Optimization of Growth and Storage Conditions for Lactic Acid Bacteria in Yogurt and Frozen Yogurt

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The optimal conditions for growth and storage of the lactic acid bacteria were studied. Both sucrose (up to 3%, w/v) and skim milk (up to 12%, w/v) increased the number of lactic acid bacteria. However, the effect of sucrose plus skim milk was not superior to that of skim milk alone. The cell number was maximal at pH 5.0-5.5 during the growth of bacteria and at pH 4.63 during the storage period of yogurt. The final cell number of yogurt stored for 150 days at -18 ~ -12°C was 1/3 of the initial cell number.

Key words: *frozen yogurt, lactic acid bacteria, shelf life, yogurt*

Yogurt has been introduced as a safe and healthy food. The shelf life of yogurt was strongly affected by the cell number of lactic acid bacteria (10^8 lactic acid bacteria/mL) [KFIO, 1995]. During storage and distribution, the cell number significantly decreases due to the overproduced lactic acid [Sun and Griffiths, 2000]. Therefore, proper control of the yogurt production can prolong the shelf life. Ingredient supplementation also significantly affected the viability of the lactic acid bacteria in the yogurt [Dave and Shah, 1998]. The total solid content of milk whey increased the viability of the lactic acid bacteria [Almeidaa *et al.,* 2009]. H^+-ATPase-defective mutants of the lactic acid bacteria were also used to prolong the viability of the lactic acid bacteria in the yogurt during refrigeration storage [Ongol *et al.,* 2007]. The addition of inulin stimulated the growth of the lactic acid bacteria in the probiotic ice cream [Akin *et al.,* 2007]. Therefore, the objective of the present study was to optimize the cell number during the production and storage. The factors used were pH, components of yogurt, and storage conditions.

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Abbreviations: CFU, colony-forming unit

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Materials and Methods

Lactic acid bacteria and yogurt fermentation. The lactic acid bacteria used in this study, *Bifidobacterium longum subsp. longum, Lactobacillus acidophilus, Streptococcus salivarius subsp. thermophilus, Lactobacillus delbrueckii subsp. Bulgaricus*, were purchased as a mixed starter (ABY-2, Rhone-poulenc, Courbevoie, France). The basic components of yogurt were milk (Seoulmilk. Seoul, Korea) and 0.5% (w/v milk) stabilizer (SL-1366, Rhone-poulenc). Skim milk, sucrose, and potassium phosphate were used to examine their influences on prolonging the viability of the lactic acid bacteria. The mixture was homogenized and incubated at 80°C for 30 min [Babel, 1976]. After cooling down to 40°C, the starter culture was added. The fermentation was performed at 40°C for 4 h without shaking. After fermentation, the yogurt was stored at 4°C.

Frozen Yogurt. After homogenization at 4°C with the overrun (80%, v/v), the yogurt was frozen in a –3°C freezer. The content of the total solid was controlled at 36.5% (w/v). Aging was performed at –36°C for 1 day. Frozen yogurt was stored in a freezer at –12~–18°C for up to 6 months.

Bacterial cell counting. The AMC, Arroyo, Martin, and Cotton agar medium [Payne *et al*., 1999] was used to count *Bifidobacterium*. To attain a complete anaerobic condition, the GasPak anaerobic system (Becton, Dickinson

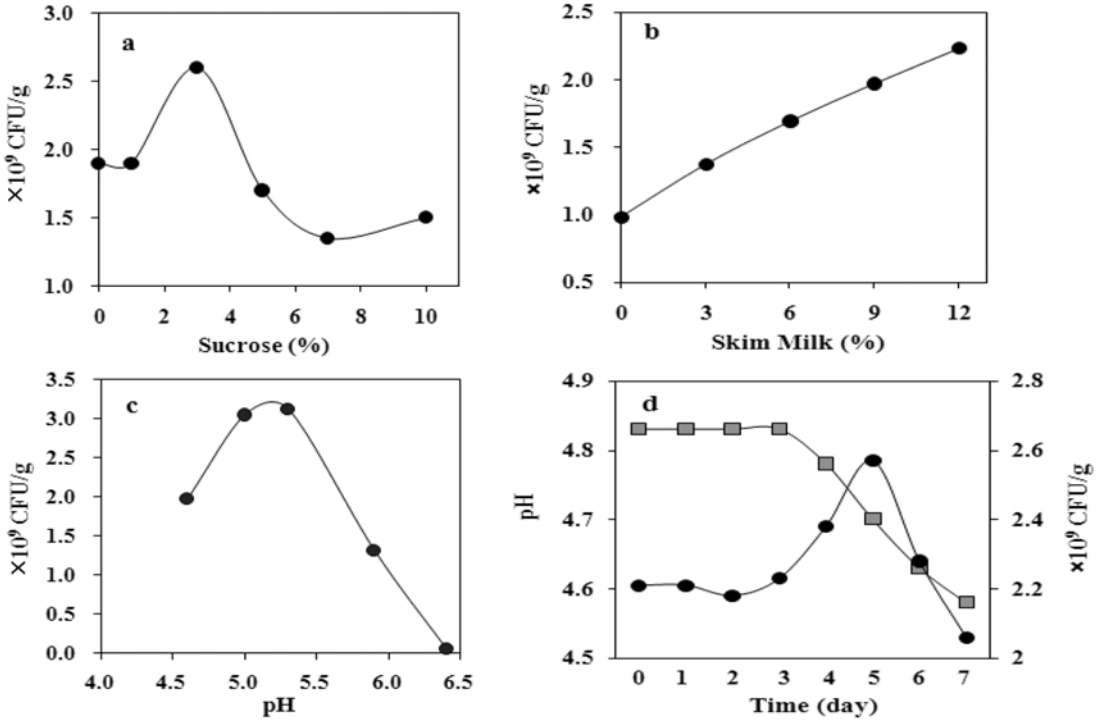

Fig. 1. Bacterial cell number in yogurt. (a) Effect of sucrose on bacterial cell number; (b) Effect of skim milk on bacterial cell number; (c) pH and bacterial cell number profiles during yogurt production; (d) pH and bacterial cell number profiles during the storage of yogurt. ●, Bacterial cell number; ■, pH

and Company, Franklin Lakes, NJ) with H^2 and CO^2 generator and palladium pellet catalyst was used to remove O^2, and the bacterium was grown in the GasPak chamber. The plate count agar medium stained with brom cresol purple showed blue color at neutral pH and yellow color at acidic pH. The bacteria was mixed with the agar medium at 45°C and incubated for 1 day at 37°C. Subsequently, the number of lactic acid bacteria was measured and expressed in CFU. All measurements were the averages of triplicate samples.

Results and Discussion

Yogurt fermentation. Because sucrose is used to improve the sensory quality food [Guinard *et al*., 1994; Trindade *et al*., 2001] and as a nutrient by the lactic acid bacteria, the effect of sucrose on the growth of the lactic acid bacteria was observed. Addition of sucrose (<3%, w/v) before fermentation increased the cell number by about 50%, compared to the control (Fig 1a). However, sucrose >5% (w/v) did not accelerate the bacterial growth (Fig. 1a), but instead prolonged the fermentation period, probably due to the osmotic pressure (data not shown). Recently, the use of skim milk for yogurt fermentation has increased due to its low fat content and improvement of the yogurt quality [Mistry and Hassan, 1992; Park *et al*., 2005]. Addition of the skim milk powder (12%, w/v) resulted in a twofold increase of the lactic acid bacterial cell number (Fig. 1b), and improved the yogurt texture and flavor [Sodini *et al*., 2002]. No significant difference in the cell numbers were observed between the whole milk powder-based and the skim milk powder-based yogurts (data not shown). The lactic acid bacteria were sensitive to pH [Martin and Chou 1992]. The maximum bacterial cell count was obtained at pH 5.0-5.5 (Fig. 1c). The bacterial cell count decreased at pH 5.0 and under. To measure the effect of pH during storage, the yogurt was kept at 4°C for 7 days. After 3 days, the cell number of lactic acid bacteria started to increase and pH started to decrease. The Cell number of lactic acid bacteria started to decrease at pH 4.7 (Fig. 1d). The contents of organic acids in yogurt during the fermentation and cold storage of yogurt continuously changed, and this affected pH of the yogurt during storage [Fernandez-Garcia *et al*., 1994].

To determine the optimal conditions, a factorial design with the combination of the above three factors was set. The effect of sucrose on the bacterial growth was not additive to that of skim milk. Addition of the sucrose factor (3%, w/v) together with the skim milk factor (12%, w/v) did not increase the cell number (Table 1), whereas the sucrose factor alone increased the cell number of the lactic acid bacteria. This result suggested that one of the

Table 1. Effect of yogurt components on bacterial cell number

Skim milk (%)	Sucrose (%)	pH	Bacterial cell number ($\times 10^9$ CFU/g)
6	0	5.15	1.88±0.03
12	0	5.15	2.40±0.08
6	3	5.15	2.00±0.04
12	3	5.15	2.39±0.14
6	0	4.70	1.38±0.15
12	0	4.70	2.05±0.03
6	3	4.70	1.52±0.13
12	3	4.70	1.84±0.07

factors increasing the cell count in the skim milk, was a carbohydrate, lactose. The effect of pH (pH 5.0-5.5 versus <pH 4.7) was additive to those of sucrose and skim milk (Table 1). Termination of the fermentation at pH 5.0-5.5 rather than at full fermentation at <pH 4.7 inhibited the production of lactic acid, which has detrimental effect on the viability of the bacteria. The statistical results obtained by Yate's algorithm [Box *et al.*, 1978] indicated that the important factors increasing the cell number were the skim milk and the pH. The addition of skim milk at 12%, compared to 6%, increased the cell number by 0.48×10^9 CFU/g. In addition, at pH 5.15, the bacterial growth increased to 0.47×10^9 CFU/g.

Because pH affected the growth of the bacteria, phosphate buffer (pH 6.8) was used. The buffer slowed the dropping of pH and increased the CFU/g from 2.21×10^9 to 2.74×10^9 (unpublished data). However, hydrophilic amino acids, including glutamine, glutamate, aspartate, asparagines, and proline, acting as buffering factors, did not increase the cell number (data not shown).

Agitation caused clotting, deteriorated the texture of the yogurt, and delayed the growth of the lactic acid bacteria, probably due to the oxygen produced during agitation (unpublished data). The use of ascorbic acid to protect the cells from the oxygen radicals slightly increased the cell count from 2.26×10^9 to 2.57×10^9 CFU/g (unpublished data). The high amount of inoculum also caused clotting in yogurt, and thus the inoculums were maintained at 0.01% (w/v) of the starter culture.

Frozen yogurt. Because ice crystals negatively affect the bacterial viability, the cell count was performed during the frozen yogurt processing stage. The pH of the yogurt before freezing process was 5.5. Frozen yogurt was produced after fermentation by storing and mixing with detergent and stabilizer, freezing in a freezer (ice cream maker), and stabilization at –36°C for 1 day. The cell numbers of lactic acid bacteria were measured at each process. After fermentation the cell number was $>10^{10}$ CFU and, after storing and mixing, decreased by 4-fold (Fig. 2a).

During the freezing of yogurt, only 1/5 of the lactic acid bacteria survived (Fig. 2a). The cell number decreased from the initial 2.70×10^8 CFU/g to 1.95×10^8 CFU/g during the 150 days storage in a freezer (–18~–12°C) (Fig. 2b). At the steady temperature of –20°C, the cell number decreased very slowly. About 10% of the cells died after 7 weeks (data not shown). According to

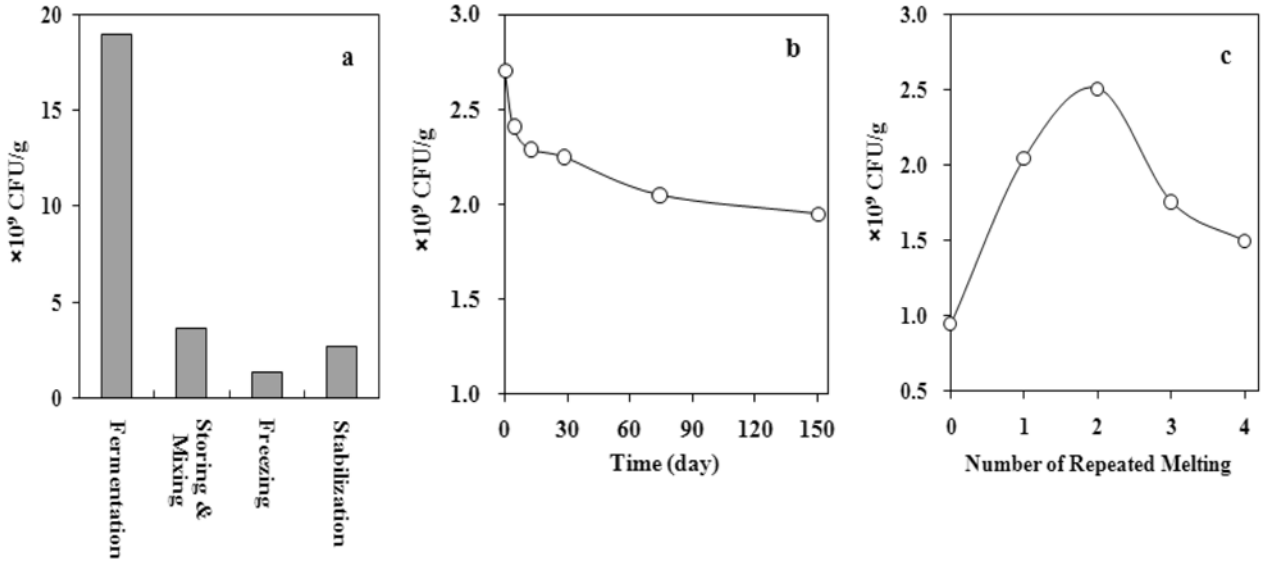

Fig. 2. Bacterial cell number in frozen yogurt. (a) Bacterial cell number during the processing of frozen yogurt; (b) Bacterial cell number during storage of frozen yogurt at –18~–12°C; (c) Bacterial cell number by repeated freezing at –18°C for 1 day and thawing at 30°C for 40 min

Hekmat and McMahon [1992], when the frozen yogurt containing 1.5×10^8 CFU/g of *L. acidophilus* and 2.5×10^8 CFU/g of *B. bifidum* was kept for 17 weeks, the cell numbers were 4×10^6 and 1×10^7 CFU/g, respectively. Different microorganisms showed different resistant to freezing [Davidson *et al*., 2000].

Since the fluctuation of temperature affected the survival rate of the bacteria, a severe condition was simulated. The frozen yogurt (80 g) was frozen at −18°C for 1 day and thawed at 30°C for 40 min. The cell number increased by up to twofold and then decreased (Fig. 2c); during the thawing, the lactic acid bacteria probably grew, because the raw yogurt had not been fully fermented. This result was also supported by the decrease of pH after repeated freezing and thawing. The pH was dropped from 5.5 to 4.9 after the second melting and to 4.5 after the forth melting (data not shown).

References

Akin MB, Akin MS, and Kirmaci Z (2007) Effects of inulin and sugar levels on the viability of yogurt and probiotic bacteria and the physical and sensory characteristics in probiotic ice-cream. *Food Chem* **104**, 93-99.

Almeidaa KE, Tamimeb AY, and Oliveiraa MN (2009) Influence of total solids contents of milk whey on the acidifying profile and viability of various lactic acid bacteria. *LWT-Food Sci Technol* **42**, 672-678.

Babel FJ (1976) Technology of dairy products manufactured with selected microorganisms. In *Dairy technology and engineering*, pp. 213-271, The AVI Publishing Company, Westport, CT, USA.

Box GEP, Hunter WG, and Hunter JS (1978) Statistics for experimenters. pp. 306-351, John Wiley & Sons, New York, NY, USA.

Dave RI and Shah NP (1998) Ingredient supplementation effects on viability of probiotic bacteria in yogurt. *J Dairy Sci* **81**, 2804-2816.

Davidson RH, Duncan SE, Hackney CR, Eigel WN, and Boling JW (2000) Probiotic culture survival and implications in fermented frozen yogurt characteristics. *J Dairy Sci* **83**, 666-673.

Fernandez-Garcia E, Vilaviciosa C, and McGregor JU (1994) Determination of organic acids during the fermentation and cold storage of yogurt. *J Dairy Sci* **77**, 2934-2939.

Guinard JX, Little C, Marty C, and Palchak TR (1994) Effect of sugar and acid on the acceptability of frozen yogurt to a student population. *J Dairy Sci* **77**, 1232-1238

Hekmat S and McMahon DJ (1992) Survival of *Lactobacillus acidophilus* and *Bifido-bacterium bifidum* in ice cream for use as a probiotic food. *J Dairy Sci* **75**, 1415-1422.

Korean Food Industry Organization (1999) Food Codex. pp. 169-171, Moonyoung Co., Seoul, Korea.

Martin JH and Chou KM (1992) Selection of bifidobacteria for use as dietary adjuncts in cultured dairy foods: I-Tolerance to pH of yogurt. *Cult Dairy Prod J* **27**, 23-26.

Mistry VV and Hassan HN (1992) Manufacture of nonfat yogurt from a high milk protein powder. *J Dairy Sci* **75**, 947-957.

Ongol MP, Sawatari Y, Ebina Y, Sone T, Tanaka M, Tomita F, Yokota A, and Asano K (2007) Yoghurt fermented by *Lactobacillus delbrueckii subsp. Bulgaricus* H^+-ATPase-defective mutants exhibits enhanced viability of *Bifidobacterium breve* during storage. *Int J Food Microbiol* **116**, 358-366.

Park DJ, Oh S, Ku KH, Mok C, Kim SH, and Imm JY (2005) Characteristics of yogurt-like products prepared from the combination of skim milk and soymilk containing saccharified-rice solution. *Int J Food Sci Nutr* **56**, 23-34.

Payne JF, Morris AE, and Beers P (1999) Evaluation of selective media for the enumeration of *Bifidobacterium* sp. in milk. *J Appl Microbiol* **86**, 353-358.

Sodini I, Lucas A, Oliveira MN, Remeuf F, and Corrieu G (2002) Effect of milk base and starter culture on acidification, texture, and probiotic cell counts in fermented milk processing. *J Dairy Sci* **85**, 2479-2488.

Sun W and Griffiths MW (2000) Survival of bifidobacteria in yogurt and simulated gastric juice following immobilization in gellan-xanthan beads. *Int J Food Microbiol* **61**, 17-25.

Trindade CS, Terzi SC, Trugo LC, Della Modesta RC, and Couri S (2001) Development and sensory evaluation of soy milk based yoghurt. *Arch Latinoam Nutr* **51**, 100-104.