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EFFECT OF TEMPERATURE, BACTERIAL PROPORTION AND INOCULUM SIZE ON THE FERMENTATION OF GOAT YOGURT WITH *BIFIDOBACTRIUM BIFIDUM*

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ABSTRACT

The effect of incubation temperature, bacterial proportion and inoculum size on goat milk fermented by probiotic culture containing *Bifidobactrium bifidum* was investigated by measuring the acidity, pH value, and viable counts during fermentation. Incubation temperature was 35℃, 37℃, 39℃, 41℃and 43℃, the ratio of *B. bifidum* to common starter cultures was 1:2, 1:1, 2:1, 3:1, 4:1, the inoculum size was 1%, 3%, 5%, 7% and 9%, respectively. The results showed that incubation temperature, bacterial proportion and inoculum size had significant impact on fermentation of goat milk. The optimum temperature was 39℃, the acidity, pH, the viable counts of *B. bifidum* and the total viable counts were 98.4^{0}T, 4.03, 7.60×10^{7}cfu/ml and 1.40×10^{9}cfu/ml, respectively. The optimum bacterial proportion of *B. bifidum* to common starter cultures was 4:1, the acidity, pH, the viable counts of *B. bifidum* and the total viable counts were 95^{0}T, 4.3, 7.40×10^{7}cfu/ml and 2.30×10^{9}cfu/ml respectively. The optimum inoculum size was 5%, the acidity, pH, the viable counts of *B. bifidum* and the total viable counts were 86^{0}T, 4.6, 1.61×10^{8}cfu/ml and 1.32×10^{9}cfu/ml respectively.

1. Introduction

Goat milk contains various nutrients needed by human body. Because of its peculiar taste and nutritional properties and its recognition as a healthy food, goat milk has received special attention by researchers and dairy industry. Some properties of goat's milk are known to be advantageous compared with those of cow's milk, such as higher tolerance by allergic children, which is related to the amount of and structural differences in whey proteins (*α-lactalbumin* and *β-lactalbumin*) and the high proportion of small fat globules (1.5 mm), which provide better digestibility (Albenzio and Santillo, 2011; Haenlein, 2004; Raynal-Ljutovac et al. 2005; Sheehan et al. 2009).In addition, the clotting behavior of goat milk was also different from cow milk. (Alloggio et al., 2000). In term of causing allergy, goat milk has been reported to have less allergenicity than cow milk (Sanz Ceballos et al., 2009).

Dairy foods are the main types of food matrices supplemented with probiotic bacteria and they have a positive reputation among consumers (Granato et al., 2010). Among the dairy products, yogurt/fermented milks have been the subject of several studies all over the world and different benefits for human health have been reported after their ingestion (Wang et al., 2012). According to FAO/WHO (2002), probiotics are live microorganisms which,

when administered in adequate amounts, confer health benefits on the host. Available evidence indicates that ingestion of probiotic bacteria may reduce the severity and frequency of diarrheal diseases and improve lactose digestibility among lactose-intolerant individuals (Mattila-Sandholm et al., 2002). Yogurts containing probiotics are claimed to provide several health benefits such as improve lactose utilization (De Vrese et al., 2001), prevent cancer (Rafter, 2003), maintain intestinal microflora balance (Mainville et al., 2005) and reduce serum cholesterol level (Baroutkoub et al., 2010). These therapeutic effects are the reason for which most probiotics are used in yogurts, fermented milks, ice creams and nutraceutical products (Cruz et al., 2010; De Oliveira and Jurkiewicz, 2009). Bifidobacteria are natural habitants of human gastrointestinal tract and can exert several beneficial effects to the host (Julio et al.,2010). They have been incorporated into a variety of food products,mainly daily, such as fermented milk and yogurts (Sanchez,de los et al.,2009)

In our previous study, the process of fermentation set-style 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 he 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, bacterial proportion and inoculum size on the goat yogurt fermented by *B. bifidum* on the basis of *S. thermophilus* and *L. bulgaricus* as common starter cultures.

2. Materials and methods

Raw materials and reagents Fresh goat milk was purchased from local farmers (Xi'an Weiyang, China)，All chemicals used were of analytical grade unless otherwise specified.

Microorganism, *S. thermophilus, L. Bulgaricus and Bifidobactrium bifidum(BB)* were provided by School of Food and Biological Engineering, shaanxi university of science and technology, they were inoculated three successive times with rehydrated de Mann Rogosa Sharpe (MRS) broth (Haibo media, Qindao, China) for *L. bulgaricus* , MRS broth with 0.5% Cys-HCl for *B. bifidum* and M17 broth (Haibo media, Qindao, China) for *S. thermophilus* to obtain fresh culture. The activated, *B. bifidum* were inoculated respectively into sterilized goat milk at 5% inoculum size, mixed and cultivated at 42°C for *S. thermophilus and L. bulgaricus* (37°C for *B. bifidum*) until coagulation. They would be used for the production of goat yogurt containing *B. bifidum*.

Fermentation process of goat yogurt The fresh goat milk was pasteurized at 95℃ for 10 minutes, cooled, then goat milk was fermented with different temperature, different ratio of *B. bifidum* and common starter cultures or inoculated different inoculum size, The acidity, pH value, viable counts of *B. bifidum* and total viable bacteria were determined every other 1.5h, then gave a sensory evaluation after 12h.

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 *B. bifidum* by MRS agar containing 0.10% LiCl (Chen et al, 2011). The process was as follows: the agar medium packed in 250ml flask was sterilized, and15-20ml poured onto the plates in a clean bench after cooling to 50℃, 0.1ml aliquot bacteria dilutions were coated on it after coagulation, and then inoculated anaerobic 2-3d at 37℃, plates containing 30-300cfu/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 BB-goat milk was determined by sodium hydroxide titration and expressed in Jill Nieer degrees (^{0}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.

3. Results and discussions

Effect of temperature on the fermentation of BB-goat yogurt

The starter culture of 5% inoculum size was inoculated in the goat yogurt; the ratio of *B. bifidum* to common starter cultures was 1:1. Inocubation temperature was 35℃, 37℃, 39℃, 41℃ and 43℃ respectively. The results were shown in Figure1 and table 1.

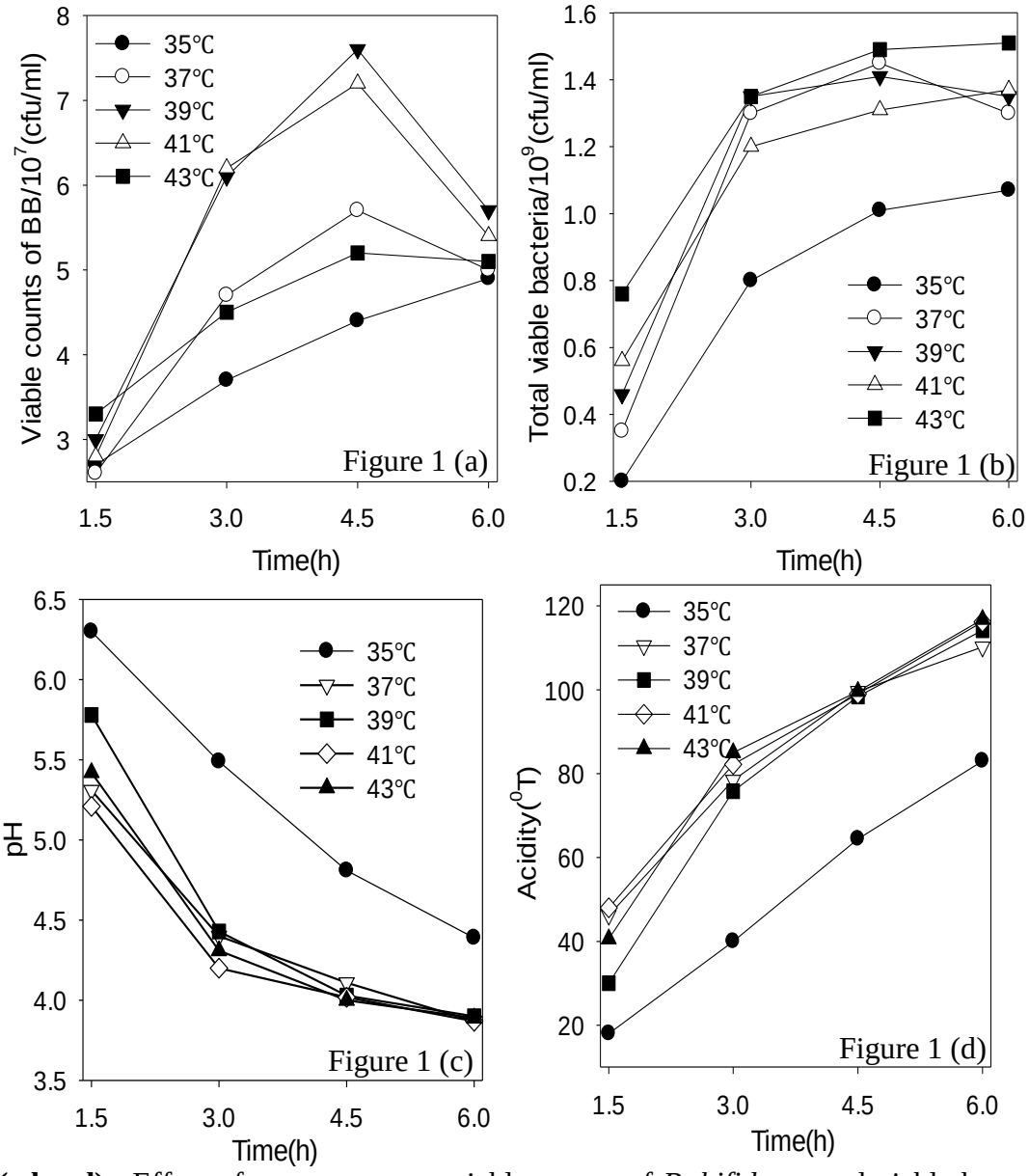

Figure 1(a,b,c,d). Effect of temperature on viable counts of *B. bifidum*, total viable bacteria, pH and acidity in BB-goat yogurt

Figure 1 (a) showed the viable counts of *B. bifidum* increased slowly at 35℃, 37℃and 43℃, but when the fermentation temperature was 39℃ and 43 ℃, the viable counts of *B. bifidum* increased rapidly within 4.5h,then presented a gradual decrease, that is because the acidity resistance of *B. bifidum* is not very well. Among then, the viable counts of *B. bifidum* at 39℃

reached the maximum, 7.60×10^7cfu/ml, the viable counts of *B. bifidum* at 35℃ was the lowest,4.40×10^7cfu/ml.

From figure 1 (b), each temperature of the total viable counts of goat yogurt had a fast growth within 3h, and increased slowly during 3-4.5h, then tended to be stable. Among then, the total viable counts of goat yogurt at 39℃, 41℃ and 43 ℃ were higher then that at other temperatures, the total viable counts of goat yogurt for 4.5h at 39 ℃, 41 ℃, 43 ℃ were 1.40×10^9cfu/ml,1.45×10^9cfu/ml and 1.49×10^9 cfu/ml respectively, that is because the common strains of goat yogurt was in the highest flight, the total viable counts of goat yogurt at 35℃ was the lowest, 1.01×10^9cfu/ml. From figure1(c) and 1(d), each temperature of the acidity and the total viable counts were basically same, that is because the *Lactobacillus bulgaricus* and *Lactobacillus acidophilus* were important acid producing bacteria. Among then, the acidity and pH value of the goat yogurt at 39℃, 41℃ and 43 ℃ for 4.5h were 98.4^0T, 4.03, 99^0T, 4.02, 99.6^0T and 4.00 respectively, the acidity and pH value of goat yogurt at 35℃ for 4.5h were 64.4^0T and 4.81.

Table 1. Sensory evaluation of BB-goat yogurt under different incubation temperature

Temperature (℃)	Color	Smell	Taste	State	CE*
35	0.98	2.15	1.53	2.11	6.77
37	0.98	1.92	2.02	2.20	7.12
39	0.99	2.19	1.98	2.20	7.36
41	0.99	2.22	2.00	2.14	7.35
43	0.99	2.28	2.13	2.23	7.63

*: Comprehensive evaluation

From table 1, when the incubation temperature was 35℃-37℃ ,the temperature was too low and the coagulation time was long, the sour of goat yogurt was slight and can not cover the goaty flavor, while the sour and sweet of goat yogurt at 39℃-41℃ were moderate and the state was well.

Effect of bacterial proportion on the fermentation of BB-goat yogurt

The inoculum size of starter culture was 5%, the ratio of *B. bifidum* to common starter cultures were 1:2, 1:1, 2:1, 3:1 and 4:1, then gave a constant temperature fermentation at 39℃, the results were shown in figure 2 and table 2.

Based on the figure 2 (a), the viable counts of *B. bifidum* at the ratio of 1:2 increased slowly at the whole fermentation process, maybe because much acid and H_2O_2 produced by *L. bulgaricus* at the common starter culture were virulent to *B. bifidum*. At the ratio of 1:1, 2:1 and 3:1,the viable counts of *B. bifidum* increased slowly at the first 3h, and came to accelerate from 3h to 4.5h,then began to decreased. At the ratio of 4:1, the viable counts of *B. bifidum* increased fast at the initial stage of fermentation, reached the peak at 4.5h, then tended to be stable. Among then, the viable counts of *B. bifidum* at the ratio of 4:1 was the highest, 7.4×10^7cfu/ml, while at the ratio of 1:2,the viable counts of *B. bifidum* was the lowest, 4×10^7cfu/ml.

Figure 2(b) presented the variation tendency of the total viable counts at the different bacterial proportion were almost same, they were all increased rapidly at the first 4.5h,then started to decreased, among then, the total viable counts at the ratio of 4:1 reached the highest, 2.30×10^9cfu/ml, followed by the ratio of 1:2, 1:1, they were 1.99×10^9cfu/ml and 1.92×10^9cfu/ml, respectively, the relatively low total viable counts were presented at the ratio of 2:1 and 3:1, which were 1.74×10^9cfu/ml and 1.52×10^9cfu/ml respectively.

From figure2(c)and 2(d), the variation tendency of acidity and the total viable counts seemed to be same, among then, the acidity and pH value at the ratio of 4:1 for 4.5h were 95^0T and 4.3. when the ratio was 1:2 and 1:1,the acidity and pH for 4.5h were 94.6^0T, 4.26, 94.4^0T, 4.28, respectively, while the acidity and pH value at the ratio of 2:1 and 3:1 for 4.5h were 103.3^0T, 4.3, 96^0T and 4.28 respectively.

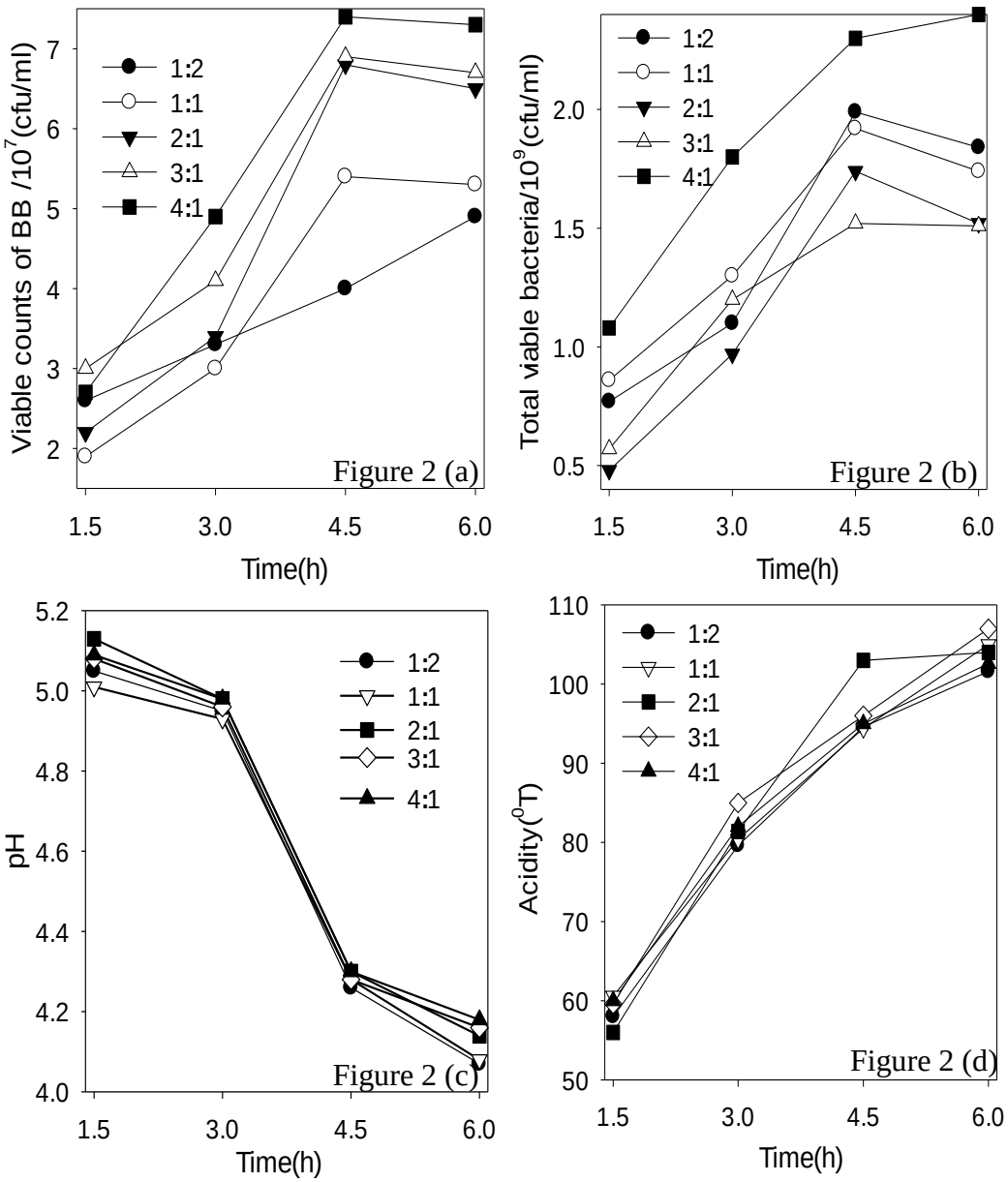

Figure 2(a,b,c,d). Effect of bacterial proportion on viable counts of *B. bifidum*, total viable bacteria, pH and acidity in BB-goat yogurt

Table 2. Sensory evaluation of BB-goat yogurt with different bacteria ratio

Inoculum size (%)	Color	Smell	Taste	State	CE*
1:2	0.98	2.28	1.80	2.33	7.38
1:1	0.98	2.28	1.98	2.35	7.59
2:1	0.98	2.30	2.17	2.29	7.73
3:1	0.98	2.23	2.16	2.31	7.67
4:1	0.98	2.32	2.16	2.32	7.75

*: Comprehensive evaluation

Table 2 presented the sensory evaluation of BB-goat yogurt, it can be suggested that each ratio had no obvious influence on the color, smell and the state of goat yogurt, but had a significant influence on the taste of goat yogurt, among then, the goat yogurt tasted sour at the ratio of 1:2, when the ratio was 1:1, 2:1, 3:1 and 4:1, the sour and sweet of goat yogurt were moderate and there was no goaty flavor.

Effect of inoculum size on the fermentation of BB-goat yogurt

The mixed liquid starter cultures of different inoculum size (1%, 3%, 5%, 7% and

9%) were inoculated in the fresh goat milk, the ratio of *B. bifidum* and common starter cultures was 4:1, and then gave constant temperature fermentation at 39℃, The results were shown in figure 3 and table 3.

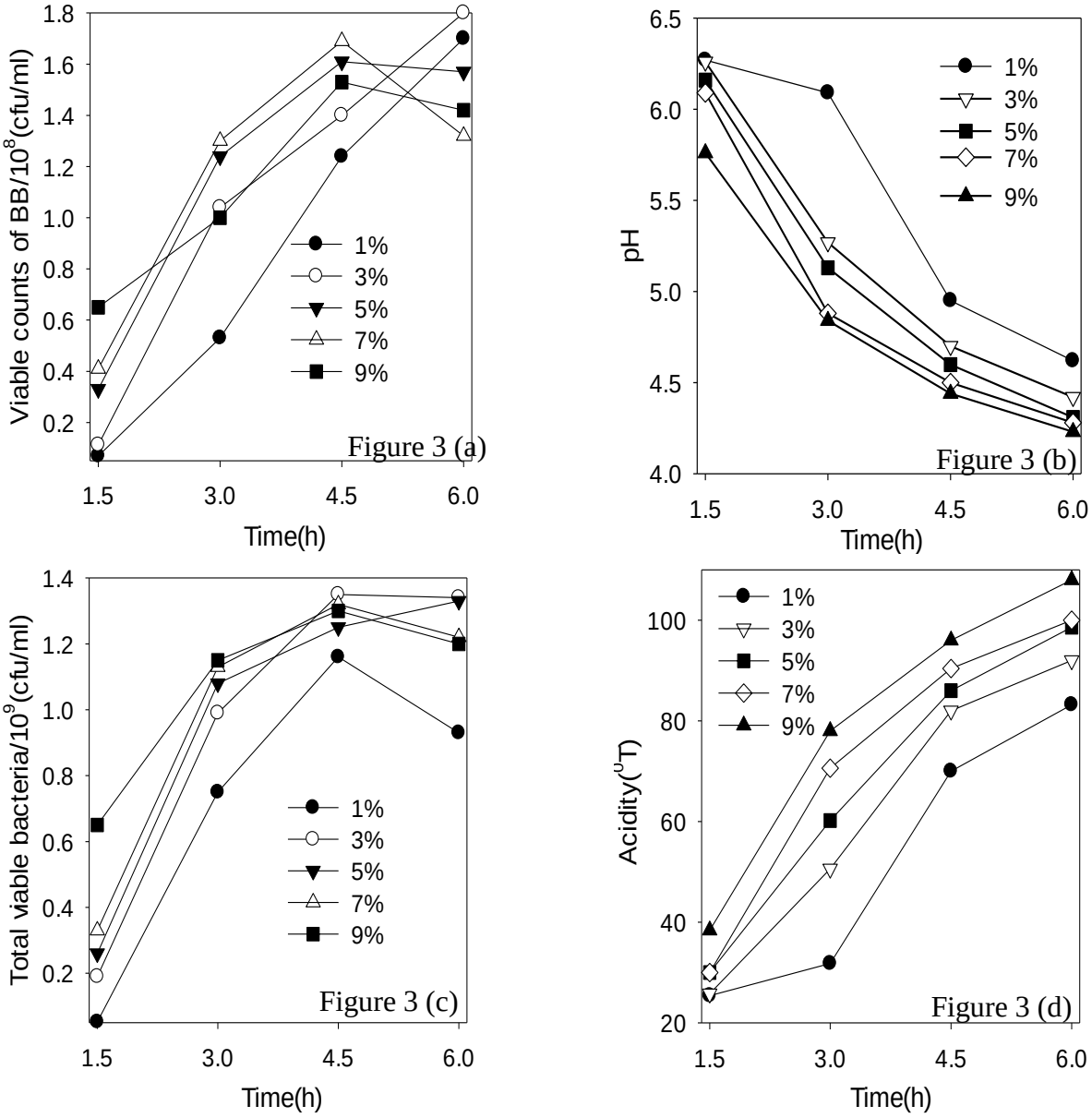

Figure 3 (a,b,c,d). Effect of inoculum size on viable counts of *B. bifidum*, total viable bacteria, pH and acidity in BB-goat yogurt

Figure 3(a) showed the viable counts of *B. bifidum* had an upward trend at the ratio of 1% and 3% at the whole fermentation progress, reached the maximum at 6h,when the inoculum size was 5%, 7% and 9%,the viable counts of *B. bifidum* increased fast at the initial stage of the fermentation progress, reached the peak at 4.5h,among then, the viable counts of *B. bifidum* at the ratio of 7% reached the highest, 1.69×10^8cfu/ml, followed by 5%, 1.61×10^8 cfu/ml, when the ratio was 1% and 9%, the viable counts of *B. bifidum* were low, 1.55×10^8cfu/ml, 1.53×10^8cfu/ml.

From figure 3(b), each inoculum size of the total viable counts increased fast within 3h, then began to slowed down from 3h to 4.5h, reached the peak at 4.5h, then tended to be stable. Among then, the total viable counts of goat yogurt at 7%

inoculum size was the highest, 1.35×10^9cfu/ml, followed by 5%, 1.32×10^9cfu/ml, while the total viable counts of goat yogurt at 1% was the lowest, 9.30×10^8cfu/ml.

From figure 3(c) and 3(d), the acidity of goat yogurt at 1% inoculum size had no obvious change within 3h, increased slowly during 3h-4.5h,then tended to be stable, the acidity at 3%, 5%, 7% and 9% had the same variation trend as the total viable counts at the same inoculum size. Among then, the acidity and pH value at 5% and 7% inoculum size for 4.5h were 86^0T, 4.6, 90.4^0T, and 4.5, respectively, while the acidity and pH value at 1% inoculum size for 6h were 70^0T and 4.62.

Table 3. Sensory evaluation of BB-goat yogurt with different inoculum size

Inoculum size (%)	Color	Smell	Taste	State	CE*
1:2	0.98	2.28	1.80	2.33	7.38
1:1	0.98	2.28	1.98	2.35	7.59
2:1	0.98	2.30	2.17	2.29	7.73
3:1	0.98	2.23	2.16	2.31	7.67
4:1	0.98	2.32	2.16	2.32	7.75

*: Comprehensive evaluation

From table 3, the inoculum size had no obvious influence on the color and state of goat yogurt, but had significant influence on the taste and smell of goat yogurt, among then, the goat yogurt at 1% and 3% inoculum size tasted not sour, and had slight goaty flavor and a little bit astringent and the coagulation was soft, the goat yogurt at 7% and 9% inoculum size was sour and had slight goaty flavor, when the inoculum size was 5%, the sour and sweet of goat yogurt was moderate and had no goaty flavor.

4. Conclusions

Based on the experiments, the temperature, Ratio of *B. bifidum* to common starter cultures, inoculum size had significant influence on the goat yogurt fermented by *B. bifidum*. The optimum temperature for BB-goat yogurt was 39℃, the acidity, pH value, the viable counts of *B. bifidum* and the total viable counts were 98.4^0T, 4.03, 7.60×10^7cfu/ml and 1.40×10^9 cfu/ml respectively and the score of sensory evaluation was 7.36.The optimum ratio of *B. bifidum* to common starter cultures was 4:1, the acidity, pH, viable count of *B. bifidum* and the total viable counts were 95^0T, 4.3, 7.40×10^7 cfu/ml and 2.30×10^9cfu/ml respectively and the score of sensory evaluation was 7.36.The optimum inoculum size was 5%, the acidity, pH value, viable counts of *B. bifidum* and the total viable counts were 86^0T,4.6,1.61×10^8cfu/ml, 1.32×10^9cfu/ml respectively, and the score of the sensory evaluation was 7.91.

5. References

Albenzio, M., & Santillo, A. (2011). Biochemical characteristics of ewe and goat milk: effect on the quality of dairy products. *Small Ruminant Research*, 101, 33-40.

Alloggio, V., Caponio, F, Pasqualone, A, and Gomes, T. (2000).Effect of heat treatment on the rennet clotting time of goat and cow milk. *Food Chemistry*, 70(1), 51-55.

Baroutkoub, A., Mehdi, R.Z., Beglarian, R., Hassan, J., Zahra, S., Mohammad, M.S. (2010). Effects of probiotic yoghurt consumption on the serum cholesterol levels in hypercholestromic cases in Shiraz, *Southern Iran. Science Resources*, Essays 5 (16), 2206–2209.

Chen He, Ji Liyuan, Shu Guowei, Wang Zhaowei, (2011). Effect of Lithium Chloride and Sodium Propionate on Growth of Selected Probiotics. *Key Engineering Materials*, 480-481,66-69.

Chen He, Wang Changfeng, Shu Guowei, (2010). Technological optimization of set-style goat yogurt fermentation. *Food science and technology*, 35(12), 71-74.

Chen He, Zhang Qian, Wan Hongchang, Shu Guowei and Li Hong, 2015. Effect of total inoculum size containing lactobacillus acidophilus or lactobacillus casei on fermentation of goat milk. *Advance Journal of Food Science and Technology*, 7(3),183-186.

Cruz, A. G., Walter, E. H. M., Cadena, R. S., Faria, J. A. F., Bolini, H. M. A., Pinheiro, H. P.,et al. (2010). Survival analysis methodology to predict the shelf-life of probioticflavored yogurt. *Food Research International*, 43, 1444–1448.

De Vrese, M., Steglman, A., Richter, B., Fenselau, S., Laue, C.,Scherezenmeir, J., (2001). Probiotics-compensation for lactase insufficiency. *The American Journal of Clinical Nutrition*. 73, 421–429.

De Oliveira, L. B., & Jurkiewicz, C. H. (2009). Influence of inulin and acacia gum on the viability of probiotic bacteria in symbiotic fermented milk. *Brazilian Journal of Food Technology*, 12, 138–144.

FAO/WHO (2002). Guidelines for the evaluation of probiotics in food, Joint FAO/WHO working group report on drafting guidelines for the evaluation of probiotics in food. London, ON, Canada, April 30th May 1st. ftp://ftp.fao.org/es/esn/food/ wgreport2.pdf Accessed August 4th, 2011.

Granato, D., Branco, G. F., Cruz, A. G., Faria, J. A. F., & Shah, N. P. (2010). Probiotic dairy products as functional foods. *Comprehensive Reviews in Food Science and Food Safety*, 9,455–470.

Haenlein, G. F. W. (2004). Goat milk in human nutrition. *Small Ruminant Research*,51, 155-163.

Julio, A., Patricia, A., Fabienne,B., Monique, Z., Marie-Christine,C.V., Marie-Jose, B. (2010). Proteomic comparison of the cytosolic proteins of three Bifidobacterium longum human isolates and B.longum NCC2705. *BMC Microbiology*, 10, 29.

Mainville, I., Arcand, Y., Farnworth, E.R., (2005). A dynamic model that simulates the human upper gastrointestinal tract for the study of probiotics. *International Journal of Food Microbiology*, 99 (3), 287–296.

Mattila-Sandholm, T., Myllärinen, P., Crittenden, R., Mogensen, G., Fondén, R., & Saarela, M. (2002). Technological challenges for future probiotic foods. *International Dairy Journal*, 12, 173–182.

Rafter, J., (2003). Probiotics and colon cancer. *Best Practice & Research Clinical Gastroenterology*. 17, 849–859.

Raynal-Ljutovac, K., Gaborit, P., & Lauret, A. (2005). The relationship between quality criteria of goat milk, its technological properties and the quality of the final products. *Small Ruminant Research*, 60, 167e177.

Sanchez,B.,de los, Reyes-Gavilan, C.G., Margolles,A.,Gueimonde,M. (2009).Probiotic fermented milks:Present and future. *International journal of Dairy technology*, 62,472-483.

Sanz, Ceballos, L, Sanz, Sampelayo, M., Gil, Extremera, F., Rodriguez, Osorio, M. (2009) Evaluation of the allergenicity of goat milk, cow milk, and their lactosera in a guinea pig model. *Journal of Dairy Science*, 92(3), 837-846.

Sheehan, J. J., Drake, M. A., & Mcsweenwy, P. L. H. (2009). Effect of partial or total substitution of bovine for caprine milk on the compositional, volatile, nonvolatile and sensory characteristics of semi-hard cheeses. *International Dairy Journal*, 19, 498-509.

Shu, G., Li, C., Chen, H. and Wang C.(2014). Effect of inoculum and temperature on the fermentation of goat yogurt. *Advance Journal of Food Science and Technology*, 6, 1, 68-71

Wang, S., Zhu, H., Lu, C., Kang, Z., Luo, Y., Feng, L. (2012). Fermented milk supplemented with probiotics and prebiotics can effectively alter the intestinal microbiota and immunity of host animals. *Journal of Dairy Science*, 95, 4813–4822.

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