Influence of temperature and sugar addition on soymilk fermentation by probiotic bacteria

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Summary

Fermented soymilk beverages were produced by fermentation with monocultures of probiotic bacteria: Bifidobacterium lactis Bb12, Lactobacillus acidophilus La5 and Lactobacillus casei Lc01 (Chr. Hansen's, Denmark), at two different temperatures (37 °C and 43 °C), with and without 5 % sugar addition (sucrose for Bb12 and La5, and glucose for Lc01). pH-values and numbers of viable cells were measured throughout the fermentation. For all tested probiotic bacteria temperature 37 °C was more appropriated than 43 °C. The fermentation process in the case of all three monocultures were shortened by an addition of sugars and viable counts at the end of the fermentation were increased. The influence of sugar on acid production and viable cells count was greatest in soymilk fermented with monoculture L. casei Lc-01. The choice of monoculture did not have a significant influence on the appearance and consistency of the fermented soymilk, apart when the achieved acidity was not enough and soymilk did not coagulate, like in the case of fermentation with strain Lc-01 without glucose addition.

Key words: Bifidobacterium lactis, fermentation, Lactobacillus acidophilus, Lactobacillus casei, soymilk

Introduction

In the Far East, soybean had the reputation of therapeutic food from ancient time. Today, when the composition of soybean is well described, it is known that soybean is cheap foodstuff, rich in valuable proteins, unsaturated fatty acids, soluble and insoluble fibers and isoflavones, which are very important in daily nutrition. Soybean proteins, although it is not well-known how and how much, support reduction of cholesterol level, alleviate menopause symptoms, reduce the risk of certain cancers, osteoporosis and gall-stones. Additionally, soybean is found to contain high amount of isoflavones, with a structure homology to human estrogens (Chien et al., 2006). Soybean isoflavones belong to a group of phytoestrogens, and their metabolism in human organism is subject of extensive investigation, because it is supposed that reduction of cancer risk is related to estrogen activity (breast, uterus or prostate cancer) (Badger et al., 2005), while in other tissue it can help in maintenance of bones density (Messina and Messina, 2000) and reduce blood cholesterol level (Friedman and Brandon, 2001). Difficulties of soybean utilization in bulk consumption are due to presence of indigestible oligosaccharides and specific taste of raw soybean. That soybean's imperfections can simply be removed by fermentation.

In western countries soymilk is allocated to population that cannot consume cow's milk, due to lactose intolerance, allergy to milk proteins or beliefs. The idea of soymilk and probiotic bacteria association has imposed by itself, and produced beverage can be multiple functional food. By the fermentation of soymilk with probiotic bacteria, the nutritional value of this product is enlarged considerably and the uptake of probiotic bacteria for the population that does not consume milk is allowed.

Because of all above mentioned the growth of probiotic bacteria *Bifidobacterium lactis*, *Lactobacillus acidophilus* and *Lactobacillus casei* in soymilk at different temperatures (37 and 43 °C), and with or without sugar addition was investigated.

Materials and methods

The commercial long life soymilk (Alnatura, Germany) was fermented with three probiotic bacteria: *Bifidobacterium lactis* Bb-12, *Lactobacillus acidophilus* La-5 and *Lactobacillus casei* Lc-1 (Chr. Hansen's, Denmark), at two different temperatures (37 and 43 °C), with and without sugar addition (5 % of sucrose for *B. lactis* Bb-12 and *Lb. acidophilus* La-5; and 5 % of glucose for *Lb. casei* Lc-1). pH-values and numbers of viable cells were monitored throughout the fermentation. The viable count of bacteria ($cfu \cdot mL^{-1}$) was determined by the standard method counting on MRS agar plates, (Biolife, Milano, Italy) after 3 d of incubation at 37 °C (*B. lactis* in anaerobic conditions, *Lb. acidophilus* and *Lb. casei* in microaerophilic conditions). Microaerophilic conditions were obtained by a layer of MRS agar over the MRS agar inoculated with bacteria, while anaerobic conditions were obtained in an anaerobic jar with Anaerogen (Oxoid Limited, Hampshire, England).

The experiments were repeated five times. The results were statistically analyzed and are shown as means.

Results and discussion

Considering specific beany flavour of soymilk, unacceptable for many consumers, 5 % sugar was added to soymilk before fermentation. Sugar addition improved the taste of fermented product considerably. For *Lb. acidophilus* La-5 and *B. lactis* Bb-12 sugar type (sucrose or glucose) did not influence fermentation time (results were not shown), and for those samples investigations were continued with sucrose addition.

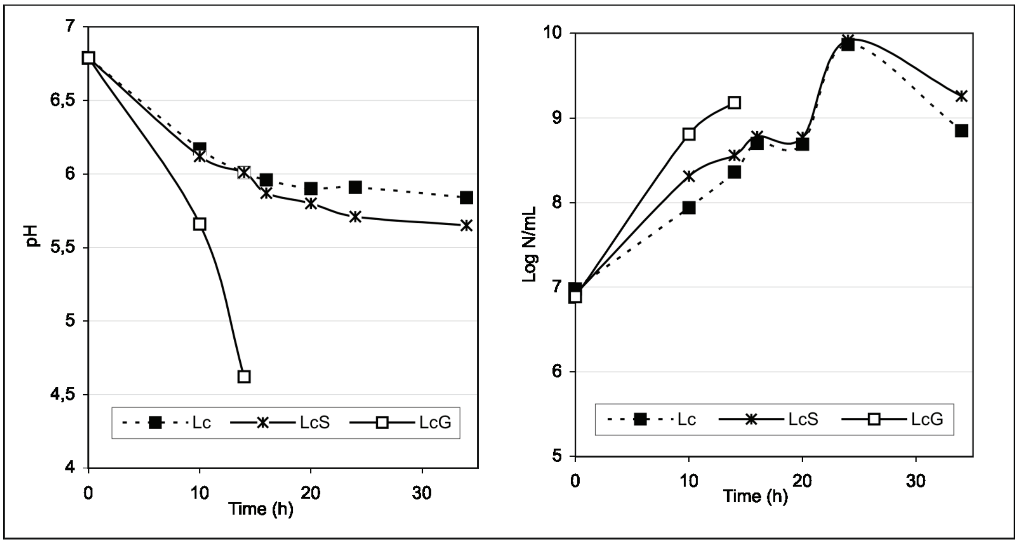

Fig. 1: pH value and viable cell count of Lb. casei Lc-01 during fermentation at 37 °C of control soymilk (Lc), and soymilk with 5 % of sucrose (LcS) and 5 % of glucose (LcG) supplementation

Grafikon 1: Promjena pH-vrijednosti i broja živih bakterija Lb. casei Lc-01 tijekom fermentacije kontrolnog sojinog mlijeka (Lc) te sojinog mlijeka obogaćenog s 5 % saharoze (LcS) i 5 % glukoze (LcG) pri temperaturi 37°C

Although *Lb. casei* Lc-01 had grown in pure soymilk and soymilk with sucrose addition, the pH-value in these samples decreased poorly (Fig. 1).

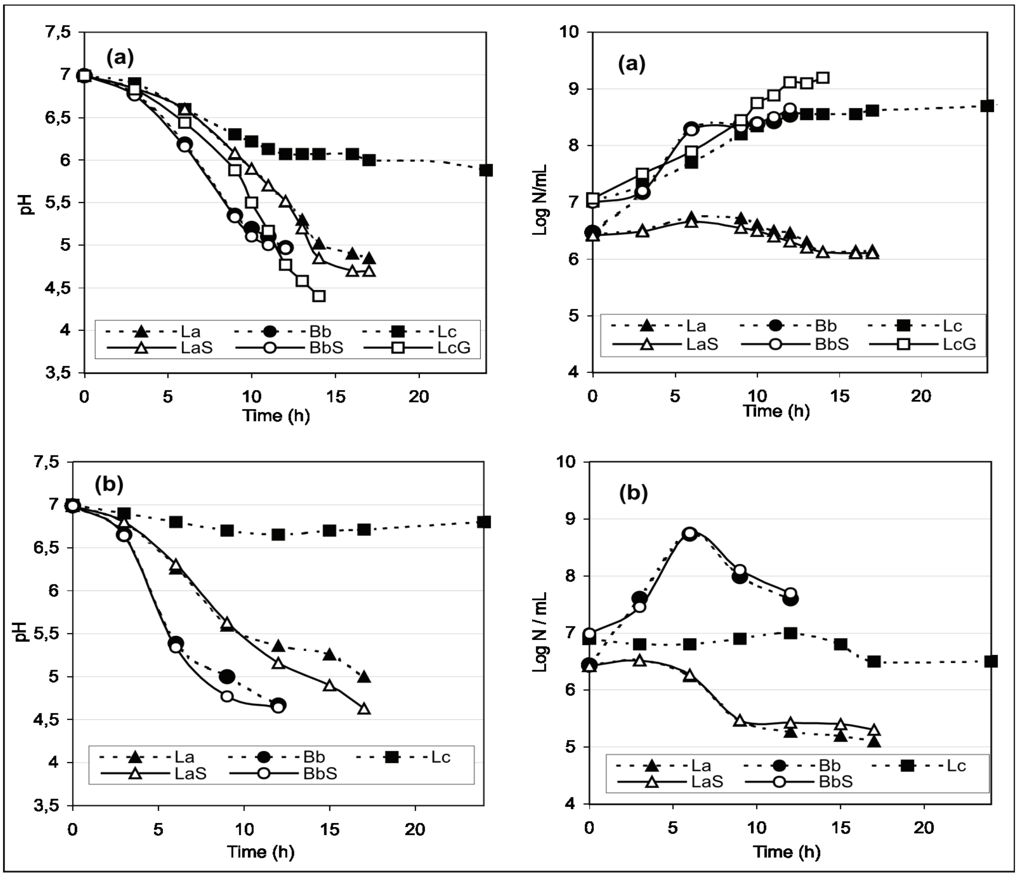

Fig. 2: pH value and viable cell count of Lb. acidophilus La-5 (La), B. lactis Bb-12 (Bb) and Lb. casei Lc-01 (Lc) during fermentation of soymilk (La, Bb, Lc) and soymilk supplemented with 5 % of sugar (LaS, BbS, LcG) at 37 °C (a) and 43 °C (b)

Grafikon 2: Promjena pH-vrijednosti i broja živih bakterija Lb. acidophilus La-5 (La), B. lactis Bb-12 (Bb) i Lb. casei Lc-01 (Lc) tijekom fermentacije sojinog mlijeka (La, Bb, Lc) sa i bez dodatka 5 % šećera, pri temperaturi 37°C (a) i 43 °C (b)

After 34 h of fermentation at 37 °C, pH-value was around 5.7, and soymilk did not coagulate although viable cells count was high (N=7.08 x 10^8 - 1.82 x 10^9/mL). In soymilk with glucose addition after 14 hours of fermentation, pH-value was 4.62. The growth of *Lb. casei* Lc-01 was good in all three samples, but in soymilk with glucose addition *Lb. casei* Lc-01 had grown the fastest. For soymilk fermentation with *Lb. casei* Lc-01 temperature 43 °C was not appropriate, because after 40 h of fermentation pH decrease was negligible (ΔpH=0.33), even though the viable cells count was higher than 10^6 (N=$2.7\text{x}10^6$/mL). Adversely, according to Garro et al. (1998) *Lb. casei* can utilize sucrose from soymilk for growth and lactic acid production. In their investigation after 24 h of pure soymilk fermentation at 37 °C with *Lb. casei* CRL 207 the pH-value has decreased to 4. According to Behrens et al. (2004) *Lb. casei* is suitable for soymilk fermentation because its responsibility for the formation of flavour and aroma precursors, contributing to the elimination of the off-flavour caused by n-hexanal.

B. lactis Bb-12 in all samples had grown the fastest, regardless of sugar addition (Fig. 2). Soymilk is suitable for the growth of lactic acid bacteria, especially bifidobacteria. Stimulative factors for growth are considered to be oligosaccharides, amino acids and peptides (Shimakawa et al., 2003). The growth of bifidobacteria is not limited with low concentration of monosaccharides, e.g. arabinose and glucose, nor high concentration of oligosaccharides: raffinose and stachyose. These strains have enzyme α-galactosidase, capable to hydrolise α-D-galactosides bonds in those oligosaccharides (Roy et al., 1992). Nevertheless, oligosaccharides have been shown to possess prebiotic properties as they have increased the populations of indigenous bifidobacteria in colon and have suppressed the growth and activity of putrefactive bacteria (Tsangalis and Shah, 2004). However, during fermentation, bifidobacteria for growth and multiplication mainly use sucrose, notable minor stachyose, and negligible little fructose and raffinose (Kwon et al., 2002). Interesting, exactly the same is the share of single sugars in soymilk: the most is sucrose (41 - 67 % of total sugars), then stachyose (12 - 35 % from total sugars), and very little fructose and raffinose (5 - 16 % of total sugars) (Božanić, 2006). *Bifidobacterium* spp. has ability to reduce the off-flavour of n-hexanal (Tsangalis and Shah, 2004). It was reported that soymilk fermented with *Bifidobacterium* Bb-12 or Bb-46 significantly decreased the levels of total plasma cholesterol and low-density lipoprotein cholesterol in rats (El-Gawad et al., 2005).

All three investigated bacterial strains have grown better at 37 than at 43 °C (Fig. 2). The growth of *Lactobacillus acidophilus* La5 in all samples was the worst. It is well-known that lactobacilli have complex growth requirements. They require low oxygen tension, fermentable carbohydrates, proteins and their breakdown products, a number of B-complex vitamins, nucleic acid derivatives, unsaturated free fatty acids, and minerals such as magnesium, manganese and iron for their growth. Soymilk contents almost all that requirements, except iron, which can be the reason for poor growth of *Lb. acidophilus* in soymilk. Moreover, vigorous stimulators of growth of *Lb. acidophilus* increase amount of thiol groups, whereas peptone and trypsin stimulate its acid production (De Vuyst, 2000). Because soymilk is scarce in amino-acids containing sulphur, that also can be a reason for poor growth of *Lb. acidophilus* La-5 in this medium.

The influence of sugar addition on *Lb. acidophilus* La-5 and *B. lactis* Bb-12 growth was negligible. Although pH-value in supplemented soymilk had decreased faster, the viable cells were only negligibly higher, and fermentation time was the same.

Probiotic foods with health claim should contain at least 10^6 CFU/mL of the bacteria because 10^8 - 10^9 of viable cells is the minimum therapeutic dose per day. In this experiment at 37 °C all bacterial strains reached that level in the end of fermentation, but it is not known how they survive during refrigerated storage. In further investigations mixed cultures will be used (to reduce fermentation time) and surviving capability during storage time will be investigated.

Conclusion

Bacterial cultures had negligible influence on appearance and consistency of fermented soymilk, although when appropriate acidity was not reached, and curd was not obtained. The growth of investigated monocultures (*Lb. acidophilus* La-5, *Lb. casei* Lc-01 and *B. lactis* Bb-12) in soymilk, with and without sugar addition, was better at 37 °C than at 43 °C. Supplementation of soymilk with sugar considerably improved taste of fermented products, and had less or more positive influence on bacterial growth. The influence of sugar addition on pH decrease and viable cells count was the greatest in fermentation of soymilk with *Lb. casei* Lc-01. In all fermented samples maximal viable cells count (N/mL) was obtained in the end of fermentation at 37 °C, and with sugar supplementation (*Lb. casei* Lc-01 = 5.00 x 10^9; *B. lactis* Bb-12 = 5.24 x 10^8; *Lb. acidophilus* La-5 = 4.43 x 10^6). Fermentation of soymilk with *Lb. casei* Lc-01at 43 °C was not appropriate, because pH decrease after 40 h of fermentation was negligible (ΔpH = 0.33), regardless of viable cells count which was higher than 10^6 (N = 2.7 x 10^6/mL). *Lb. acidophilus* La-5 did not grow during fermentation of soymilk regardless of sugar addition or fermentation temperature.

UTJECAJ TEMPERATURE I DODATKA ŠEĆERA NA FERMENTACIJU SOJINOG MLIJEKA PROBIOTIČKIM BAKTERIJAMA

Sažetak

Sojino mlijeko fermentirano je s tri komercijalne monokulture probiotičkih bakterija (Bifidobacterium lactis Bb12, Lactobacillus acidophilus La5 i Lactobacillus casei Lc01) (Chr. Hansen's, Danska), pri dvije različite temperature (37 °C i 43 °C) te sa i bez dodatka 5 % šećera (saharoze za Bb12 i La5 i glukoze za Lc01). Tijekom fermentacije praćena je promjena pH-vrijednosti i broja živih bakterijskih stanica. Sve tri monokulture bolje su rasle pri temperaturi 37 °C nego 43 °C. Dodatkom šećera fermentacija sa sve tri monokulture bila je kraća, a broj živih bakterijskih stanica na kraju fermentacije bio je veći. Utjecaj dodatka šećera bio je najveći u fermentaciji s monokulturom L. casei Lc01. Izbor bakterijske monokulture nije značajno utjecao na izgled i konzistenciju fermentiranog sojinog mlijeka, osim kada nije postignuta odgovarajuća kiselost pa nije došlo ni do koagulacije kao u slučaju fermentacije sojem Lc01 bez dodatka glukoze.

Ključne riječi: Bifidobacterium lactis, fermentacija, Lactobacillus acidophilus, Lactobacillus casei, sojino mlijeko

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