Survivability of *Lactobacillus rhamnosus* during the Preparation of Soy Cheese

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Summary

The aim of this study was to develop a new probiotic soy cheese on the basis of chinese sufu. The soy cheese was made from soymilk fermented with soy cheese bacterial starter cultures (DH_1 and GH_4) and *L. rhamnosus* 6013. After ripening, probiotic soy cheese sensory scores (standard SB/T 10170-93) were compared to the control. The changes in pH, bacterial growth and the survivability of the potential probiotic *L. rhamnosus* 6013 during fermentation and storage at 10 °C were examined. After 6 h of fermentation, *L. rhamnosus* 6013 was capable of growing in soymilk as high as 10^8–10^9 CFU/mL. After being stored for 30 days at 10 °C, slight decrease in pH and the viable counts of the strain was noticed. The viable counts of *L. rhamnosus* 6013, DH_1 and GH_4 were 10^7, 10^6 and 10^6 CFU/g, respectively, after storage for 30 days. The levels of stachyose, raffinose and sucrose in soy cheese were determined by high performance liquid chromatography. The results indicated that *L. rhamnosus* 6013 could utilize the soybean oligosaccharides as carbon sources. In addition, 2–4 % of NaCl had little effect on the survivability of *L. rhamnosus* 6013. It indicated that *L. rhamnosus* 6013 could withstand the technological processing of soy cheese and had no negative effect on the fermentation and the sensory properties of the soy cheese.

Key words: *Lactobacillus casei* ssp. *rhamnosus* 6013, fermentation, survivability, probiotic soy cheese, HPLC

Introduction

Soy products play an important role in prevention of chronic diseases such as menopausal disorder, cancer, atherosclerosis and osteoporosis (*1–2*). Soymilk is rich in high quality proteins and suitable for the growth of bifidobacteria (*3–6*) because this product contains sucrose, raffinose and stachyose, used by most of the strains that belong to this genus during fermentation (*7*). It is difficult for mammals to digest the two latter sugars, which tend to cause flatulence in human body (*8*). These bacteria appear promising starter cultures for the production of quality fermented soymilk products containing reduced quantities of antinutritional factors.

Preparation of a cheese-like product from soymilk coagulated by lactic acid bacteria gained much interest in the past (*9–11*). The Chinese cheese (sufu), produced in many different forms by various processes in different localities in China (*12–13*), occurred there many centuries ago. It is a soft creamy cheese-type product that possesses a characteristic flavour and a relatively high protein level, and it can be used in the same way as cheese (*14*).

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Probiotic lactic acid bacteria are widely used in dairy products but not in soy cheese products. Probiotics are live microbial food ingredients that are beneficial to the health (*15*). Consumption of probiotic bacteria *via* food products is an ideal way to reestablish the intestinal microflora balance. Recently, the potential probiotic strains *L. gasseri* JCM113 (*16–17*), *L. rhamnosus* FERM P-15120 and *L. paracasei* ssp. *paracasei* FERM P-15121 (*18*) were used for meat fermentation. The potentially probiotic *L. casei* LC-01 and probiotic *Bifidobacterium lactis* Bb-12 were used in fermenting dry sausages (*19*). However, little information about *L. rhamnosus* used in soy cheese production is available.

In this study, soy cheese with *L. rhamnosus* 6013 and two soy cheese strains (DH$_1$ and GH$_4$) was made. Moreover, the technological properties of the potentially probiotic *L. rhamnosus* 6013 during soy cheese processing were examined, as well as some of the technological properties during soymilk fermentation and storage. Apart from that, changes in the sugar content by HPLC, and the effect of NaCl concentration on survivability were evaluated. Sensory evaluation was developed according to the corresponding standard (SB/T 10170-93).

Materials and Methods

Bacterial strains, soymilk, and chemicals

Lactobacillus casei ssp. *rhamnosus* 6013 (*L. rhamnosus* 6013) was obtained from China Center of Industrial Culture Collection (CICC). We screened over 250 bacterial strains from Chinese sufu products in our laboratory. After selecting those strains most likely to degrade soy protein and produce proteinase, 10 strains were screened for their ability to ferment soy cheese. The promising strains were further investigated for coagulation in soymilk and flavour in soy cheese. Two strains (DH$_1$ and GH$_4$) were used in this study.

Dehulled soybeans were soaked in deionized water (water/bean ratio=6:1) overnight. After rinsing, the soaked beans were ground and soymilk was obtained after sieving through 200 mesh and was sterilized (0.1 MPa, 121 °C, 20 min). The soymilk contained (in g/100 mL): protein 4.80, fat 2.50, sugars 3.50 (sucrose 2.20, stachyose 0.54 and raffinose 0.23) and ash 0.50.

Cultivation of L. rhamnosus 6013 in soymilk

Cultures of *L. rhamnosus* 6013 were grown in MRS anaerobically at 37 °C for 48 h. After successive transfers of *L. rhamnosus* 6013 into the sterilized soymilk at 37 °C for 48 h, this cell suspension was inoculated at 5 % into the sterilized soymilk.

Preparation of soy cheese

The soy cheese fermented by bacteria was made according to the method by Han *et al.* (*20*). After cooling the sterilized soymilk to 37 °C, *L. rhamnosus* 6013, DH$_1$ and GH$_4$ were inoculated at 5 % (soy cheese A – *L. rhamnosus* 6013; soy cheese B – *L. rhamnosus* 6013 and DH$_1$; soy cheese C – *L. rhamnosus* 6013 and GH$_4$). Soymilk was fully coagulated after 6 h of fermentation, and then incubated at 55–60 °C for 30 min to accelerate coagulation.

When the soft curd was transferred to moulds for draining and pressing, cake was cut into cubes of the size 3.0×3.0×1.5 cm. The cubes were placed in jars containing NaCl brine and salted for 3 days at 4 °C. After the brine was drained, the cubes were placed in sealed jars for storage at 10 °C for 30 days.

Microbiological analysis

Samples were taken (at 0, 1, 2, 3, 4, 5 and 6 h) and the growth of bacteria was determined as follows: *L. rhamnosus* 6013 was determined by plating suitable dilutions on MRS agar, while DH$_1$ and GH$_4$ were determined on LB agar. Serial dilutions of each sample in sterile saline solution (γ(NaCl)=9 g/L in deionized water) were prepared. A volume of 0.4 mL of diluted samples was spread onto MRS (Oxoid, England) (*21*) and LB (Huankai Microbial Sci. & Tech. Co., Ltd., Guangdong) agar plates. Colonies on MRS and LB plates were counted after incubation at 37 and 28 °C, respectively, for 48 h. The numbers of viable cells (expressed in CFU/mL for soymilk and in CFU/g for soy cheese) were the means of four repeated experiments. The pH change during growth was measured.

A mass of 10 g of soy cheese was taken aseptically from a jar at 0, 5, 10, 15, 20, 25 and 30 days, homogenized with 100 mL of sterile deionized water, and decimal dilutions in 0.9 % of sterile saline solution were prepared. The viability of the samples was assessed by the method mentioned above, and the pH value was measured.

Physical and chemical analyses

NaCl content of soy cheese

After the cubes were salted in different NaCl solutions for 3 days at 4 °C, the brine solution was drained and the cubes were placed in sealed jars for storage at 10 °C for 30 days. Soy cheese was taken and homogenized with 100 mL of sterile deionized water, then it was brought to boil and cooled to ambient temperature. Tenfold diluted soy cheese was titrated with 0.1 mol/L of AgNO$_3$, and 10 % (*m/V*) of K$_2$CrO$_4$ solution was used as an indicator (*20*). A volume of 5 mL of supernatants was removed to 250-mL conical flask and 25 mL of deionized water and 10 drops of 10 % (*m/V*) of K$_2$CrO$_4$ solution were added. The following equation was used: % salt as $w(NaCl)/\%=V \times M \times 0.0584 \times 100 \times 20/m$; where V and M are the volume and molarity of AgNO$_3$ used, and m is the mass of fresh sample.

Measurement of sugars

The HPLC was used to determine the contents of sugars (sucrose, stachyose and raffinose) using Waters Spherisorb NH$_2$ column (4.6 mm×250 mm, 5 μm). Operational conditions were as follows: mobile phase: 70 % acetonitrile (Kermel Chemical Reagents Development Centre, Tianjin, China) in distilled deionized water; flow rate: 0.6 mL/min; column temperature: 40 °C; a refractive index detector (model 830-RI, Jasco). Reference sucrose, stachyose and raffinose (Sigma-Aldrich, Shanghai, China) were chromatographed to determine their retention times. Samples were deproteinized (*22*) and centrifuged for 20 min at 8000 rpm. Supernatant fractions

were filtered through a 0.45-μm membrane, and then subjected to HPLC analysis. External standards were prepared by diluting specific amounts of sugars in deionized water. Standard curves were constructed for each carbohydrate. Least squares regression analysis was used to derive equations from the values reported for each carbohydrate.

Sensory evaluation of soy cheese

Quality standard and examination for fermented bean curd SB/T 10170-93 (*13*) was used for soy cheese grading. Evaluation was based on five features, such as appearance, colour, flavour, aroma and texture, resulting in a maximum of five points for each characteristic. According to this system, there are four classes of cheese, such as excellent (4.50 to 5.00 points), standard (4.00 to 4.49 points), II class (3.50 to 3.99 points), and III class (under 3.50 points). Soy cheese A, soy cheese B, soy cheese C and control were graded in a blind manner after 30 days of ripening by five specialists familiar with the sensory evaluation of soy cheese from a local sufu manufacturing plant and our center. Control was commonly mould-fermented sufu from China.

Statistical analysis

All the tests were done four times and the data were averaged. Standard deviation was also calculated. Microcal Origin V.7.0 was used to evaluate significantly different (p<0.05) means for each sample.

Results and Discussion

The pH change of soymilk in cheese and growth of *L. rhamnosus* 6013 during cheese fermentation

Changes in the pH during fermentation of soy cheese are shown in Fig. 1. The initial pH of all soymilk samples after inoculation was 5.5–5.9. At 37 °C, the fastest pH decline was observed in soy cheese C, while soy cheese A and B showed a slower decrease after 3 h of fermentation. After 6 h of fermentation, the pH values of soy cheese C reached 4.7, whereas those of cheese A and B remained at 5.1–5.2. All the samples were coagulated after 6 h of fermentation. The viable cell counts of *L. rhamnosus* 6013 are presented in Fig. 2. The viable
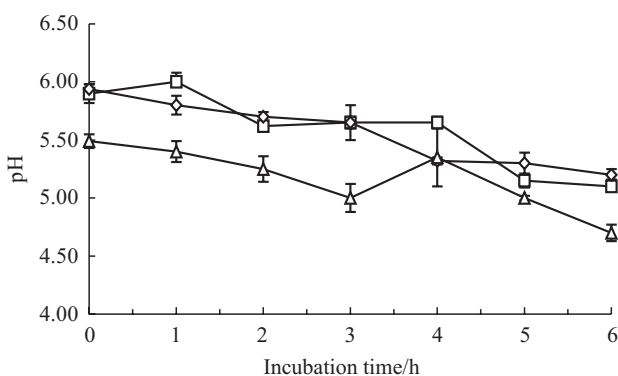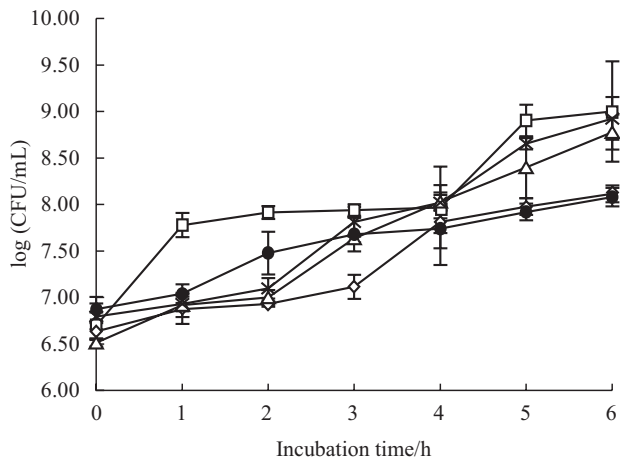

Fig. 2. Bacterial growth during soy cheese fermentation inoculated with *L. rhamnosus* 6013 and starters
□ *L. rhamnosus* 6013 in cheese A, ◇ *L. rhamnosus* 6013 in cheese B, △ *L. rhamnosus* 6013 in cheese C, × DH_1 in cheese B, ● GH_4 in cheese C

count of *L. rhamnosus* 6013 in these soy cheeses increased significantly (p<0.05) from 6.7, 6.6, and 6.5 log (CFU/mL) to 9.0, 8.1 and 8.8 log (CFU/mL), respectively, after 6 h of fermentation. The viable count of DH_1 of soy cheese B, and GH_4 of soy cheese C increased significantly (p<0.05) from 6.8 and 6.9 log (CFU/mL) to 8.9 and 8.1 log (CFU/mL), respectively. Besides China, many other countries such as Vietnam, Philippines, Thailand and Korea produce various soy cheeses, most of which depend on heat, salts (magnesium sulphate, calcium sulphate or glucono-δ--lactone) and lactic acid to produce the gel, but the texture of the final products is often unsatisfactory (*23*). However, if using lactic acid bacteria for fermentation in soymilk, the slower coagulation might obtain a product with more consistent texture product. The lactic acid bacteria metabolize carbohydrates in soymilk and produce lactic acid that coagulates the soymilk protein. The significant (p<0.05) decrease in pH causes a decrease in the water binding capacity of the soymilk, which accelerates the coagulation process of soy cheese (*6*).

Survivability of *L. rhamnosus* 6013 in the fermented soymilk during storage

Fig. 3 shows that pH decreases slightly in these three soy cheese samples during storage. The pH of the soy cheese A decreased from 4.5 to 4.3, while the pH of the soy cheese B and C decreased significantly (p<0.05) from 4.8 to 4.4 in both cases. The pH values of these three soy cheese samples remained 4.3–4.4 after 30 days of storage at 10 °C. Han *et al.* (*24*) investigated 23 samples of three different types of soy cheese in China and some in the Netherlands, and they found that the pH value was almost between 5.25–7.45. The lower pH of this novel probiotic soy cheese assured the food safety, because the lactic acid disturbed the homeostasis of the bacterial cells, including pathogens (clostridia, salmonellae) and spoilage bacteria and therefore limited their growth (*25*).

Fig. 4 shows that the viable numbers of *L. rhamnosus* 6013 in soy cheese A, B and C decreased significantly (p<0.05) from 8.9, 8.7 and 8.8 log (CFU/g) to 7.9, 7.8 and 7.9 log (CFU/g), respectively, in 30 days of storage at 10
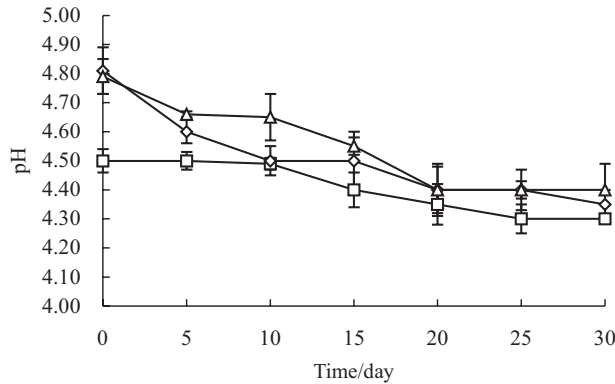

Fig. 1. pH change during soy cheese fermentation inoculated with *L. rhamnosus* 6013 and starters
□ cheese A, ◇ cheese B, △ cheese C

Fig. 3. pH change during storage for 30 days at 10 °C
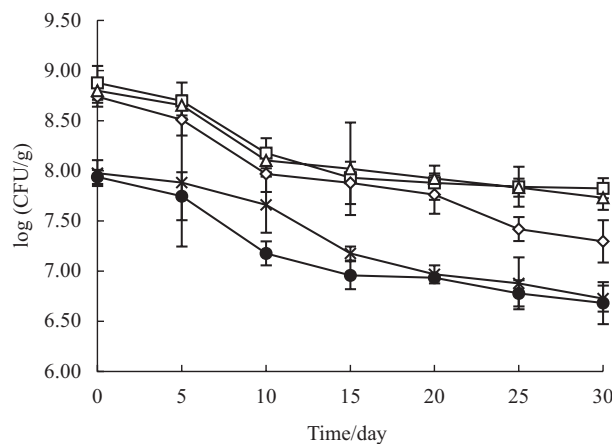□ cheese A, ◇ cheese B, △ cheese C

Fig. 4. Survivability of bacteria during storage for 30 days at 10 °C
□ *L. rhamnosus* 6013 in cheese A, ◇ *L. rhamnosus* 6013 in cheese B, △ *L. rhamnosus* 6013 in cheese C, × DH_1 in cheese B, ● GH_4 in cheese C

°C. After storage, slight decrease in pH and the viable counts of the strain was noticed. The viable counts of *L. rhamnosus* 6013, DH_1 and GH_4 were 10^7, 10^6 and 10^6 CFU/g, respectively. In order to exert the maximum probiotic effect of *L. rhamnosus* 6013, it is important to keep high live bacterial numbers. Kurmann and Rasic (*26*) asserted that probiotic foods with health claim should contain at least 10^6 CFU/mL of the bacteria on expiry date because 10^8–10^9 cells is the minimum therapeutic dose per day. The potential probiotic strain, *L. rhamnosus* 6013, could withstand the cheese manufacturing process and survive during ripening and storage without negative effects on the quality of cheese.

The effect of NaCl concentration on survivability of *L. rhamnosus* 6013 in the soy cheese during storage

Table 1 shows that 2–4 % NaCl had little effect on survivability of *L. rhamnosus* 6013 when NaCl concentration of brine was 4 %, and the viable counts of *L. rhamnosus* 6013 in soy cheese A, B and C were kept as high as 10^7 CFU/g. But the addition of 10 % of NaCl to the curd not only decreased significantly (p<0.05) the survivability of *L. rhamnosus* 6013 (10^6 CFU/g), but also led to a decrease in hardness and cohesiveness of the cheese. Salt plays a very important role in soy cheese, which imparts a salty taste to the product as well as serves to control the enzyme activity. It inhibits the survival and/or growth of spoilage-causing, pathogenic and toxin-producing microorganisms. Han *et al*. (*24*) found that most types of sufu contain considerable levels of the antimicrobial NaCl (8–15 %) that could prevent the survival or growth of pathogens. For public health reasons, the reduction of the salt level to 2–4 % could cause a reduction of safety of soy cheese. Therefore, the addition of lactic acid bacteria, especially probiotics, was expected to ensure a rapid initiation of the fermentation process and decline in pH. In addition, the low pH of the soy cheese should reduce spore-formers during storage (*27*).

Changes in the quantities of stachyose, raffinose and sucrose after 6 h of soy cheese fermentation

The behaviour of *L. rhamnosus* 6013 in soymilk was studied with respect to hydrolysis of sucrose, raffinose and stachyose. It was established by HPLC that the predominant sugars in unfermented soymilk were (in g/L): sucrose 22.00, raffinose 2.30 and stachyose 5.40. The results show that the antinutritional factors raffinose and stachyose can be used by *L. rhamnosus* 6013. In particular, raffinose was completely metabolized after 6 h (p<0.05), whereas stachyose was significantly (p<0.05) reduced to 2.70, 1.76 g/L and 1.49 g/L in soymilk fermented by *L. rhamnosus* 6013, *L. rhamnosus* 6013+DH_1 and *L. rhamnosus* 6013+GH_4, respectively. Sucrose concentrations also decreased significantly (p<0.05) from 22.00 to 10.50, 8.45 and 4.38 g/L by *L. rhamnosus* 6013, *L. rhamnosus* 6013+DH_1 and *L. rhamnosus* 6013+GH_4, respectively. Stachyose and raffinose, the principal oligosaccharides in soymilk, are believed to cause flatulence in humans after the consumption of soybean foods. These oligosaccharides can be hydrolysed by α-galactosidase (*7*), which is capable of hydrolyzing α-1,6 linked α-galactoside residues. The production of galactosidase by lactic acid bacteria and bifidobacterium has been reported (*28,29*). Changes in the content

Table 1. Effect of NaCl solution concentration on survivability of *L. rhamnosus* 6013

$\frac{m}{V}$ (NaCl)/%	Soy cheese A	Soy cheese B	Soy cheese C
0	0.04±0.01/8.0±0.15[a]	0.04±0.10/7.9±0.22	0.04±0.01/7.9±0.08
2	1.40±0.11/7.9±0.85	1.30±0.15/7.6±0.31	1.35±0.11/7.9±0.14
4	3.00±0.21/7.8±0.25	2.76±0.23/7.5±0.13	2.81±0.22/7.7±0.08
10	8.83±0.27/6.4±0.08	8.37±0.65/6.3±0.29	8.14±0.65/6.7±0.20

[a] $\frac{m}{V}$ (NaCl)/%/log (CFU/g)

of stachyose and raffinose in soymilk fermented with *L. acidophilus* or *S. thermophilus* or with *Bifidobacteria* have been reported (*30*). The ability of *L. fermentum*, *L. casei* and *S. salivarius* ssp. *thermophilus* to utilize soybean oligosaccharides has been evaluated by Garro *et al.* (*22,31, 32*). The changes of stachyose, raffinose and sucrose contents were related to the pH drop and fast growth noted in Figs. 1 and 2, respectively. The lactic acid bacteria metabolize carbohydrates in soymilk and produce lactic acid that coagulates the soymilk protein. The *L. rhamnosus* 6013 inoculated into soymilk can metabolize oligosaccharides and produce organic acids that coagulate the soymilk protein.

Sensory evaluation

When trying to introduce a probiotic strain into soy cheese products, a probiotic strain should withstand the manufacturing process without the loss of viability or negative effect on the sensory properties of the soy cheese. Soy cheese variants with probiotic additive were found to have flavour and texture comparable to the control. Soy cheese B, C and the control were described to be of commercial grade with respect to sensory criteria after 30 days of ripening. The score value of the control was 4.5 points. Soy cheese B and C achieved the grades of 3.7 and 3.9 points, respectively, due to their harder texture and less strong flavour (Fig. 5). The score value of
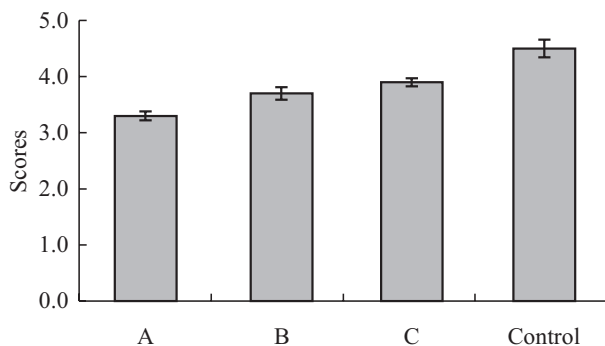

Fig. 5. Sensory examination of three soy cheese samples and mould-fermented sufu from China based on appearance, colour, flavour, aroma and texture. Values in this figure are means of expert panel's scoring in a blind manner (A – *L. rhamnosus* 6013, B – *L. rhamnosus* 6013 and DH$_1$, C – *L. rhamnosus* 6013 and GH$_4$, Control – mould-fermented sufu)

soy cheese A was only 3.3 points because it had a harder consistency and the moisture content was too low (28.65 %). The interactions between probiotic and starter do not have an impact on the product sensory properties. In this study, it is possible to produce soy cheese with good sensory properties and good survival of *L. rhamnosus* 6013 and starters (DH$_1$ and GH$_4$).

Conclusions

The incorporation of probiotics into cheese during processing is expected to result in the functional foods if the cultures maintain viability during ripening and do not adversely affect composition, texture or sensory criteria of the products. *L. rhamnosus* 6013 can favourably utilize soy oligosaccharides as carbon sources. This strain

is capable of withstanding the technological processing of soy cheese. No negative effect on fermentation and the sensory properties of the soy cheese has been observed in this study, which indicates that this strain can be used as a promising starter culture in producing a soy cheese of good taste.

References

1. J.J.B. Anderson, M. Anthony, M. Messina, S.C. Garner, Effects of phyto-oestrogens on tissues, *Nutr. Res. Rev. 12* (1999) 75–116.

2. K.D.R. Setchell, A. Cassidy, Dietary isoflavones: Biological effects and relevance to human health, *J. Nutr.* (Suppl.), *129* (1999) 758–767.

3. Y. Shimakawa, S. Matsubara, N. Yuki, M. Ikeda, F. Ishikawa, Evaluation of *Bifidobacterium breve* strain Yakult-fermented soymilk as a probiotic food, *Int. J. Food Microbiol. 81* (2003) 131–136.

4. J. Matsuyama, H. Hirata, T. Yamagishi, K. Hayashi, Y. Hirano, K. Kuwata, I. Kiyosawa, T. Nagasawa, Fermentation profiles and utilization of sugars of *Bifidobacteria* in soymilk, *Nippon Shokuhin Kogyo Gakkaishi, 39* (1992) 887–893 (in Japanese).

5. C.C. Chou, J.W. Hou, Growth of *Bifidobacteria* in soymilk and survival in the fermented soymilk drink during storage, *Int. J. Food Microbiol. 56* (2000) 113–121.

6. S. Chumchuere, R.K. Robinson, Selection of starter cultures for the fermentation of soy milk, *Food Microbiol. 16* (1999) 129–137.

7. P. Scalabrini, M. Rossi, P. Spettoli, D. Matteuzzi, Characterization of *Bifidobacterium* strains for use in soymilk fermentation, *Int. J. Food Microbiol. 39* (1998) 213–219.

8. R. Gitzelmann, S. Auricchio, The handling of soy alpha-galactosides by a normal and a galactosemic child, *Pediatrics, 36* (1965) 231–235.

9. Y.D. Hang, H. Jackson, Preparation of soybean cheese using lactic starter organisms: I. General characteristics of the finished cheese, *Food Technol. 21* (1967) 95–96.

10. Y.D. Hang, H. Jackson, Preparation of soybean cheese using lactic starter organisms: II. Effects of addition of extract and skim-milk, *Food Technol. 21* (1967) 97–100.

11. W.M. El-Ella, Hard cheese substitute from soy milk, *J. Food Sci. 45* (1980) 1777–1778.

12. Y.J. Li, Soy cheese – A health soybean food, *J. China Brew. Ind. 4* (1997) 1–4 (in Chinese).

13. *The Production of Soy Cheese in China*, R.Z. Wang, X.X. Du (Eds.), China Light Industry Press, Beijing (1998) (in Chinese).

14. Y.C. Su: Sufu. In: *Legume-Based Fermented Foods*, N.R. Reddy, M.D. Pierson, D.K. Salunkhe (Eds.), CRC Press, Boca Raton, Florida, USA (1986) pp. 69–83.

15. S. Salminen, A. Ouwehand, Y. Benno, Y. Lee, Probiotics: How should they be defined?, *Trends Food Sci. Technol. 10* (1999) 107–110.

16. K. Arihara, H. Ota, M. Itoh, Y. Kondo, T. Sameshima, H. Yamanaka, M. Akimoto, S. Kanai, T. Miki, Utilization of probiotic lactic acid bacteria for meat products, *Proceedings of the 42th ICoMST Congress*, Lillehammer, Norway (1996) pp. 501–502.

17. K. Arihara, H. Ota, M. Itoh, Y. Kondo, T. Sameshima, H. Yamanaka, M. Akimoto, S. Kanai, T. Miki, *Lactobacillus acidophilus* group lactic acid bacteria applied to meat fermentation, *J. Food Sci. 63* (1998) 544–547.

18. T. Sameshima, C. Magome, K. Takeshita, K. Arihara, M. Itoh, Y. Kondo, Effect of intestinal *Lactobacillus* starter cultures on the behaviour of *Staphylococcus aureus* in fermented sausage, *Int. J. Food Microbiol. 41* (1998) 1–7.

19. S. Erkkilä, M.L. Suihko, S. Eerola, E. Petäjä, T. Mattila-Sandholm, Dry sausage fermented by *Lactobacillus rhamnosus* strains, *Int. J. Food Microbiol. 64* (2000) 205–210.

20. B.Z. Han, F.M. Rombouts, M.J.R. Nout, A Chinese fermented soybean food, *Int. J. Food Microbiol. 65* (2001) 1–10.

21. J. Prasad, H. Gill, J. Smart, P.K. Gopal, Selection and characterisation of *Lactobacillus* and *Bifidobacterium* strains for use as probiotics, *Int. Dairy J. 8* (1999) 993–1002.

22. M.S. Garro, G.F. De Valdez, G. Oliver, G.S. De Giori, Growth characteristics and fermentation products of *Streptococcus salivarius* subsp. *thermophilus*, *Lactobacillus casei* and *L. fermentum* in soymilk, *Z. Lebensm. Unters. Forsch. A, 206* (1998) 72–75.

23. K. Kohyama, K. Nishinari, Rheological studies on the gelation process of soybean 7S and 11S proteins in the presence of glucono-δ-lactone, *J. Agric. Food Chem. 41* (1993) 8–14.

24. B.Z. Han, R.R. Beumer, F.M. Rombouts, M.J.R. Nout, Microbiological safety and quality of commercial sufu – A Chinese fermented soybean food, *Food Control, 12* (2001) 541–547.

25. L. Leistner, Basic aspects of food preservation by hurdle technology, *Int. J. Food Microbiol. 55* (2000) 181–186.

26. J.A. Kurmann, J.L. Rasic: The Health Potential of Products Containing *Bifidobacteria*. In: *Therapeutic of Fermented Milks*, R.K. Robinson (Ed.), Elsevier, London, UK (1991) pp. 117–158.

27. S. Työppöen, A. Markkula, E. Petäjä, M.L. Suihko, T. Mattila-Sandholm, Survival of *Listeria monocytogenes* in North European type dry sausages fermented by bioprotective meat starter cultures, *Food Control, 14* (2003) 181–185.

28. D.B. Hughes, D.G. Hoover, Viability and enzymatic activity of *Bifidobacteria* in milk, *J. Dairy Sci. 78* (1995) 268–276.

29. B.K. Mital, R.S. Shallenberger, K.H. Steinkraus, α-galactosidase activity of lactobacilli, *Appl. Microbiol. 26* (1973) 783–788.

30. Y.C. Wang, R.C. Yu, H.Y. Yang, C.C. Chou, Sugar and acid contents in soymilk fermented with lactic acid bacteria alone or simultaneously with *Bifidobacteria*, *Food Microbiol. 20* (2003) 333–338.

31. M.S. Garro, G.S. De Giori, G.F. De Valdez, G. Oliver, Utilization of soymilk galactosaccharides by lactobacilli, *Microbiol. Alim. Nutr. 12* (1994) 61–66.

32. M.S. Garro, G.F. De Valdez, G. Oliver, G.S. De Giori, Hydrolysis of soya milk oligosaccharides by *Bifidobacterium longum* CRL849, *Z. Lebensm. Unters. Forsch. A, 208* (1999) 57–59.