Advance Journal of Food Science and Technology 7(3): 183-186, 2015
DOI:10.19026/ajfst.7.1290
ISSN: 2042-4868; e-ISSN: 2042-4876

Submitted: August 31, 2014 Accepted: October 11, 2014 Published: February 05, 2015

Research Article

Effect of Total Inoculum Size Containing *Lactobacillus acidophilus* or *Lactobacillus casei* on Fermentation of Goat Milk

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Abstract: In order to obtain the optimum inoculum size of goat milk fermented by probiotics, the total inoculum size containing *L. acidophilus* or *L. casei* on pH, acidity and viable counts and sensory during fermentation were studied on the basis of *S. thermophilus* and *L. bulgaricus* as starter cultures. The results showed as follows: the optimum inoculum size of *L. acidophilus* and *L. casei* were all 7% and goat milk was fermented at 39°C for 4.5 h. The pH, acidity, the viable counts of *L. acidophilus* and the total viable counts were respectively 4.48, 91°T, 1.60×10^7 cfu/mL and 1.69×10^9 cfu/mL. The pH, acidity, the viable counts of *L. casei* and the total viable counts were respectively 4.38, 96°T, 2.80×10^8 cfu/mL and 2.20×10^9 cfu/mL.

Keywords: Goat milk, inoculum size, *L. acidophilus*, *L. casei*, yogurt

INTRODUCTION

Probiotic was defined the microorganism which can promote the ecological balance of host intestinal microbial flora, beneficial effects on host health and physiological function (Golden, 1998). Its physiological functions include adjusting the intestinal flora, improving the metabolism of protein and vitamin, preventing constipation, producing antimicrobial substances, relieving the lactose intolerance, curing liver injury, reducing the incidence of colon cancer, Anti-tumor, enhancing the immune function, lowering cholesterol and treating of urinary system infected, etc (Lina *et al*., 2000; Rial, 2000).

Goat milk is a unique dairy resource, which is known as "the king of milk" in the world (Agnihotri and Prasad, 1993). Its nutritional value is more than milk, performance of protein, fat, vitamins and minerals (Keogh and O'Kennedy, 1998; Haenlein, 2004). Goat yogurt is recognized as the closest to human milk (Rafter, 2003; Saarela *et al*., 2002). And goat milk production of yogurt flavor is unique, organization delicate smooth. But above all, it can adapt to people suffering from cow milk allergies, bronchitis and other gastrointestinal (Betoret *et al*., 2003). At present, yogurt in the market most was fermented by *S. thermophilus* and *L. bulgaricus*, but these two kinds of bacteria can not tolerant of hydrochloric acid in gastric juice and bile and not colonize the intestinal, the survival rate was only 0.065~0.01%, its beneficial effect was limited (Gao, 2004). In recent years, with the improvement of consciousness of people for health care, in order to improve the health benefits of yogurt by add probiotics in yogurt production (Tharmaraj and Shah, 2000).

L. casei is one kind of probiotics, that can tolerate defense mechanism of organism, including oral enzymes, low pH in gastric juice and bile acid in small intestine, etc., so when *L. casei* can in intestine of live, can rise to adjust balance, promote human digestion and absorption and so on (Vinderola *et al*., 2002). *L. acidophilus* is also a kind of important beneficial bacteria; it can regulate intestinal microbial flora balance, enhance immunity, lower cholesterol levels, relieve the lactose intolerance, etc. and plays an important role in human health and normal physiological function (Jack *et al*., 1995; Kashket, 1985).

In our previous study, the process of fermentation set-style type goat yogurts was optimized by *S. thermophilus* and *L. bulgaricus* (Chen *et al*., 2010) and the effect of Inoculum and Temperature on the fermentation of goat yogurt by *Lactobacillus bulgaricus* and *Streptococcus thermophilus* (Shu *et al*., 2014). The purpose of the present work was to study effect of inoculum size on goat milk fermented by *Lactobacillus acidophilus* or *Lactobacillus casei* for further optimizing process parameters of goat yogurt containing probiotics.

MATERIALS AND METHODS

Microorganism *L. acidophilus (LA), L. casei (LC), S. thermophilus* and *L. bulgaricus* were obtained from

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College of Life Science and Engineering, Shaanxi University of Science and Technology. They were inoculated three successive times with MRS (for *L. acidophilus*, *L. casei* and *L. bulgaricus)* and M17 (for *S. thermophilus*) broth (Hopebio, Qindao, China) in order to obtain fresh culture.

Preparation of goat yogurts Goat milk was heated at 90°C for 10 min, cooled to 45°C. And then was divided into two equal portions in order to the single-factor test of total inoculum siz (1, 3, 5, 7, 9%), The mixed liquid starter cultures of different inoculum size were inoculated in the fresh goat milk, *L. casei* and common goat yogurt starter cultures ratio was 1:1 at 39°C constant temperature fermentation. *L. acidophilus* was the same too. The acidity, pH value and viable counts of *L. acidophilus* or *L. casei*, total viable bacteria were determined every other 1.5 h, then given a sensory evaluation after 12 h so as to study the influence of of *L. casei* and *L. acidophilus in total* inoculum size on the fermentation of goat yogurt.

Analysis methods Plate coating method was used to determinate the viable counts. Among then, the viable counts were determinate by modified Tomato Juice medium, determination of *L. acidophilus* and *L. casei* were MRS agar containing 0.06% bile salt (Shu *et al*., 2011). The pH of culture media was directly determined through a pH-meter (pHS-3c) at the room temperature. Acidity was determined by Sodium hydroxide titration and express in Jill Nieer degree (°T). The samples were organoleptically assessed by five panelists, tasted and described the texture of the product: color organizational state, taste, smell, who was trained on the basis of normal sensory acuity and consistency.

RESULTS AND DISCUSSION

Effect of inoculum size on goat milk fermented by *L. acidophilus*: The effect of inoculum size on goat milk fermented by *L. acidophilus* was shown in Fig. 1 and Table 1.

Figure 1a showed *L. acidophilus* viable counts increased slowly in the whole fermentation process when the 1% inoculum size of the goat yogurt, reached the maximum value of 6 h. 3, 5, 7, 9% inoculum size of *L. acidophilus* growth slow at the initial stage of fermentation, but began to accelerate after 3 h, reached

Table 1: The sensory evaluation of LA-yogurt fermented from goat milk with different inoculum size

Total inoculum size (%)	Color	Smell	Taste	State	CE*
1	0.98	1.81	1.68	2.31	6.78
3	0.95	1.83	1.89	2.29	6.95
5	0.93	1.95	1.93	2.25	7.05
7	0.95	2.05	2.08	2.30	7.38
9	0.98	1.93	1.99	2.33	7.21

*: Comprehensive evaluation

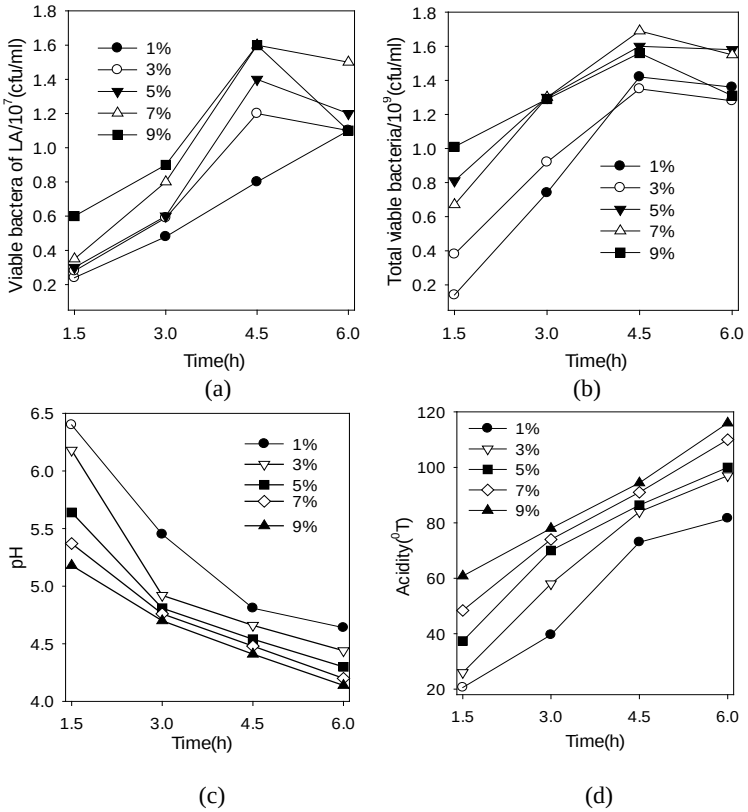

Fig. 1: Effect of total inoculum size on viable counts of *L. acidophilus*, total viable bacteria, pH and acidity in goat yogurt

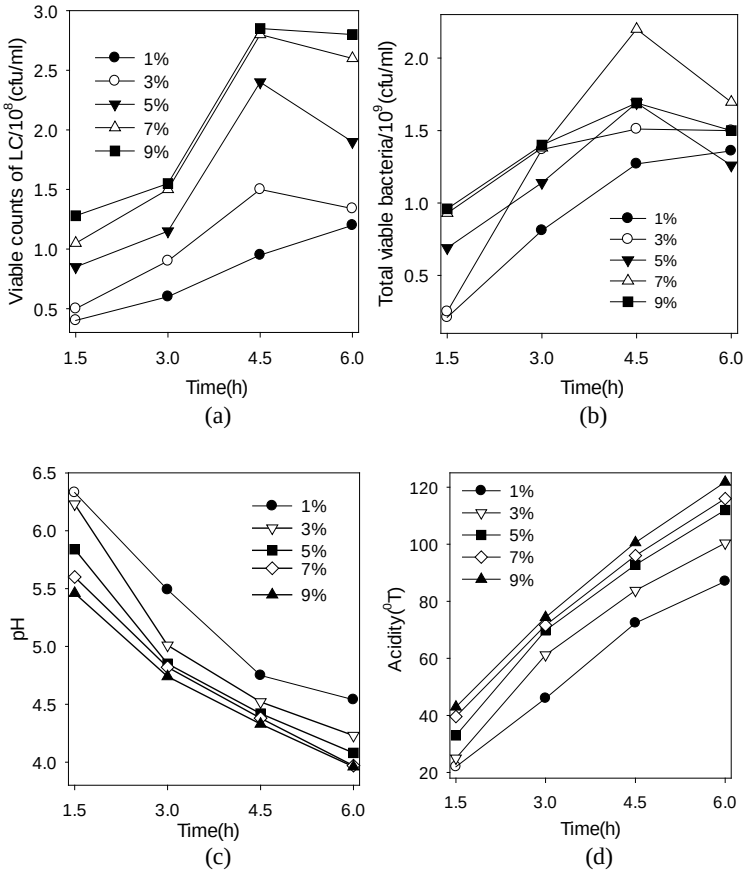

Fig. 2: Effect of total inoculum size on viable counts of *L. casei*, total viable bacteria, pH and acidity in goat yogurt

Table 2: The sensory evaluation of LC-yogurt fermented from goat milk with different inoculum size

Total inoculum size (%)	Color	Smell	Taste	State	CE*
1	0.96	2.21	1.60	2.16	6.94
3	0.96	2.20	1.69	2.19	7.04
5	0.96	2.19	1.85	2.19	7.19
7	0.96	2.24	2.25	2.21	7.66
9	0.96	2.31	1.93	2.19	7.39

*: Comprehensive evaluation

a peak at 4.5 h, then tended to be stable. Among then, the viable counts of 7, 9% inoculum size reached the maximum 1.60×10^7 cfu/mL, the viable counts of 1% inoculum size was the lowest, 1.10×10^7 cfu/mL.

Figure 1b showed each inoculum size of the total viable counts of goat yogurt the fast growth within 4.5 h, reached a maximum at 4.5 h, then tended to be stable. Among them, the total viable counts of 7% inoculum size reached the maximum 1.69×10^9 cfu/mL, the total viable counts of 1% inoculum size reached the minimum 1.35×10^9 cfu/mL. From Fig. 1c and d, 1% inoculum size of goat yogurt pH decreased rapidly within 4.5 h, then began to slow down. 3, 5, 7, 9% inoculum size of goat yogurt decreased rapidly before 3 h and then began to slow down. Each inoculum size of the acidity of goat yogurt showed rise at a constant speed trend in the fermentation process. Among them, the acidity and pH of 7, 9% inoculum size was 91°T, 94.4°T and 4.48, 4.41 for 4.5 h, respectively. The acidity and pH of 1% inoculum size were 73°T, 81.6°T and 4.81, 4.64 for 4 h and 6 h, respectively.

Table 1 showed the total inoculum size had no obvious influence on the color, organization state of goat yogurt. However, had a significant effect on goat yogurt odor and taste. Among them, the sour of goat yogurt taste a little pale and has slight goaty flavor in 1, 3, 5% inoculum size; sweet and sour of goat yogurt had moderate and texture was better in 7% inoculum size; 9% inoculum size of goat yogurt had a little acid.

Effect of inoculum size on goat milk fermented by *L. casei*: The effect of inoculum size on goat milk fermented by *L. casei* was shown in Fig. 2 and Table 2.

Figure 2a showed *L. casei* viable counts increased slowly in the whole fermentation process when the 1% inoculum size of the goat yogurt, reached the maximum value of 6 h. 3, 5, 7, 9% inoculum size of *L. casei* growth slow at the initial stage of fermentation, but began to accelerate after 3 h, reached a peak at 4.5 h, then tended to be stable. Among then, the viable counts of 7, 9% inoculum size reached the maximum 2.80×10^8 cfu/mL and 2.85×10^8 cfu/mL, respectively. The viable counts of 1% inoculum size was the lowest, 1.20×10^8 cfu/mL.

Figure 2b showed each inoculum size of the total viable counts of goat yogurt the rapid growth at the initial stage of fermentation, reached a maximum at 4.5 h, then tended to be stable. Among them, the total viable counts of 7% inoculum size reached the maximum 2.20×10^9 cfu/mL, the total viable counts of 1% inoculum size reached the minimum 1.27×10^9 cfu/mL. From Fig. 2c and d, each inoculum size of the acidity (pH) of goat yogurt in the rapid growth (decrease) trend. Among them, the acidity and pH of 7, 9% inoculum size were respectively 96°T, 100.6°T and 4.38, 4.33 for 4.5 h, the acidity and pH of 1% inoculum size were 72.4°T and 4.75 for 4.5 h.

Table 2 showed the total inoculum size had no obvious influence on the goat yogurt color, smell and texture. However, it had a significant effect on goat yogurt taste. Among them, the sour of goat yogurt tasted a little pale and has slight goaty flavor and texture was soft in 1%, 3% inoculum size; sweet and sour of goat yogurt had moderate and texture was better and quality of a material was uniform fine in 7, 5; but 5% inoculum size of goat yogurt had goaty flavor; 9% inoculum size of goat yogurt had a little acid.

CONCLUSION

Inoculum size of *L. acidophilus* and *L. casei* had a significant effect on pH, acidity, the viable counts and tasty. The optimum temperature on fermentation of *L. acidophilus* and *L. casei* were 39°C for 4.5 h. The pH, acidity, the viable counts of *L. acidophilus* and the total viable counts were 4.48, 91°T, 1.60×10^7 cfu/mL and 1.69×10^9 cfu/mL, respectively. The pH, acidity, the viable counts of *L. casei* and the total viable counts were 4.38, 96°T, 2.80×10^8 cfu/mL and 2.20×10^9 cfu/mL, respectively.

ACKNOWLEDGMENT

The project was partly supported by the science and technology plan project of Xi'an city [No. NC1317 (1)], the Science and Technology Research Development plan project of Shaanxi Province, China (No. 2014K01-17-07) and Science and Technology Bureau of Weiyang District, Xi'an city (No.201208).

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