Influence of temperature and glucose addition on growth and survival of bacteria from BCT culture in soymilk

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Summary

Soymilk was fermented using culture composed of Lactobacillus casei, Bifidobacterium spp. and Streptococcus thermophilus (BCT culture, Chr. Hansen's, Denmark) at two temperatures 37 °C and 41 °C with and without 5% of glucose addition. Fermentation was conducted until pH value 4.6 was reached. During fermentation and storage time (28 days at +4 °C) changes of pH values and viable cells counts were observed. All fermentations lasted between 6 and 7.5 hours. At temperature 41 °C fermentation was approximately 1 hour shorter, while glucose addition had reduced fermentation time for 30 minutes. Higher temperature of fermentation, as well as glucose addition, had negligible influence on portions and viable cells count of particular bacterial species in the product. In all fermented soymilk samples the viable cells count of particular bacterial strains was roughly equal: Str. thermophilus have grown the best (~ 10^8 CFU/mL) while lactobacilli have grown the weakest (~ 10^5 - 10^6 CFU/mL). Generally, for soymilk fermentation mainly Str. thermophilus was responsible. On its growth rate glucose addition and higher fermentation temperature had strong positive influence. During 28 days of refrigerated storage, the product was stable. A remarkable decrease of viable cells count of lactobacilli was noticed in the last week of storage.

Keywords: soymilk, Bifidobacterium spp., Lactobacillus casei, Streptococcus thermophilus, fermentation

Introduction

Although the soy in the past has been known only in east countries, especially in China, today it has a great significance also in other parts of the world, mostly because of its numerous positive characteristics and nutrional value. Soy proteins often serve as a substitute for proteins with animal origin because of their abundance of essential amino acids. Many vegetarians often

use soy as an alternative to animal food. Additionally, soybean is found to contain high amount of isoflavones, with a structure homological to human estrogens (Bannwart et al., 1984) and with possible effect on human health (Coward et al., 1993; Booth et al., 1999). One of the advantages of soy food is absence of cholesterol and saturated fatty acids that can be associated with higher blood pressure and diseases like arteriosclerosis. Unlike cow milk, soymilk does not contain lactose, so it is convenient for people who suffer from lactose intolerance. Like cow milk it is abundant in vitamins and minerals. Many studies have been published which point out beneficial influence of soy food on health. It is considered that soymilk reduces cholesterol levels in blood (Rosel et al., 2004), reduces the risk of cancer, osteoporosis and other similar illness (Rosenthal et al., 2003; De Luis et al., 2006; Messina, 1995; Rivas et al., 2002).

Today, fermented soy products are becoming more and more important. Because of greater antioxidative actions (Wang et al., 2006) they are considered healthier than pure soymilk. The purpose of fermentation is to remove undesirable beany taste (Wang et al., 2002, 2003, 2006) that mostly comes from presence of n-hexanal and pentanal (Scalabrini et al., 1998) and to improve nutritional characteristics of soymilk. Fermentation, especially with *Bifidobacteria*, also makes present proteins more digestible (Hughes and Dallas, 1991; Ishisashi and Shimura, 1993) and reduces the content of soy oligosaccharides, stachyose and raffinose, which can cause digestive problems (Cruz et al., 1981). Fermentation is usually performed with lactic acid bacteria (Garro et al., 1997; Hsiang et al., 2006), but also with some types of moulds.

In our previous investigation it was reported that monoculture of bifidobacteria and *Lactobacillus casei* can grow well in soymilk, but fermentation time was relatively long (12 to 15 hours) (Božanić et al., 2008). In this study possible reduction of soymilk fermentation time was investigated using BCT culture (*Bifidobacteria* spp., *Streptococcus thermophilus, Lactobaccilus casei*) and supplementing the soymilk with 5% of glucose. In addition to fermentation time, in this study the growth of bacteria from BCT culture in soymilk will be determined as well as the influence of temperature and glucose addition on fermentation time, viable cells counts and stability during 28 days of refrigerated storage.

Materials and methods

The commercial long life soymilk (Alnatura, Germany) was fermented with commercial frozen BCT culture which is composed of *Bifidobacteria* spp., *Streptococcus thermophilus* and *Lactobaccilus casei* (Chr. Hansen's, Denmark) at two temperatures 37 °C and 43 °C, with and without 5% of glucose addition. Soymilk was inoculated with 0.2 mg/mL of BCT culture, according to producer's recommendation. Fermentation was conducted until pH value 4.6 was reached. Fermented soymilk samples were stored in refrigerator at 4 °C during 28 days. Microbial analyses and pH value have been performed during fermentation and every week during storing time.

The viable count of bacteria ($CFU \cdot mL^{-1}$) was determined by standard plate methods. Incubations were performed at 37 °C/3 days for *Lb. casei* and *Bifidobacteria* spp. and at 41 °C/2 days for *Streptococcus thermophilus*. For *Str. thermophilus* M17 media was used. *Bifidobacteria* spp. and *Lb. casei* were cultivated on MRS agar plates (Biolife, Milano, Italy) with NNLP (Božanić et al., 2002) in anaerobic conditions in anaerobic jar with Anaerogen (Oxoid Limited, England). *Lb. casei* grew in a form of round, milky-white colonies with diameter around 3 - 4 mm, while the bifidobacteria colonies were small, transparently white coloured, round to oval shape.

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

Results and discussion

Soymilk was fermented with BCT culture at two different temperatures (37°C and 41°C) with and without 5% of glucose addition. Fermentation was performed till pH 4.6 was reached. All fermentations lasted between 6 and 7.5 hours (Fig. 1). With supplementation of soymilk with 5% of glucose at both temperatures, fermentation was reduced for roughly 30 minutes, while at 41°C fermentation was 60 minutes shorter than at 37 °C.

Temperature and presence of glucose did not have considerable influence on viable cells count. According to our previous results (Božanić et al., 2008) greater influence of glucose addition on fermentation time and viable cells count was expected. In this paper only negligible effect of glucose addition on fermentation time was observed. Thereby, the reason for shorter fermentation time could be caused by presence of *Str. thermophilus* that has grown very fast (at the first step of fermentation) and reduced pH value. Because of that probiotic bacterias *Lb. casei* and bifidobacteria did not have

enough time for growth and multiplication. When soymilk was fermented with monoculture of probiotic bacteria *Lb. casei* Lc-1 and *Bifidobacteria animalis* ssp. *lactis* Bb-12, fermentation lasted much longer (~12-15 hours) and greater viable cells count was achieved (~10^8-10^9 CFU/mL) (Božanić et al., 2008).

As mentioned earlier, in all experiments *Str. thermophilus* have grown the best of all used species. Viable cells count of streptococci at the end of fermentation was about 10^8 CFU/mL which corresponds with information found in literature (Wang et al., 2002). Generally, for soymilk fermentation mainly *Str. thermophilus* was responsible. On its growth rate glucose addition and higher temperature of fermentation had strong positive influence (Fig. 1). Also in other studies based on fermentation with mixed culture containing streptococci, on various types of milk (cow, goat) *Str. thermophilus* have always shown the best growth than other lactic acid bacteria species (Božanić et al., 2002). These results have been expected regarding to streptococcus as non-demanding bacteria for cultivation and soymilk obviously contains all required nutrients for their growth and multiplication.

The viable cells counts of both probiotic bacteria did not increased importantly during fermentation time (Fig. 1). Slightly better growth was observed at 37 °C for both probiotic species. *Lb. casei* does not ferment sucrose, dominant sugar in soymilk. Because of that fact soymilk was supplemented with glucose, but it had negligible influence on viable cells count of lactobacili, probably because of short soymilk fermentation time.

During 28 days of storage time bifidobacteria survived much better than *Lb. casei* (Fig. 2). The viable cells count of bifidobacteria was stable, around 7.5 $\log_{10}$/mL for all fermented soymilk samples. This results match literature data, showing that soymilk is a convenient media for growth of these bacteria (Shimakawava et al., 2003). According to the literature the most soymilk fermentations for beverage production is performed with bifidobacteria (Scalabrini et al., 1998; Shimakawa et al., 2003; Wang et al., 2002, 2003, 2006). Additionally, bifidobacteria can reduce the content of soymilk oligosaccharides, stachyose and raffinose, which can cause digestive problems (Cruz et al., 1981). Their growth is not limited by low monosacharides concentration i.e. arabinose and glucose, nor high concentration of oligosacharides. Moreover, it is proved that oral consumption of stachyose and raffinose increase bifidobacteria population in the colon. Bifidobacteria are capable to decrease unacceptable beany taste of soymilk caused by n-hexanal (Scalabrini et al., 1998). Contrary, lactobacilli are described as very demanding organisms that require for their growth and reproduction the

number of factors like: fermentable carbohydrates, proteins, unsatured fatty acids, derivates of nucleic acids, minerals and corresponding temperature and low oxygen concentration (De Vuyst, 2000).

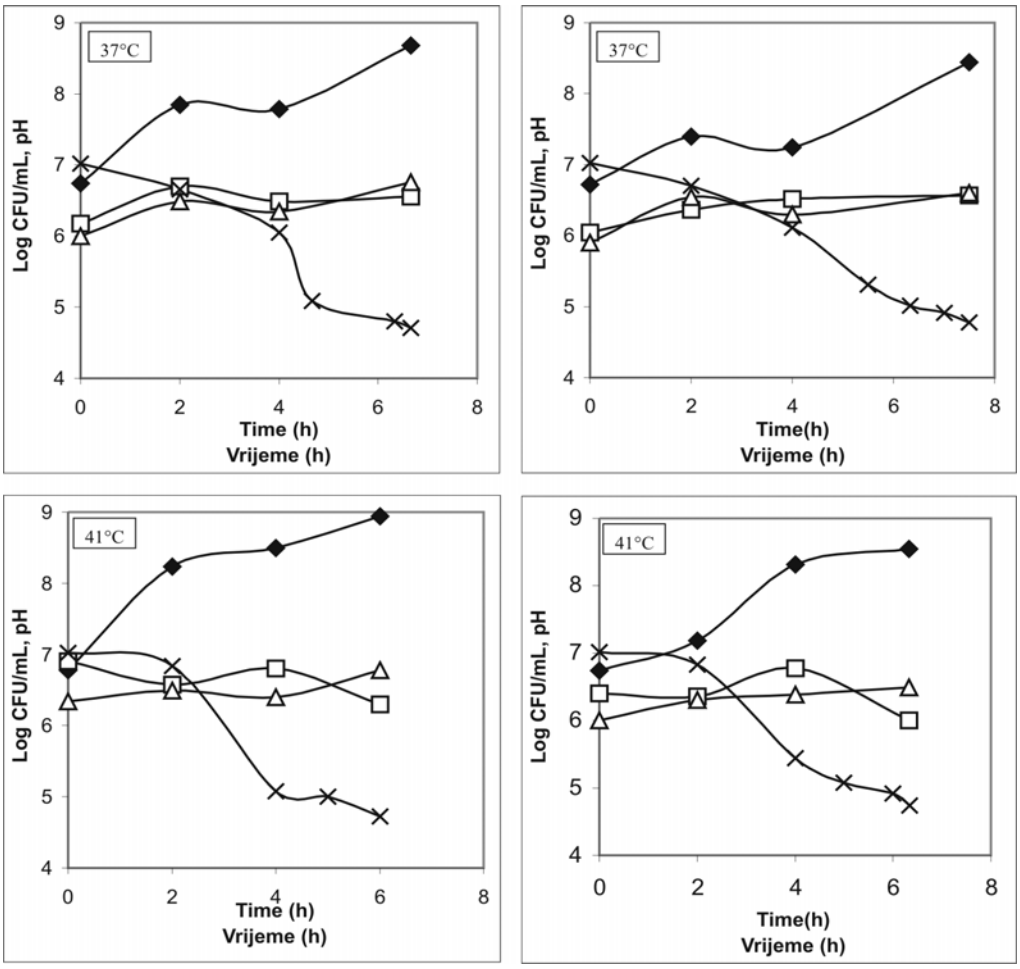

Figure 1: pH value (X) and viable cells count (LogCFU/mL) of Streptococcus thermophilus (♦), Lactobacillus casei (□) and Bifidobacteria spp. (Δ) during soymilk fermentation at 37 °C and 41 °C with and without 5 % of glucose addition

Slika 1: pH-vrijednost (X) i broj živih stanica (LogCFU/mL) Streptococcus thermophilus (♦), Lactobacillus casei (□) i Bifidobacteria spp. (Δ) tijekom fermentacije sojinog mlijeka pri 37 i 41 °C sa i bez dodatka 5 % glukoze

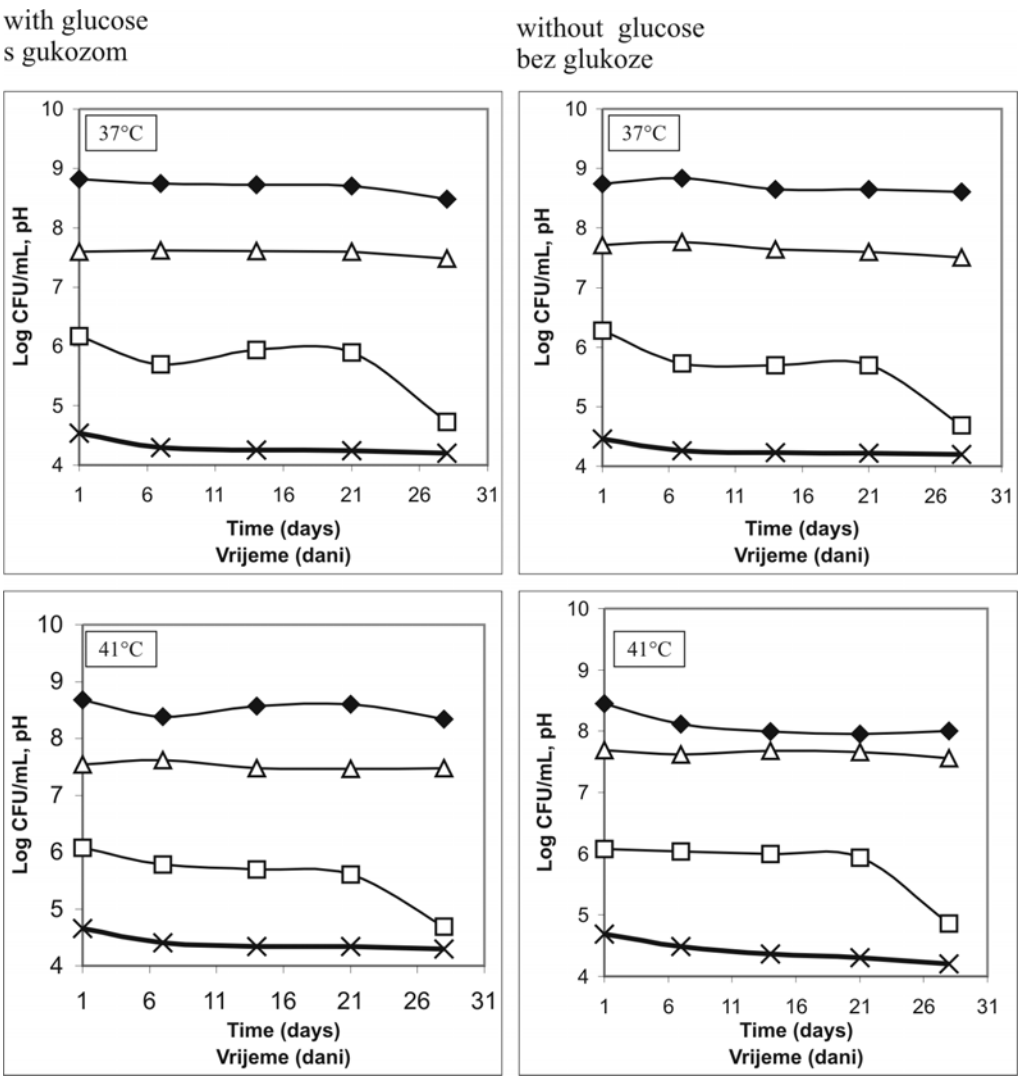

Figure 2: pH value (X) and viable cells count (LogCFU/mL) of Streptococcus thermophilus (♦), Lactobacillus casei (□) and Bifidobacteria spp. (Δ) during 28 days of storage time at 4 °C soymilk fermented at 37 °C and 41 °C with and without 5 % of glucose addition

Slika 2: pH-vrijednost (X) i broj živih stanica (LogCFU/mL) Streptococcus thermophilus (♦), Lactobacillus casei (□) i Bifidobacteria spp. (Δ) tijekom 28 dana čuvanja pri 4 °C sojinog mlijeka fermentiranog pri 37 i 41 °C sa i bez dodatka 5 % glukoze

During 28 days of refrigerated storage all fermented soymilk samples were stable, without considerable variation in pH value and in viable cells counts. Only in the last week, after 21 days of cool storage, what is usually shelf life for fermented beverage, the viable cells count of lactobacillus decreased in all samples. Mild but constant decrease in pH value has been monitored during storage time.

Fermented soymilk had an appearance of pudding, with smooth consistency, without intense flagrance. It was coloured like white coffee with porcelain gleam. Beany taste was not so intense like in pure soymilk. Sample with added glucose had slightly better taste, with less sourness than in fermented soymilk without glucose addition. Samples fermented on different temperature did not differ in taste and consistency.

UTJECAJ TEMPERATURE I DODATKA GLUKOZE NA RAST I PREŽIVLJAVANJE BAKTERIJA BCT KULTURE U SOJINOM MLIJEKU

Sažetak

Sojino je mlijeko fermentirano BCT kulturom (Lactobacillus casei, Bifidobacterium spp. i Streptococcus thermophilus) pri dvije temperature 37 i 41 °C s i bez dodatka 5 % glukoze. Fermentacija je vođena do pH-vrijednosti 4,6. Tijekom fermentacije i vremena čuvanja (28 dana pri temperaturi od +4 °C) praćena je promjena pH-vrijednosti i broja živih bakterijskih stanica. Sve su fermentacije trajale između 6 i 7,5 sati. Pri temperaturi 41 °C fermentacija je bila oko 1 sat kraća, dok je dodatak glukoze skratio vrijeme fermentacije za oko 30 minuta. Viša temperatura fermentacije, kao i dodatak glukoze, imali su neznatan utjecaj na udjel i broj živih stanica pojedinačne bakterijske vrste u proizvodu. Str. thermophilus je rasao najbolje (~ 10^8 CFU/mL) dok je Lactobacillus casei rastao najslabije (~ 10^5 - 10^6 CFU/mL). Za fermentaciju sojinog mlijeka uglavnom je bio odgovoran Str. thermophilus. Na specifičnu brzinu rasta tog mikroorganizma pozitivno je utjecao dodatak glukoze kao i povišenje temperature inkubacije na 41 °C. Tijekom 28 dana hladnog čuvanja proizvod je bio stabilan. Značajno smanjenje broja živih bakterijskih stanica laktobacila zapaženo je u posljednjem tjednu čuvanja.

Ključne riječi: sojino mlijeko, Bifidobacterium spp., Lactobacillus casei, Streptococcus thermophilus, fermentacija

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