

Research Article

Effect of Temperature and Inoculum Size on Fermentation of Goat Yogurt Containing *Lactobacillus acidophilus* and *Bifidobacterium bifidum*

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Abstract: The objective of this study was to investigate the effect of Incubation temperature (35, 37, 39, 41 and 43°C, respectively) and inoculum size (1, 3, 5, 7 and 9%, respectively) on the acidity, pH and viable counts in fermented goat milk containing *L. acidophilus* and *Bifidobacterium bifidum* by single factor test, respectively. The results showed that the optimum incubation temperature was 37°C, the acidity, pH, the viable counts of *Bifidobacterium bifidum*, *L. acidophilus* and the total viable counts were 81.8°T, 4.55, 3.60×10^7 , 3.8×10^7 and 1.67×10^9 cfu/mL, respectively. The optimum inoculum size was 5%, the acidity, pH, the viable counts of *Bifidobacterium bifidum*, *L. acidophilus* and the total viable counts were 98.2°T, 4.51, 5.2×10^7 , 7.7×10^7 and 1.89×10^9 cfu/mL, respectively.

Keywords: *Bifidobacterium bifidum*, goat milk, incubation temperature, inoculum size, *L. acidophilus*

INTRODUCTION

Probiotics have been defined as live microorganisms which when administered in adequate amounts confer a health benefit on the host (FAO/WHO, 2006). *Bifidobacteria* and *Lactobacilli*, which can be considered the most common probiotics (Farnworth *et al.*, 2007; Lourens-Hattingh and Viljoen, 2001), are associated with maintaining optimum microbial balance in the digestive tract with a number of well documented health benefits, including enhancement of the immune system (Lourens-Hattingh and Viljoen, 2001), reduction of lactose intolerance (Kim and Gilliland, 1983) reduction of serum cholesterol level and possible anti carcinogenic properties (Rasic, 2003). Thus, probiotics have been extensively incorporated into dairy foods over the last decade and yogurts containing *Lactobacillus acidophilus* and/or *Bifidobacteria* species are widely marketed (Shah, 2000).

L. acidophilus is a kind of important beneficial bacteria; it can regulate intestinal flora balance, enhance immunity, lower cholesterol, relieve the lactose intolerance etc and plays an important role in human health and normal physiological function (Jack *et al.*, 1995; Kashket, 1985). *Bifidobacteria* can reduce the incidence of colon cancer, anti-tumor, enhance the

immune function etc., it is also a beneficial probiotic for human.

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), the effect of inoculum and temperature on the fermentation of goat yogurt by *L. bulgaricus* and *S. thermophilus* (Shu *et al.*, 2014) was investigated, the effect of the total inoculum size containing *L. acidophilus* or *L. casei* on the fermentation of goat milk was studied on the basis of *S. thermophilus* and *L. bulgaricus* as starter cultures (Chen *et al.*, 2015). The purpose of this study was to study the effect of incubation temperature and inoculum size on the goat yogurt fermented by *L. acidophilus* and *Bifidobacterium* on the basis of *S. thermophilus* and *L. bulgaricus* as common starter cultures for developing AB-goat yogurt.

MATERIALS AND METHODS

Microorganism: *L. acidophilus*, *Bifidobacterium bifidum*, *S. thermophilus* and *L. bulgaricus* were provided from School of Food and Biological Engineering, Shaanxi university of science and technology, they inoculated three successive times with MRS (for *L. acidophilus* and *L. bulgaricus*), MRS with

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0.5% Cysteine Hydrochloride (for *Bifidobacterium bifidum*) and M17 (for *S. thermophilus*) to obtain fresh culture.

Fermentation process of goat yogurt: Fresh goat milk → filtration → physical and chemical properties → heating and mixing → packing → sterilization (90~95°C, 10 min) → cooling → inoculation → fermentation → refrigeration and post acidification → product.

Analysis method: Plate coating method was used to determine the viable counts. the total viable counts were determinate by modified Tomato Juice medium, determination of *L. acidophilus* by MRS agar containing 0.06% bile salt and determination of *B. bifidum* by MRS agar containing 0.10% LiCl (Chen *et al.*, 2011; Shu *et al.*, 2011). The process was as follows: the agar medium packed in 250 mL flask was sterilized and 15-20 mL poured onto the plates in a clean bench after cooling to 60°C, 0.1 mL aliquot

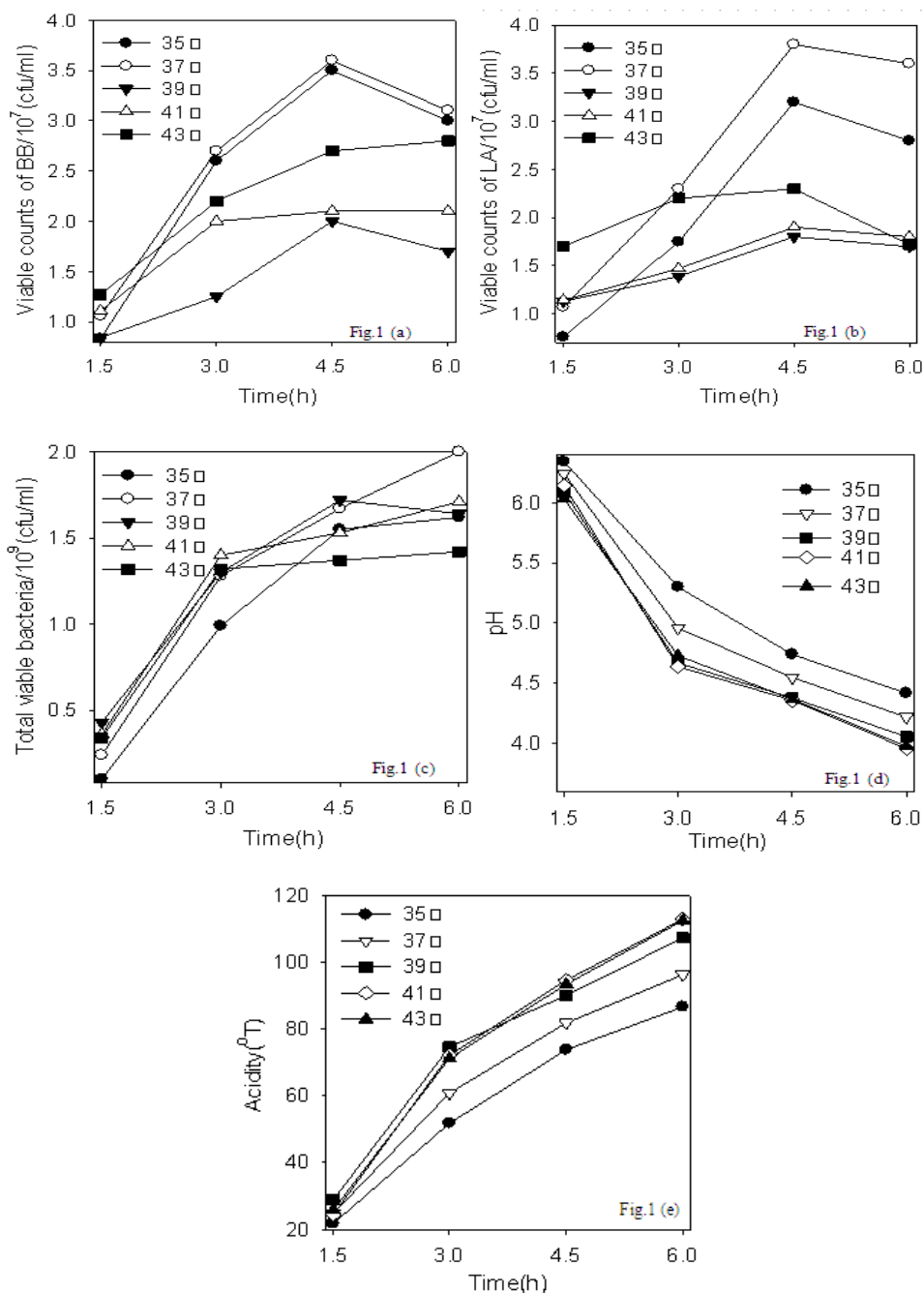


Fig. 1: Effect of bacteria proportion on viable counts of *B. bifidum*, *L. acidophilus*, total viable bacteria, pH and acidity in AB-goat yogurt

Table 1: The sensory evaluation of AB-goat yogurt under different temperature

Temperature (°C)	Color	Smell	Taste	State	CE*
35	0.99	2.00	1.70	2.49	7.18
37	0.99	2.11	1.98	2.52	7.60
39	0.99	2.18	2.14	2.48	7.79
41	0.99	2.20	2.28	2.52	7.99
43	0.99	2.10	2.24	2.48	7.81

*: Comprehensive evaluation

bacteria dilutions were coated on it after coagulation and then inoculated anaerobic 2-3 day at 37°C, plates containing 30-300 cfu/mL colonies were counted and the results expressed as colony-forming units per mL of sample.

The variation of pH was evaluated using a pH-meter (pHS-3c) at room temperature. Acidity of culture media was determined by sodium hydroxide titration and expressed by Jill Nieer degrees (°T). The sensory evaluation of samples including color, smell, taste, texture were organoleptically assessed by five panelists, who was trained on the basis of normal sensory acuity and consistency.

RESULTS AND DISCUSSION

Effect of temperature on the goat milk fermented by *B. bifidum*, *L. acidophilus* and common starter cultures:

First, goat milk was heated at 90°C, cooled to 45°C after 10 min, then the starter culture of 7% inoculum size was inoculated. The ratio of *B. bifidum*, *L. acidophilus* and common starter cultures was 4:1:1. Incubation temperature was 35, 37, 39, 41 and 43°C, respectively. The acidity, pH value, viable counts of *L. acidophilus* and *Bifidobacterium bifidum*, total viable bacteria were determined every other 1.5 h, then gave a sensory evaluation after 12 h. The result was shown in Fig. 1 and Table 1.

Figure 1a showed viable counts of *Bifidobacterium bifidum* increased rapidly within 3 h at 35 and 37°C, then increased slowly, reached a peak at 4.5 h, showed a gradual decrease after 4.5 h. The viable counts of *B. bifidum* grew slow within 4.5 h at 39 and 43°C and then tended to be stable. The viable counts of *B. bifidum* at 41°C grew slow at the initial stage of fermentation, reached the maximum value at 3 h and then tended to be stable. Among then, the viable counts of *B. bifidum* at 37°C reached the maximum, 3.60×10^7 cfu/mL, the viable counts of *B. bifidum* at 41°C was the lowest, 2.00×10^7 cfu/mL.

Figure 1b showed the variation of *L. acidophilus* viable counts. It had a fast growth within 4.5 h at 35°C and 37°C, reached a maximum at 4.5 h, the viable counts increased slowly in the whole fermentation process at 39, 41 and 43°C, respectively then reached a peak at 4.5 h. It can be suggested that the viable counts of 37°C reached the maximum, 3.80×10^7 cfu/mL, the viable counts of 39°C was the lowest, 1.80×10^7 cfu/mL.

Based on the Fig. 1c, it can be observed that each temperature of the total viable counts of goat yogurt had a fast growth within 3 h. The total viable counts presented a slow increase after 3 h at 37, 39 and 41°C,

respectively and then reached the maximum at 4.5 h, while the total viable counts tended to be stable after 3 h at 35 and 43°C. With the temperature of 39°C, the total viable counts presented the highest number, 1.72×10^9 cfu/mL, followed by 37°C, 1.67×10^9 cfu/mL. When the fermentation temperature was 43°C, the total viable counts presented the lowest counting, 1.32×10^9 cfu/mL.

Figure 1d and e showed each temperature of acidity of goat yogurt increased rapidly within 3 h, then began to slow down. The pH of goat yogurt decreased rapidly within 3 h and then began to slow down. The acidity and pH of 37°C were 81.8°T and 4.55 at 4.5 h. It can be observed that the optimum temperature on fermentation of *L. acidophilus* and *B. bifidum* was 37°C.

Table 1 presents the sensory evaluation of AB-goat yogurt; it can be observed that the incubation temperature had no obvious influence on the color and texture of goat yogurt. The score of smell and taste is rowing with the increase of the temperature before 41°C, after then, the score decreased with the increase of the temperature. Among then, the goat yogurt tasted a little sweet, no sour and had slight goaty flavor at 35°C. The sour and sweet of goat yogurt were moderate and had a good coagulation at 37, 39, 41 and 43°C, respectively but there is a little goaty flavor at 37°C.

Effect of inoculum size on the goat milk fermented by *B. bifidum*, *L. acidophilus* and common starter cultures:

Goat milk was sterilized at 90°C for 10 min, cooled to 45°C, then the mixed liquid starter cultures of different inoculum size (1, 3, 5, 7 and 9%, respectively) were inoculated in the fresh goat milk, the ratio of *B. bifidum*, *L. acidophilus* and common starter cultures was 2:1:1, then gave a constant temperature fermentation at 39°C. The acidity, pH value, viable counts of *L. acidophilus* and *B. bifidum*, total viable bacteria were determined every other 1.5 h and then there was a sensory evaluation after 12 h. the result was shown in Fig. 2 and Table 2.

Figure 2a showed the viable counts of *B. bifidum* in goat yogurt had an upward trend within 4.5 h, reached a maximum at 4.5 h, then tended to be stable under different inoculum size. Among then, the viable counts of *B. bifidum* at 5% inoculum size reached the maximum, 5.20×10^7 cfu/mL, while the viable counts of *B. bifidum* at 1% inoculum size was the lowest, 2.30×10^7 cfu/mL.

From Fig. 2b, the viable counts of *L. acidophilus* increased slowly in the whole fermentation process at the 1% inoculum size. Viable counts of *L. acidophilus* of 3 and 5% increased fast within 4.5 h then began to decrease. The viable counts of *L. acidophilus* at 7 and

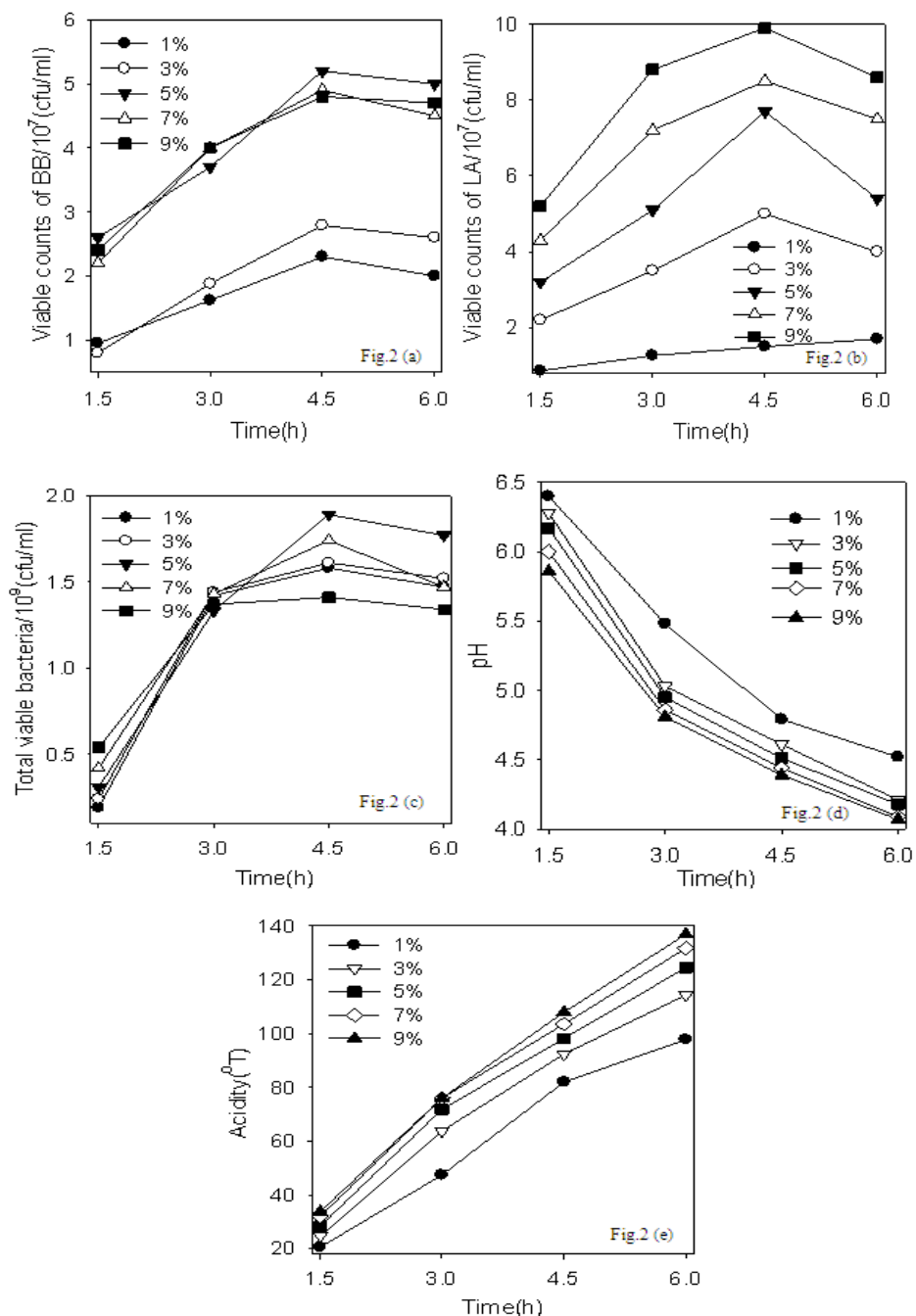


Fig. 2: Effect of inoculum size on viable counts of *B. bifidum* and *L. acidophilus*, total viable bacteria, pH and acidity in AB-goat yogurt

9% inoculum size had a rapidly growth at the initial stage of fermentation, but began to slow down after 3 h, reached the maximum at 4.5 h. Among then, the viable counts of *L. acidophilus* at 9% inoculum size presented the highest number, 9.90×10^7 cfu/mL, followed by 7 and 5% inoculum size, they were 8.50×10^7 and 7.70×10^7 cfu/mL, respectively.

Figure 2c showed the total viable counts at 1, 3 and 9%, respectively inoculum size in goat yogurt had a rapid growth at the beginning of fermentation, then

tended to be stable after 3 h, the total viable counts of 5 and 7% inoculum size increased fast at first, then started to slow down, reached the maximum at 4.5 h. Among then, the total viable counts at 5% inoculum size presented the highest number, 1.89×10^9 cfu/mL, the total viable counts at 9% inoculum size was the minimum, 1.37×10^9 cfu/mL.

From Fig. 2d and e, the acidity of goat yogurt of each inoculum size had a dramatically increased trend in the process of fermentation, while the pH had a

Table 2: The sensory evaluation of AB-goat yogurt under different inoculum size

Inoculum size (%)	Color	Smell	Taste	State	CE*
1	0.99	2.13	1.64	2.09	6.84
3	0.99	2.15	1.86	2.23	7.23
5	0.99	2.24	1.99	2.26	7.48
7	0.99	2.25	1.79	2.18	7.20
9	0.99	2.31	1.75	2.15	7.20

*: Comprehensive evaluation

decreased trend. Among then, the acidity and pH of 5, 7 and 9%, were 98.2, 103.6 and 108^oT, 4.51, 4.44 and 4.39, respectively.

Table 2 presented the sensory evaluation of AB-goat yogurt. It can be suggested that the sour of goat yogurt tasted a little pale and had slight goaty flavor in 1% inoculum size, the sweet and sour of goat yogurt were moderate and there was not goaty flavor in 3, 5% inoculum size. Seven, 9% inoculum size of goat yogurt had a little acid and slight astringent. Over all, the proper inoculum size of AB-goat yogurt was 5%, the time of coagulation was 4.5 h.

CONCLUSION

Based on the experiments, the incubation temperature and inoculation had significant effects on acidity, pH value, viable counts of *L. Acidophilus*, *B. bifidum* and the total viable counts in the AB-goat yogurt. It can be suggested that the optimum temperature on fermentation of *L. acidophilus* and *B. bifidum* was 37^oC. The acidity, pH, the viable counts of *L. acidophilus* and *B. bifidum* and the total viable counts were 81.8^oT, 4.45, 3.8×10⁷, 3.60×10⁷ and 1.67×10⁹ cfu/mL respectively, the score of sensory evaluation was 7.36. The optimum inoculum size of AB-goat yogurt was 5%, the acidity, pH, viable counts of *B. bifidum*, *L. acidophilus* viable counts and total viable counts were 98.2^oT, 4.51, 5.2×10⁷ and 1.89×10⁹ cfu/mL, respectively and the score of sensory evaluation was 7.91.

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