Optimising Fermentation of Soymilk with Probiotic Bacteria

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Abstract

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Soymilk was fermented with probiotic culture ABT5 and yoghurt culture with the addition of bifidobacteria at different temperatures (37°C and 42°C) with the aim of shortening the fermentation time and producing a probiotic fermented soymilk. During the fermentation and storage of the fermented soymilk (28 days at +4°C), the changes in pH-value and viable cells count were observed. Incubation temperature did not affect significantly fermentation time (7 h at 42°C and 8 h at 37°C, respectively), with ABT5 culture (*Lactobacillus acidophilus, Bifidobacterium* spp., and *Streptococcus thermophilus*). However, *Lactobacillus acidophilus* survived poorly during cold storage and the viable cells count was under the probiotic minimum as soon as after the first week of storage. Therefore in the consequent phase of the experiment, soymilk was fermented at 42°C with yoghurt culture YCX11 enriched with *Bifidobacterium animalis* subsp. *lactis* Bb12. Consequently, the fermentation time was shortened to 4 hours whereby the viable cells count of bifidobacteria increased during fermentation for the half of the logarithm scale approximately. During 28 days of cold storage, bacterial count remained constant and above 10^7 CFU/ml.

Keywords: soymilk; fermentation; *Lactobacillus acidophilus*; *Bifidobacterium* spp.; *Streptococcus thermophilus*; yoghurt culture; *Lactobacillus animalis* subsp. *lactis* BB12

Due to its extraordinary nutritive value and health characteristics, soymilk has become a very interesting food. It is a very rich source of highly valuable proteins, unsaturated fatty acids, soluble and insoluble dietary fibers, and isoflavones whose presence in everyday diet is very important (Božanić 2006). In western countries, soymilk is intended for population who can not digest milk for reasons like lactose intolerance, allergy to milk proteins, or vegetarian way of diet. Fermenting soymilk with lactic acid bacteria considerably increases its health value. Because of greater antioxidative actions (Wang *et al*. 2006), they are considered healthier than pure soymilk. The purpose of fermentation is to remove the undesirable beanie taste (Wang *et al*. 2002, 2003, 2006) which is mostly due to the presence of *n*-hexanal and pentanal (Scalabrini *et al.* 1998), and to improve on the nutritional characteristics of soymilk. Fermentation, especially with *Bifidobacteria*, also makes the proteins present more digestible (Hughes & Dallas 1991; Ishisashi & Shimura 1993) and reduces the contents of soy oligosaccharides, stacchiose, and raffinose, which can cause digestive problems (Cruz *et al.* 1981). The idea of affiliating soymilk and probiotic bacteria comes naturally and the drink obtained could be a unique multifunctional food.

The main probiotic microorganisms that are currently used belong to the genera *Lactobacillus* and *Bifidobacterium* (Tamime *et al.* 2005). In preliminary studies, soymilk was fermented with monocultures of probiotic strains *Lactobacil-*

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lus acidophillus La5, *Lactobacillus casei* Lc1, and *Bifidobacterium animalis* subsp. *lactis* Bb12 which developed well in soymilk while the fermentation lasted 12–17 h (Božanić *et al.* 2008a). From those three probiotic strains in question, *Lactobacillus casei* Lc1 with the addition of glucose (because *Lactobacillus casei* does not ferment sucrose which is the dominating sugar in soymilk) showed the best growth in soymilk. Therefore, to shorten the fermentation time in further research, soymilk was fermented by BCT culture (*Bifidobacterium* spp., *Lactobacillus casei,* and *Streptococcus thermophilus*) with and without glucose addition. The fermentation of soymilk with BCT culture was shorter (6–7 h) but the viable probiotic cells count (*Lactobacillus casei* and bifidobacteria) did not significantly increase (approximately for a half of logarithm) and was not above the probiotic minimum during the storage period (Božanić *et al.* 2008b). The addition of glucose did not considerably affect the growth and survival of the probiotic strains used in this case.

Thus the aim of this study was to produce fermented soymilk with probiotic bacteria within eligible time of fermentation and with the viable cells count in the final product above the probiotic minimum (10^6 CFU/ml) which would be stable during 28-day cool storage, that is the requirement for probiotic products (Tamime *et al.* 2005). Therefore, soymilk was fermented using ABT5 culture (*Lactobacillus acidophillus, Bifidobacterium* spp., and *Streptococcus thermophilus*) and also using yoghurt culture YCX11 with the addition of *Bifidobacterium animalis* subsp. *lactis* Bb12.

MATERIALS AND METHODS

Commercially long-life soymilk was fermented using two different DVS cultures (Chr. Hansen's, Denmark): ABT5 (*Lactobacillus acidophilus, Bifidobacterium* spp., and *Streptococcus thermophilus*) and yoghurt culture YCX11 (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*) with the addition of the monoculture *Bifidobacterium animalis* subsp. *lactis* Bb12. Soymilk was inoculated with 0.2 mg/ml of ABT5 culture in the first set of experiments, and with 0.2 mg/ml of YCX11 + 0.05 mg/ml of *Bifidobacterium animalis* subsp. *lactis* Bb12 in the second set of experiments. Each inoculum was prepared separately, by dissolving a culture bag (500 g) in 500 ml of soymilk, and then another volume of soymilk with 0.2 mg/ml was inoculated using this preparation. The samples were examined each two hours during the fermentation, and then each 7^{th} day during the storage time. The fermentation with ABT5 culture was conducted at two temperatures (37°C and 42°C) while the fermentation with the yoghurt culture YCX11 with the addition of the monoculture *Bifidobacterium animalis* subsp. *lactis* Bb12 was performed at 42°C. Both fermentations were conducted until reaching pH 4.6, which is the proximate isoelectrical point of soy proteins (Vissesanguan *et al*. 2005; Božanić 2006). The samples were cooled and stored for 28 days in a refrigerator at the temperature of 4°C. During fermentation and during storage of the fermented products the changes in pH-value and viable cells count were periodically observed.

The viable cells count (i.e. expressed as colony forming units (CFU)/ml) was determined by standard microbiological methods employing inoculation on nutrient agar plates. For streptococci M17 agar (Biolife, Milan, Italy) was used, and for Lactobacilli MRS agar (Biolife, Milan, Italy) whose pH value was adjusted to 5.4 by adding glacial acetic acid. Bifidobacteria were determined by MRS agar with the addition of 5% of NNLP (nalidixic acid, neomycin sulfate, lithium chloride, and paramomyicine sulfate) solution following the instructions of the culture producer, as described previously (Božanić *et al.* 2002), and were incubated under anaerobic conditions in anaerobic jars, using oxygen binding agent Anaerogen (Oxoid Limited, Hampshire, England). *Lactobacillus acidophilus* was incubated at 37°C, while *Lactobacillus delbrueckii* subsp. *bulgaricus,* streptococci, and bifidobacteria were incubated at 42°C for 48 hours.

Each experiment was replicated five times. The results were statistically analysed and are shown as means with standard deviations.

RESULTS AND DISCUSSION

In this study, soymilk was fermented at two different temperatures (37°C and 42°C) using ABT5 culture (*Lactobacillus acidophilus, Bifidobacterium* spp., *Streptococcus thermophilus*) and also using yoghurt culture with the addition of *Bifidobacterium lactis* subsp. *animalis* Bb12 at 42°C.

In our previous investigations, the monoculture of *Lactobacillus acidophilus* La5 and that of *Bifi-*

dobacterium lactis subsp. *animalis* Bb12 showed good growth in soymilk, the fermentation time being 12–17 h (Božanić *et al*. 2008b). Therefore, to shorten the fermentation time, soymilk was fermented in this research by ABT5 culture. ABT culture (*Lactobacillus acidophilus*, *Bifidobacterium* spp., and *Streptococcus thermophilus*) was chosen for soymilk fermentation also because of the positive interaction between probiotic strains *Bifidobacterium* spp., and *Lactobacillus acidophilus* (Tamime *et al.* 2005). Besides that, *Streptococcus thermophilus* is always the dominant strain in mixed cultures and shows the greatest growth regardless of the fermentable substrates, e.g. in cow milk, goat milk (Božanić *et al.* 2002), or in soymilk (Božanić *et al.* 2008b). In order to examine optimal growth temperature for particular strains included in ABT5 culture, the fermentation of soymilk was performed at two different temperatures, 37°C and 42°C (Figure 1).

Soymilk fermentation was 1 hour shorter at 42°C (7 h) than at 37°C (8 h) and also pH decreased slightly faster at 42°C. In this investigation, *Lactobacillus acidophilus* grew poorly during both fermentations (Figures 1b,c). At the end of the fermentation performed at 37°C, the viable cells count of lactobacilli was slightly higher (6.38 × 10^6 CFU/ml) than at 42°C (3.27 × 10^6 CFU/ml), but the final viable cells count was not much higher than at the beginning of the experiment (~1.1 × 10^6 CFU/ml) though above the probiotic minimum.

Bifidobacteria grew better during the fermentation at 42°C than at 37°C, and their viable cells count was approximately 10^7 CFU/ml at the end of fermentation. Soymilk is a good substrate for the growth of bifidobacteria because these assimilate well oligosaccharides as a source of energy due to the presence of β-galactosidases. These enzymes reduce oligosaccharides content during fermentation whereby the content of monosaccharides in soymilk increases (Hou *et al.* 2000; Shimakawa *et al.* 2003). The growth of bifidobacteria is not limited either by the influence of low monosaccharide concentrations (e.g. arabinose and glucose) or by high oligosaccharide concentrations (e.g. raffionse and stacchiose) (Tsangalis & Shah 2004). During the fermentation process, bifidobacteria mainly use sucrose but in considerably lower amounts also stacchiose, while the use of fructose and raffionse is negligible (Kwon *et al.* 2002). Interestingly, the sugar composition in soymilk is exactly the same: sucrose content is the highest one (41–67% of

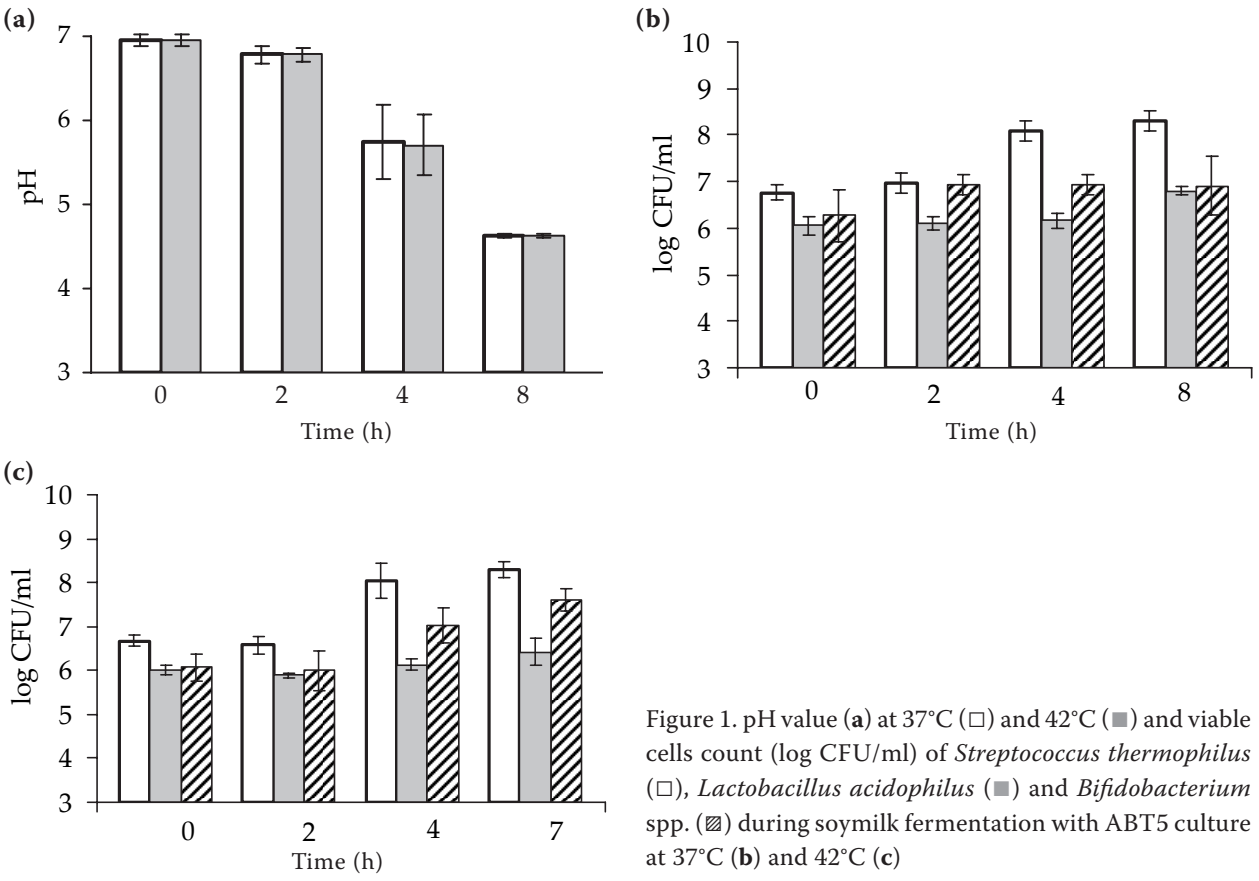

Figure 1. pH value (**a**) at 37°C (□) and 42°C (■) and viable cells count (log CFU/ml) of *Streptococcus thermophilus* (□), *Lactobacillus acidophilus* (■) and *Bifidobacterium* spp. (▨) during soymilk fermentation with ABT5 culture at 37°C (**b**) and 42°C (**c**)

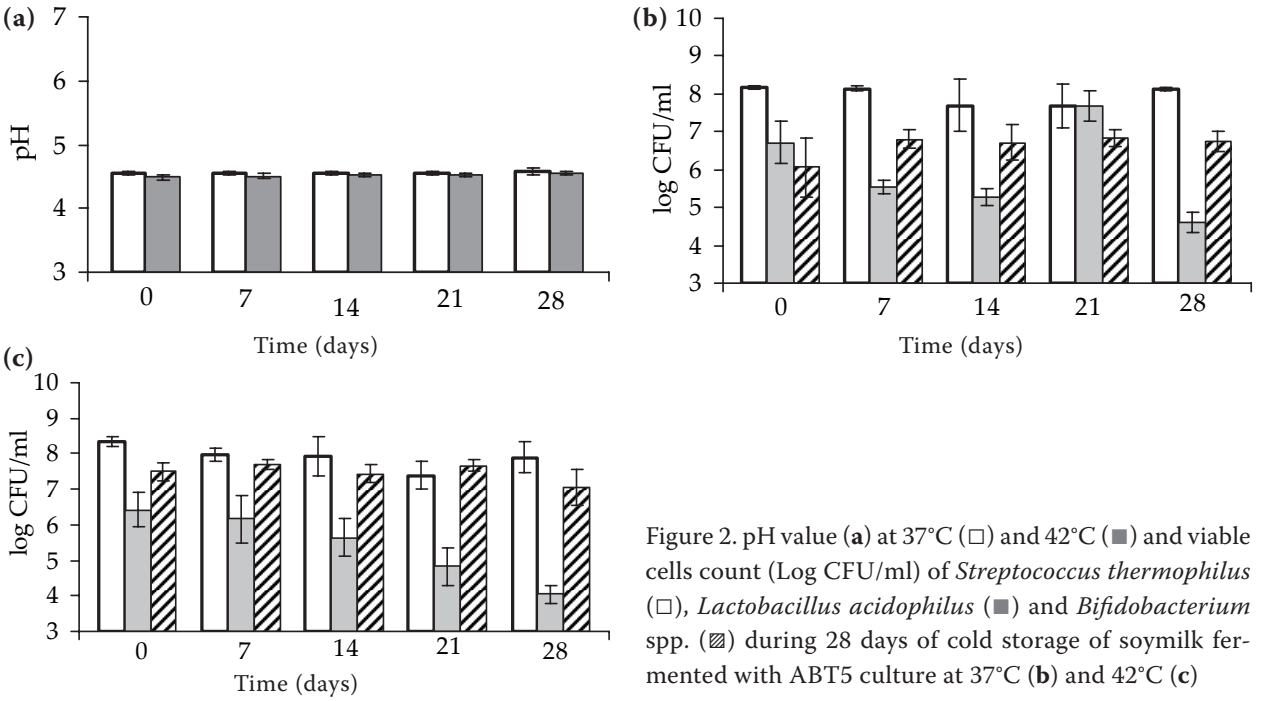

Figure 2. pH value (**a**) at 37°C (□) and 42°C (■) and viable cells count (Log CFU/ml) of *Streptococcus thermophilus* (□), *Lactobacillus acidophilus* (■) and *Bifidobacterium* spp. (▨) during 28 days of cold storage of soymilk fermented with ABT5 culture at 37°C (**b**) and 42°C (**c**)

total sugars) followed by stacchiose (ca 12–35% of total sugars) whereas fructose and raffionse are represented in the lowest amounts (ca 5–16% of total sugars) (USDA 2006).

pH value of the fermented soymilk samples was very stable during 28 days of cold storage in refrigerator (Figure 2a). To maintain confidence in probiotic products, it is important to demonstrate a good survival of bacteria in the products throughout the shelf-life of the products. Streptococci and bifidobacteria survived well during the storage time while the viable cells count of *Lactobacillus acidophilus* decreased below 10^6 CFU/ml already after the first week of storage (Figures 2b,c).

Based on the results obtained in this study (Figures 1 and 2) and also in preliminary studies (Božanić *et al.* 2008b), it is shown that lactobacilli develop poorly in soymilk. Therefore, in the consequent phase of this study soymilk was fermented using yoghurt culture with the addition of bifidobacteria. Fermentation was performed at 42°C since bifidobacteria present in ABT5 culture grew better at that temperature (Figures 1b,c), and this temperature is also optimal for the growth of yoghurt culture bacteria.

The fermentation of soymilk using yoghurt culture with the addition of bifidobacteria at 42°C lasted 4 h (Figure 3a). In this experiment, streptococci

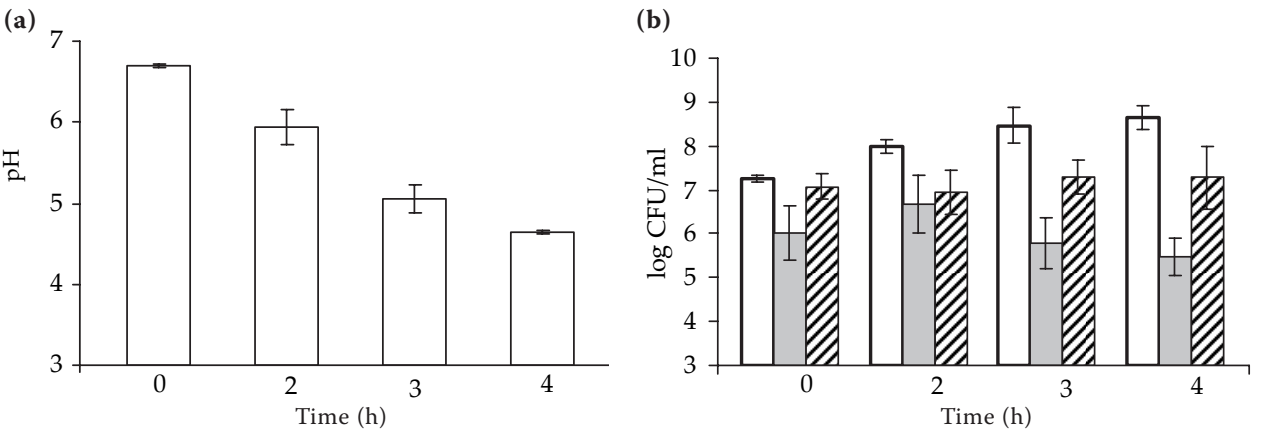

Figure 3. pH value (**a**) and viable cells count (**b**) (log CFU/ml) of *Streptococcus thermophilus* (□), *Lactobacillus delbrueckii* subsp. *bulgaricus* (■) and *Bifidobacterium animalis* subsp . *lactis* Bb12 (▨) during soymilk fermentation at 42°C with yoghurt culture YCX11 plus *Bifidobacterium animalis* subsp. *lactis* Bb12

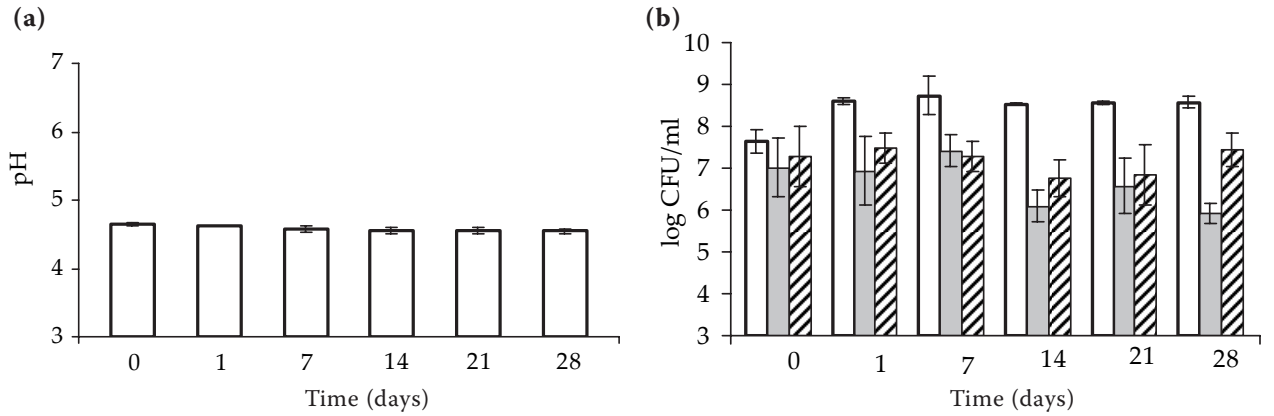

Figure 4. pH value (**a**) and viable cells count (**b**) (log CFU/ml) of *Streptococcus thermophilus* (□), *Lactobacillus delbrueckii* subsp. *buduring* (■), and *Bifidobacterium animalis* subsp. *lactis* Bb12 (▨) 28 days of cold storage of soymilk fermented with yoghurt culture YCX11 plus *Bifidobacterium animalis* subsp. *lactis* Bb12

showed again the best growth while lactobacilli the lowest one (Figure 3b), as observed also in the previous experiment with ABT5 culture (Figures 1b,c). These results comply with the results obtained in former experiments on soymilk fermentation using composite cultures of streptococci, lactobacilli, and bifidobacteria. In each experiment, lactobacilli grew poorly, regardless of *Lactobacillus casei* (Božanić *et al.* 2008), *Lactobacillus acidophilus* (Figures 1b,c), or *Lactobacillus delbrueckii* subsp. *bulgaricus* (Figure 3b) being used. The viable cells count of streptococci remained above 10^8 CFU/ml at the end of fermentation and during the entire storage period (Figure 4b). The viable cells count of bifidobacteria increased during fermentation for approximately half of logarithm (Figure 3b), and remained stable during 28 days of storage (Figure 4b), which is required for probiotic products (Tamime *et al.* 2005).

Donkor *et al.* (2007) investigated the capability of different probiotic organisms and *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* to ferment soymilk. It is dependent on their α-galactosidase activity which greatly differs, and is also strain dependent. *Lactobacillus delbrueckii* subsp. *bulgaricus* had a low α-galactosidase activity, but it grew well in a mixed culture with *Streptococcus thermophilus.* Farntworth *et al.* (2007) showed that both bacteria strains contained in yoghurt culture grew very well in soymilk. When yoghurt culture was combined with probiotic strains (*L. rhamnosus*, *L. jonsoni*, *Bifidobacteria* sp.), *Lactobacillus delbrueckii* subsp. *bulgaricus* grew very poorly. Contrary to the research of Donkor *et al.* (2005) the presence of probiotic bacteria enhanced in soy yoghurt the growth of both bacteria strains contained in yoghurt culture in comparison to the control sample produced by sole yoghurt culture. The use of yoghurt culture in conjunction with probiotic cultures resulted in an appreciable proteolytic activity, probably improving the growth of the selected probiotics. More importantly, soy yoghurt produced by probiotic strains as adjunct culture exerted an appreciable ACE (angiotensin-converting enzyme) inhibitory activity. This enzyme plays a major role in the regulation of blood pressure. The development of soy yoghurt containing higher concentrations of released bioactive ACE inhibitors and viable probiotic may deliver health benefits of these functional compounds more efficiently (Donkor *et al.* 2005)

Relating to the acceptable length of fermentation (4 h) and the viable cells count of probiotic bacteria *Bifidobacterium animalis* subsp. *lactis* Bb12 above 10^7 CFU/ml as obtained in this experiment, it can be concluded that the aim of this study was accomplished. Future studies should be focused on optimising the sensory characteristics of this product. Fermented soymilk had smooth texture and smooth consistency likewise fermented cream. It had porcelain shine and light colour, but no characteristic odour was detected. Compared to soymilk, the fermented product obtained had less expressed flavour of peas. This specific flavour of soymilk can be masked by the addition of sugars, aromas, and fruit paste. In that way, an eligible probiotic and nutritionally improved product may be yielded.

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