diseases and osteoporosis (Pilšáková *et al*., 2010). There are also drawbacks with soymilk that impede its consumption. For instance, aldehydes in soymilk produce unpleasant flavor and its oligosaccharides cause gastrointestinal bloating and discomfort (Tsangalis and Shah, 2004).

Similar to milk, soymilk is a good delivery vehicle for probiotics (Shimakawa *et al*., 2003; Tsangalis and Shah, 2004). Soymilk contains oligosaccharides, amino acids, and peptides which support the growth of probiotic bacteria. Oligosaccharides in soymilk can act as prebiotics, so addition of probiotics to soymilk makes it a synbiotic product. Besides, fermentation of soymilk by probiotics resolves unfavorable taste and flatulence and improves its nutritional value (Tsangalis and Shah, 2004). Moreover, by their β-glucosidase activity, probiotics hydrolyze isoflavone glucosides in soymilk to corresponding aglycones and therefore increase isoflavones' biological activity (Otieno *et al*., 2006).

A number of previous studies have examined microbial, textural, chemical, and sensory properties of probiotic soymilk or soy yogurt (Farnworth *et al*., 2007; Yang and Li, 2010; Ghorbani *et al*., 2012). However, flavor and aroma of soymilk is discouraging, especially when fermented with bacteria. One group of investigators tested physicochemical and organoleptic characteristics of soymilk-milk mixture fermented with *lactobacillus acidophilus* (Yeganehzad *et al*., 2009). But only 10% and 20% soymilk-milk mixtures were tested. In addition, flavor and texture were the only organoleptic properties that were assessed. In the present study, we investigated the effect of addition of 20%, 40%, and 60% soymilk on physicochemical, microbial, and sensory characteristics of milk fermented with *Lactobacillus acidophilus*. Sensory characteristics included texture, mouth sense, aroma, flavor, and color.

Materials and Methods

Preparation of soymilk

Soybeans were washed and soaked overnight in distilled water at 5°C. After separation of water, the soybeans were blended 1:5 w/v with distilled water. The resultant slurry was then filtered through a double-layered cheese cloth and sterilized for 15 min at 121°C (Wang *et al*., 2002).

Inoculation of *Lactobacillus acidophilus*

The soymilk was mixed in 20%, 40%, and 60% (v/v) proportions with 1.5% fat milk. Milk was used as the control. The resultant mixtures were then sterilized at 121°C for 15 min. After cooling to 40°C, *Lactobacillus acidophilus* LA-5 (Chr. Hansen, Hørsholm, Denmark) was added (0.01% w/v) aseptically and the samples were dispensed into 200 ml containers and inoculated at 40°C. They were then refrigerated for 14 days at 5°C. Physicochemical and microbial evaluation of the samples was performed on days 1, 7, and 14 of the refrigerated storage.

Enumeration of *Lactobacillus acidophilus*

The number of *Lactobacillus acidophilus* in the samples was counted by plating serial dilutions on MRS-Agar (Merck, Darmstadt, Germany). The plates were then incubated anaerobically using gas generating pack A (Merck, Darmstadt, Germany) for 72 h at 40°C (Shah *et al*., 2007). Total colony count was performed using colony counter and the results were reported as Log cfu/ml.

Titratable acidity and pH

The samples were titrated with 0.01 N NaOH solution and their acidity was expressed as percentage of lactic acid (AOAC, 1999). pH was determined using a pH meter 605 (Methrohm AG, Herisau, Switzerland) at room temperature.

Syneresis

Syneresis was determined by placing 50 g of the samples on a fine mesh screen (aperture size 38 µm) at the top of a funnel and leaving it for 1 h at 5°C (Mazloomi *et al*., 2011). The released whey was collected and quantified as the index of syneresis.

Sensory analysis

Color, flavor, aroma, texture/consistency, and mouth feeling of the samples were questioned on a 1-5 point scale from 30 untrained panelists in random order using identical containers coded with random numbers (Iranian National Standard, 2002).

Color assessment

The color parameters L^* (lightness/darkness), a^* (redness/greenness axis) and b^* (yellowness/blueness axis) of the samples were determined using a digital camera in a box with fluorescent light and the photos were analyzed using the Adobe Photoshop software version 8. The colorimetric values were determined in the Lab mode of the software (Yam and Papadakis, 2004).

Statistical analysis

Data were obtained from two independent experiments and each sample was analyzed in triplicate. The collected data was analyzed using SPSS version 16 (SPSS Inc., Chicago, IL).

To compare normally distributed data between groups, one-way ANOVA was used with Scheffe test as the post-hoc test. For non-normal distributed data, Kruskal-Wallis was performed for comparison of data between the four groups, and Mann-Whitney to compare data between two groups. For within-group comparisons between different days, Wilcoxon signed rank test was used. Differences were considered to be significant at $P<0.05$.

Results

The addition of 20% and 40% soymilk to milk did not cause a significant alteration in the growth and survival of *Lactobacillus acidophilus*. However, the sample with 60% soymilk showed a statistically significant decline in the number of *Lactobacillus acidophilus* during storage at 4°C on the day 14 (Fig. 1).

While titratable acidity was statistically higher in milk-20% soymilk than the control (milk) on the day 1 of the storage, it was not statistically different between milk-soymilk samples and the control during the storage times of 7 and 14 days (Fig. 2). The pH value of the samples on days 1, 7, and 14 was between 3.88 and 4.96.

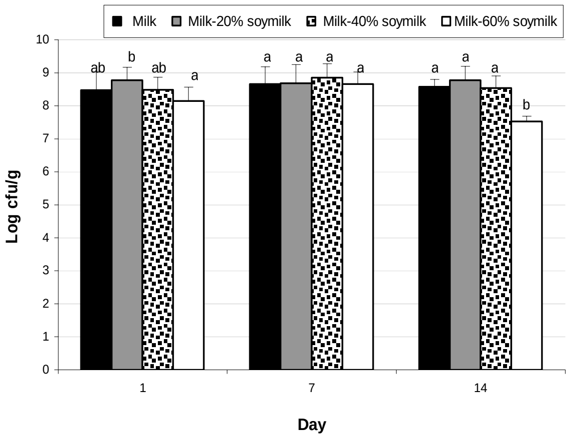

Fig. 1: Survival of *Lactobacillus acidophilus* in samples of milk and milk with 20%, 40%, and 60% soymilk during 1, 7, and 14 days of refrigeration. The error bars represent one standard deviation. In each bar series different letters represent significant differences (P<0.05)

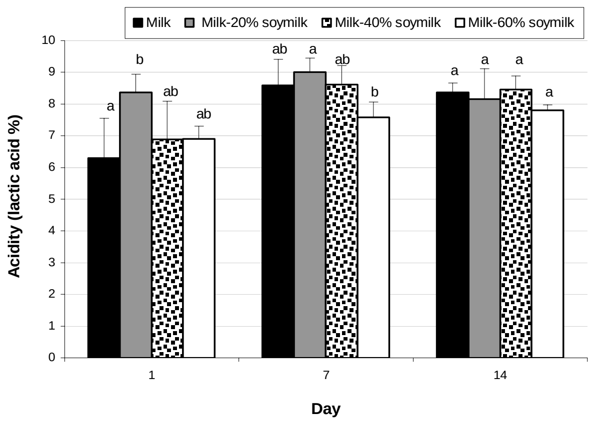

Fig. 2: Titratable acidity of samples of milk and milk with 20%, 40%, and 60% soymilk fermented with *Lactobacillus acidophilus* on days 1, 7, and 14 of refrigeration. The error bars represent one standard deviation. In each bar series different letters represent significant differences (P<0.05)

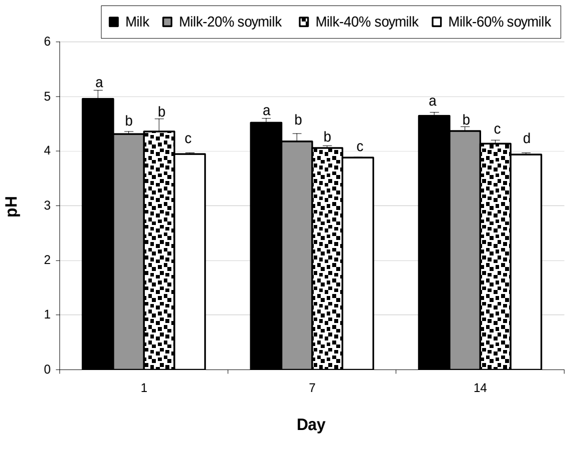

Fig. 3: The pH of samples of milk and milk with 20%, 40%, and 60% soymilk fermented with *Lactobacillus acidophilus* on days 1, 7, and 14 of refrigeration. The error bars represent one standard deviation. In each bar series different letters represent significant differences (P<0.05)

In each experimental day, there was a noticeable decrease in the pH of the samples along with increasing concentration of soymilk. Thus, the control sample had the highest and the sample with 60% soymilk had the lowest pH (P<0.05 between the highest and the lowest) (Fig. 3). On the day 14, as concentration of soymilk in the samples increased their pH significantly decreased (P<0.05 between milk and milk-60% soymilk).

Addition of soymilk to milk increased syneresis in the samples, with a statistically significant difference between milk (control) and the samples of 40% and 60% soy milk and also between 20% and 60% soy milk samples (Fig. 4). There was no statistically significant difference in syneresis between different experimental days.

Addition of soymilk affected organoleptic properties of the samples concentration-dependently (Fig. 5). Scores of texture, mouth sense, aroma, and flavor of the samples significantly decreased when milk was mixed with 40% and 60% soy milk.

For the color, the difference was significant only between milk and 60% soymilk. The 20% soymilk did not have a significant difference with milk regarding any

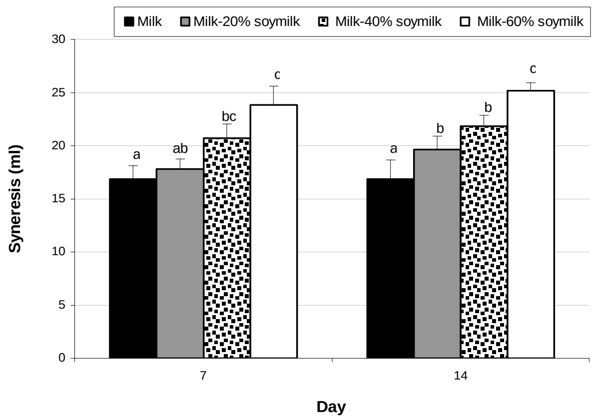

Fig. 4: Syneresis in samples of milk and milk with 20%, 40%, and 60% soymilk fermented with *Lactobacillus acidophilus* on days 1, 7, and 14 of refrigeration. The error bars represent one standard deviation. In each bar series different letters represent significant differences (P<0.05)

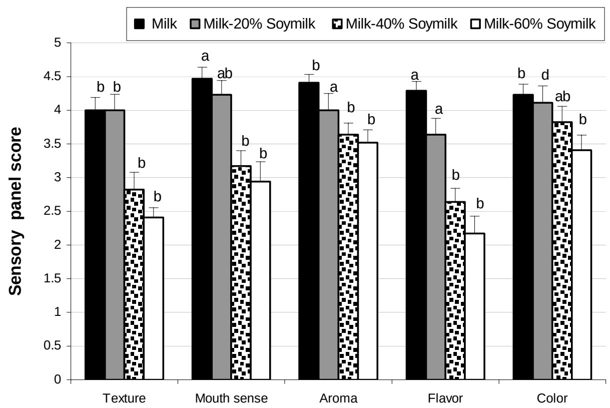

Fig. 5: Sensory properties of milk and milk with 20%, 40%, and 60% soymilk. The error bars represent one standard error of the mean. In each bar series different letters represent significant differences (P<0.05)

Table 1: Color values of the samples fermented with *Lactobacillus acidophilus* on days 7, and 14 of refrigeration

Treatments	Day 7			Day 14		
	L^*	a^*	b^*	L^*	a^*	b^*
Milk[1]	63.44 ± 1.33^{ab}	-6.22 ± 1.56	14.88 ± 4.28	60.00 ± 1.50^{a}	-7.00 ± 0.70	15.33 ± 1.32
Milk-20% soymilk	64.00 ± 1.00^{b}	-5.11 ± 1.05	14.77 ± 1.64	61.23 ± 1.58^{a}	-6.22 ± 0.66	15.44 ± 1.33
Milk-40% soymilk	62.88 ± 2.52^{ab}	-6.22 ± 1.48	16.66 ± 1.22	60.66 ± 1.11^{a}	-6.88 ± 0.78	15.44 ± 1.66
Milk-60% soymilk	61.66 ± 0.86^{a}	-5.44 ± 0.72	16.77 ± 1.20	57.33 ± 1.32^{b}	-6.33 ± 0.50	15.77 ± 1.09

[1] Data are means ± SD. L^* represents lightness/darkness, a^* shows redness/greenness, and b^* is for yellowness/blueness. Different superscript letters in each column indicate significance differences

of the mentioned sensory characteristics. In 60% soy milk, L^* (light) color significantly decreased which was more pronounced with longer storage times (Table 1). The samples, however, did not differ in a^* color (red).

Discussion

Addition of soymilk to milk did not cause a significant alteration in the growth and survival of *Lactobacillus acidophilus* during storage except for 60% soy milk on the day 14 of the storage. Despite this decrease in the survival of *Lactobacillus acidophilus* in the milk-60% soymilk sample, the number of *Lactobacillus acidophilus* was still more than the lowest number of bacteria (10^6 cfu/ml) reported to exhibit beneficial effects as probiotics (Sellars, 1991). This finding is in line with that of Drake *et al.* (2000) who found that addition of soy protein concentrate had no effect on lactic acid bacteria counts in yogurt. Survival of *Bifidobacterium infantis* has also been reported during storage of soymilk, while *Bifidobacterium longum* failed to survive in the same condition, indicating that some probiotics may not remain metabolically active under storage conditions (Chou and Hou, 2000).

Valdez and Giori (1993) compared the ability of soymilk and milk in preserving cell viability of *Lactobacillus acidophilus* and indicated that the survival rate was higher in soymilk. The higher survival rate of *Lactobacillus acidophilus* in soymilk may be due to the existence of oligosaccharides in soymilk which help the growth of lactic acid bacteria (Valdez and Giori, 1993). Probiotics have shown the potential to ferment oligosaccharides, raffinose and stachyose in soymilk, resulting in a decrease in the raffinose, stachyose, and sucrose contents along with a reduction in pH (Wang *et al.*, 2003). In higher oligosaccharide concentrations which occur in higher soymilk proportions, the reduction of pH may be large enough to inhibit the growth of lactic acid bacteria. In this context, Wang *et al.* (2003) reported the decrease of soymilk pH in parallel with increasing duration of fermentation by lactic acid bacteria alone or simultaneously with bifidobacteria. In agreement, our results demonstrated the decrease of pH in milk-soymilk mixtures concomitant with increasing concentration of soymilk. It should be noted that utilization of these oligosaccharides varied with different species of lactic acid bacteria used in different studies (Wang *et al.*, 2003).

Syneresis is the expulsion of whey from casein as occurs during formation of cheese or storage of yogurt. In the current study, addition of soymilk to milk increased syneresis in the samples. This increase is due to formation of weak casein gel with low water-binding potential as a result of the decrease in casein concentration (Shaker *et al.*, 2002; Kailasapathy, 2006).

Addition of 40% and 60% soymilk to milk significantly decreased scores of texture, mouth sense, aroma, and flavor of the samples. The alteration of textural properties following addition of soymilk is due to the decrease of casein concentration which consequently reduces gelling potential of the product (Kailasapathy, 2006). The intense impact of soymilk on organoleptic characteristics of milk is mainly due to the existence of 2-isopropyl-3-methoxypyrazine, the key aroma compound in soybean (Kaneko *et al.*, 2011). Dairy products have a mild flavor, which is easily affected by stronger flavors of other compounds. Additionally, sterilization of soymilk produces compounds with sulfur flavor such as methional, methanethiol, and dimethyl sulfide, and compounds with roasted aroma such as 2-acetyl-1-pyrroline and 2-acetyl-thiazole (Lozano *et al.*, 2007). Furthermore, addition of soymilk to milk decreases concentration of lactose (Fiocchi *et al.*, 2003). Although lactose is a sugar with low sweetness, reducing its amount in milk decreases sweetness and palatability of milk.

Regarding the effect of addition of soymilk on yogurt color, the value of L^* (light) color decreased in samples containing 60% soymilk. Lee *et al.* (1990) also reported a decrease in L^* amount in soymilk-based yogurt compared to milk-based yogurt. Likewise, Drake *et al.* (2000) found a decrease in lightness and redness of yogurt following its fortification with soy protein. The reduction in the lightness and redness of milk results from addition of soymilk to milk which gives milk a brown color.

In conclusion, the results of the present study suggest that fermentation of a mixture of milk with 20% soymilk by *Lactobacillus acidophilus* does not affect sensory characteristics of the product while retaining the minimum therapeutic dosage of probiotics (10^6 cfu/ml) up to 14 days of refrigeration. For the samples with 40% and 60% soymilk, low acceptability was reported by taste panelists regarding sensory characteristics. Also, negative physicochemical characteristics including pH, color parameter (L^*) and high syneresis were seen in the samples with 40% and 60% soymilk. Since addition of soymilk lowers lactose and cholesterol content of milk and increases its beneficial effects, results herein suggest adding low concentrations of soymilk to milk in order to produce healthier probiotic dairy-based products.

Acknowledgements

This study was funded by the grant No. 91-6364 from the Student Research Committee of Shiraz University of Medical Sciences. The authors would like to thank the Research Consultation Centers in Emam Reza Polyclinic and the Medical School for statistical analysis and manuscript editing, respectively, and Mr. M. Mohammadi for assistance in the laboratory.

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