10.2478/aucft-2018-0011

EFFECTS OF STACHYOSE ON SYNBIOTIC YOGURT OBTAINED FROM GOAT MILK WITH *LACTOBACILLUS ACIDOPHILUS* AND *LACTOBACILLUS CASEI*

– Research paper –

Guowei SHU*[1], Mengqi TIAN*, Binyun CAO**, Changfeng WANG*, Ni XIN***

**School of Food and Biological Engineering, Shaanxi University of Science and Technology, Xi'an 710021, China*
***College of Animal Science and Technology, Northwest A&F University, Yangling 712100, China*
****Department of Research and Development, Xi'an Baiyue Gaot Milk Corp., Ltd., Xi'an 710089, China*

Abstract: Development of synbiotic yogurt having multiple health benefits has become a new trend. The purpose of this study was to obtain the optimum stachyose concentration of the goat yogurt fermented by probiotics including *Lactobacillus acidophilus* (LA) or *Lactobacillus casei* (LC) besides *Streptococcus thermophilus* and *Lactobacillus bulgaricus* by measuring pH, acidity, viable counts and the change of stachyose content. The optimal stachyose concentration of goat LA-yogurt and goat LC-yogurt were 0.4% and 0.8%. Under the above concentrations, the total bacteria number and the viable counts of LA were 2.02×10^9cfu/mL, 3×10^7cfu/mL, respectively; the total bacteria number and the viable counts of LC were 2.55×10^9cfu/mL, 2.53×10^8cfu/mL, respectively. The results indicate that stachyose can effectively improve the vitality and the viable counts of strains in goat yogurt.

Keywords: stachyose; synbiotic yogurt; goat milk; viable counts; *Lactobacillus acidophilus*; *Lactobacillus casei*.

INTRODUCTION

Probiotic milk is very popular among consumers for the delicate taste, high nutritional value, easy to digest and other characteristics. There has been an increased interest in the role of probiotic bacteria in human health. This is due to the probiotics with strong vitality can improve digestion, promote appetite, to alleviate the symptoms of lactose malabsorption, increase the natural resistance to infectious diseases of the intestinal tract, which is beneficial to the health of the human body.
Goat milk has been proved by modern nutritional science to have a more similar nutritional composition and function to breast milk (Marion et al., 2015). In addition, the improvement of flavor and variety of products also make it more and more popular. Nowadays, the production of goat milk products has been emerging as a possible alternative to dairy products due to their nutritional and functional properties. Goat yogurt, which is fermented by probiotics with fresh goat milk as raw material, has better nutritional ingredients than fresh goat milk and has that above function. Therefore, probiotic goat yogurt has been popular by people at home and abroad in recent years.
However, lactobacilli in goat yogurt are sensitive to acid or oxygen, and the viable bacteria is low during their Passage through the gastrointestinal tract and their processing and storage period, thus affecting the quality and health function of goat yogurt (Zhang et al., 2014). A study found that stachyose could promote the growth of *S. thermophilus* and *L. bulgaricus* in yogurt (Wang et al., 2012). Other researchers also found that prebiotics such as stachyose have superior lactobacillus and bifidobacteria proliferative function (García-Rebollar et al., 2016). Inulin and fructooligosaccharides have an significant effect on the growth of B. bifidum BB01 (Chen et al., 2016). Meanwhile, stachyose can not only increase probiotics, protection of intestinal and enhance the body immunity (Huang et al., 2010). It also has other beneficial effects on the body, such as anti-aging, detoxification, prevention of constipation, anti-senile dementia, reduction of cholesterol after meals, protection of the liver and adjuvant therapy for patients using antibiotics, (Hu, 2005; García-Rebollar et al., 2016; Li et al., 2006; Roberfroide et al., 2010). Based on that, it is commonly used in

[1] Corresponding author. E-Mail address: shuguowei@gmail.com

functional health foods and is very popular in many countries such as Japan and Europe. For example, it can partly or completely replace sucrose to develop suitable health food and regulate human function (Liang et al., 2009). Stachyose can prevent the formation of dental caries and is increasingly used in children's food (Duan, 2016).

In recent years, demand on food quality and diversification has increased, and the development of multi-health benefits of synbiotic yogurt has become a new trend. Synbiotics consists of probiotics and prebiotics, so it has the dual function of both probiotics and prebiotics. Gustaw et al. (2011) found that adding inulin during yogurt fermentation can promote the growth of lactobacilli. Studies have shown that, stachyose can increase the growth speed of bifidobacteria to 40 times, far superior to the growth-promoting effect of isomalto- and fructooligosaccharides (Li et al., 2016). However, there are few studies in China and there is a lack of related products in the market at present.

In our previous study, *L. bulgaricus* and *S. thermophilus* were screened out to ferment goat yogurt and the optimal conditions were optimized (Chen et al., 2010). Then new types of fermented goat yogurt were studied by adding probiotics including *L. acidophilus* and *L. casei* to the goat yogurt prepared above. On the basis of *S. thermophilus* or *L. bulgaricus* as starter cultures, the effect of the total inoculum size and bacteria proportion containing *L. acidophilus* and *L. casei* on the fermentation of goat milk was studied (Chen et al., 2015; Shu et al., 2015). Based on it, we further added stachyose to increase the functionality of fermented goat milk and the aim of the present work was to study the effects of stachyose on goat milk fermented by probiotics including *L. acidophilus* or *L. casei* besides *S. thermophilus* and *L. bulgaricus* for developing goat yogurt containing *L. acidophilus* (goat LA-yogurt) and *L. casei* (goat LC-yogurt), which lays a foundation for the development of the future synbiotic products.

MATERIALS AND METHODS

Microorganism and cultivate preparation: *S. thermophilus* (ST), *L. bulgaricus* (LB), *L. acidophilus* (LA) and *L. casei* (LC) were supplied by School of Food and Biological Engineering, Shaanxi University of Science and Technology. Goat milk powder supplied from Xi'an Baiyue Gaot Milk Corp., Ltd. (Shaanxi, China) was used.

The above freeze-dried bacteria powder was inoculated in an anaerobic tube filled with sterilized skim milk and grown at 37 ℃ until the curd (24 h), and a viable strain was obtained after repeated activation three times, 3%-5% (v/v) inoculum was inserted into a flask containing sterilized whole goat milk after the curd was vigorously crumbled and incubated at 37 ℃ until the curd, and then stored at 4 degrees until use.

Preparation of synbiotic goat yogurts: Various amounts of stachyose were added in the whole condensed milk and the stachyose concentration of 0%, 2.5%, 5%, 7.5% and 10% w/w was obtained. Then 7% mixture bacteria was inoculated into goat milk and cultured at 37 ºC in triplicate. The ordinary yogurt starter was consisted of *L. bulgaricus* (LB) and *S. thermophilus* (ST). Goat LA-yogurt was fermented by starter and *L. acidophilus* (LA) with a ratio of LA:(LB:ST=2:1)=1:1, the bacteria proportion (v/v) of starter and *L. casei* (LC) for goat LC-yogurt was the same as goat LA-yogurt. Acidity, pH and viable bacteria of all samples were measured at 1.5h intervals, and the content of stachyose was measured before and after the fermentation process. Each sample was measured by triplicate and the results are the average.

Determination of viable counts: Plate coating method and top agar method were used to obtain the viable counts. Among the various media developed for the selective counting of probiotics, the total viable counts were determinate by using modified Tomato Juice medium (TJA), MRS agar medium (containing 0.06% bile salt or 0.10% LiCl) was used to obtain viable counts of LA and LC (Shu et al., 2011; Chen et al., 2011).

Determination of acidity and pH: The pH of each sample was directly measured using a pH-meter (pHs-3c) at the room temperature (Lv et al., 1999), acidity (ºT) was measured by the method of sodium hydroxide titration (Zhao et al., 2009).

Determination of stachyose content: ELSD-HPLC was used to obtain the stachyose content. Stachyose was separated on a Hypersil NH_2 column at ambient temperature with methanol: water (80:20) as mobile phase. The flow rate was 1.0 mL/min. The parameters of

ELSD were set as follows: drift tube temperature was 85℃, Gas pressure was 30Psi. Take 1.5 mL of synbiotic goat yogurt and centrifuge it at 15,000 rpm for 30 min, then take the supernatant and continue centrifugation until it is clear. Take 1 mL to 10 mL with ultra-pure water and the sample was obtained after over 0.45 μm filter. 20 μl sample was injected to measure peak area, then substitute it into a linear regression equation ($y=12.204x-47.478$, $R^2=0.9918$) to calculate the stachyose content (Song et al., 2012).

RESULTS AND ANALYSIS

Effects of stachyose on fermentation of goat LA-yogurt

Under the optimal fermentation conditions of goat LA-yogurt, stachyose (0.4%, 0.6%, 0.8% and 1.0%) was added to explore the effect of stachyose on the fermentation of goat LA-yogurt. The results are shown in Figure 1.

Figure 1(a) shows different concentrations of stachyose almost all have a positive effect on the growth of *L. acidophilus* (LA) throughout the fermentation period. In the concentration range from 0.4% to 0.6%, the viable counts of LA increased significantly, but the stachyose concentration had no significant influence on the viable counts of LA in the range of 0.8%-1.0%. The optimal fermentation time for goat LA-yogurt was 4.5h. At this time, 0.4% stachyose had the best enriching effect on LA, and the viable counts of LA reached the maximum (3.00×10^7 cfu/mL). With the increasing concentration of stachyose, the viable counts of LA decreased from 2.20×10^7 cfu/mL at stachyose 0.6% to 1.70×10^7 cfu/mL at stachyose 0.8%, then dropped to 1.61×10^7 cfu/mL at 1.0%, which was very close to the viable counts of control sample (1.59×10^7 cfu/mL) at 4.5h. The above data indicated that a lower concentration of stachyose has a better enriching effect on LA. The stachyose concentration is appropriate to be 0.4%~0.6%.

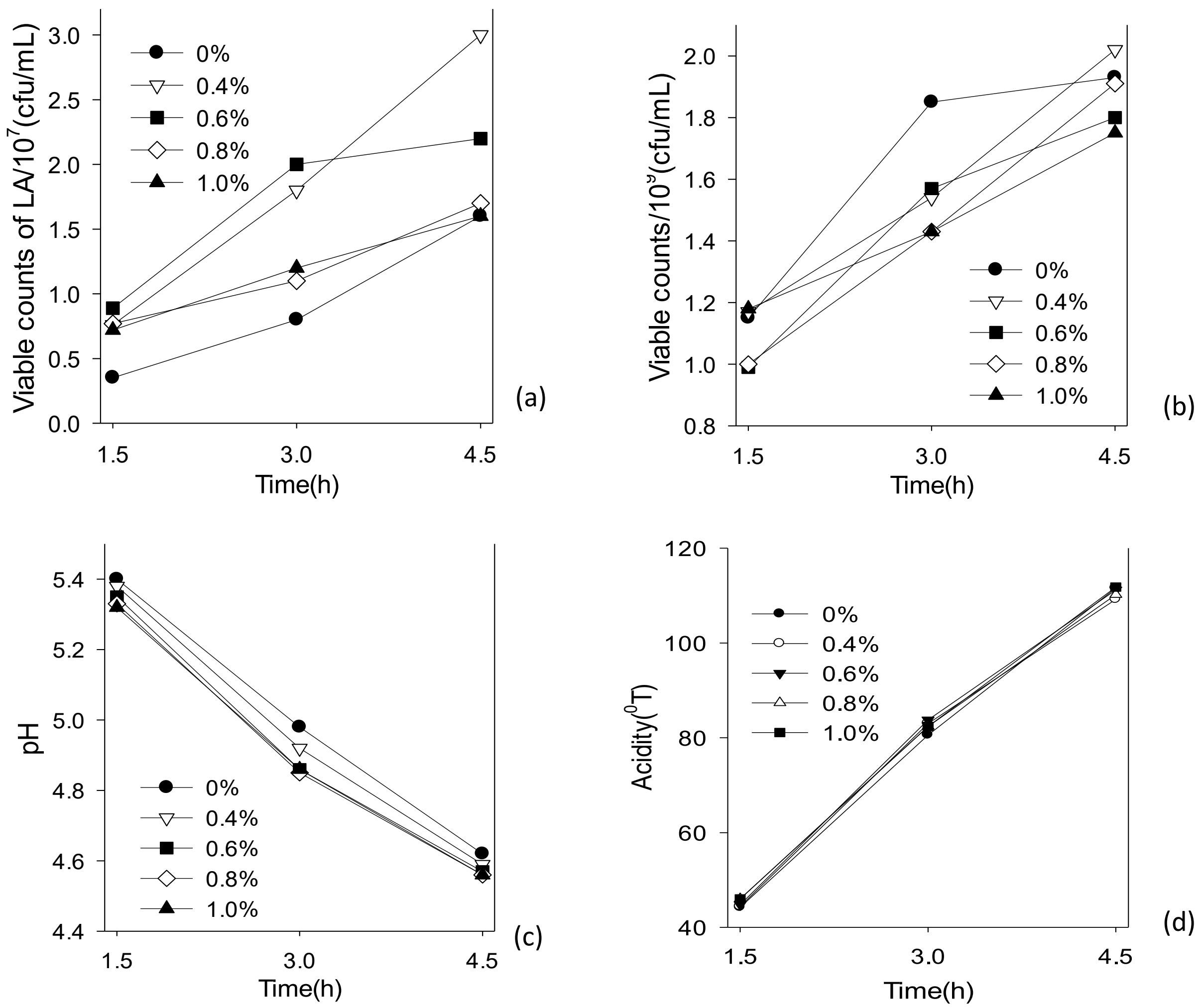

Figure 1. Effect of stachyose on viable counts of *L. acidophilus*, viable counts, pH and acidity in goat LA-yogurt

As Figure 1(b) shows, stachyose with different concentrations almost have no positive effect on the increase of viable counts. During the first three hours, stachyose with all concentrations inhibited the increase of viable counts in goat LA-yogurt, then the inhibitory effect decreased with time until 4.5 hours, and at this time, the viable counts in goat LA-yogurt with different concentrations of stachyose other than 0.4% (0.6%, 0.8%, 1.0%) decreased very little, were 1.80×10^9 cfu/mL, 1.91×10^9 cfu/mL and 1.75×10^9 cfu/mL, except that, the total number of bacteria added with 0.4% stachyose was slightly higher (2.02×10^9 cfu/mL) than that of the control group (1.93×10^9 cfu/mL). These indicates the viable count in goat LA-yogurt with different concentration stachyose did not change much compared with the control sample without stachyose. Though high concentrations of stachyose still showed the little inhibitory effect on the increse of total viable counts, stachyose at a lower concentration (0.4%) exhibited an enriching effect.

From Fig.1(c) and (d), the effect of stachyose on acidity (pH) and the change of viable counts have a correspondence relation in all goat LA-yogurt. The acidity were all first increased rapidly then began to slow down as total viable counts with time increasing, while the variation trend of pH in all goat LA-yogurt was opposite to acidity. From 0.4% to 0.6%, the pH was decresed from 4.59 to 4.57 while the acidity increased from 109.2 ^{o}T to 111.2 ^{o}T as the addition of stachyose. Without the addition of stachyose, the pH and acidity were 4.62 and 111.6 ^{o}T.

As shown in Figure 2, the amount of stachyose in goat LA-yogurt with different stachyose concentration were all reduced at the end of fermentation, and the change in stachyose content of yogurt increased with the increasing additive amount of stachyose. This indicates that there is stachyose consumption across the whole yogurt fermentation process. Among then, the consumption of stachyose at 0.4% was the minimum (0.76 mg/mL).

According to these data above, the optimum additive amount of stachyose for goat LA-yogurt is 0.4%. At this time, the viable counts of LA reached the maximum (3.00×10^7 cfu/mL), the total viable bacteria is 2.02×10^9 cfu/mL, the pH and acidity were 4.59 and 109.2 ^{o}T, and stachyose consumed at least at the same time.

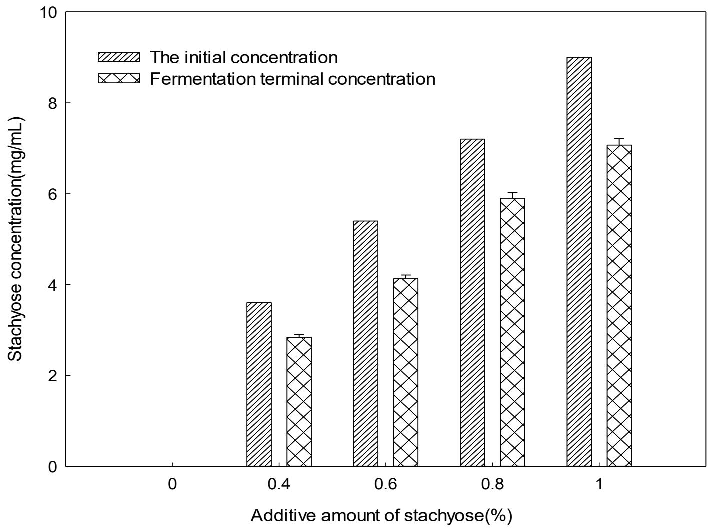

Figure 2. Change in stachyose content of goat LA-yogurt

Effect of stachyose on fermentation of goat LC-yogurt

As Figure 3(a) shows, the viable counts of *L. casei* (LC) with all concentrations stachyose except at 0.8% (0.4%, 0.6%, 1.0%) were respectively 2.45×10^8 cfu/mL, 2.40×10^8 cfu/mL and 2.40×10^8 cfu/mL, both of which were slightly higher than the control group (2.40×10^8 cfu/mL) at the end of fermentation. This indicated that the effect of stachyose from 0.4% to 0.6% had little influence on the increase of LC throughout the fermentation period. However, the work showed that the effect on the growth of LC is more obvious when the stachyose at 0.8%, and the viable counts of LC reached the maximum (2.53×10^8 cfu/mL) at the end of fermentation.

As Figure 3(b) shows, the stachyose with all concentrations except at 0.4% had significant effect on the increase of viable counts in goat LC-yogurt. During the first three hours, stachyose with concentrations at 0.6%, 0.8%, 1.0% had a positive effect on the increase of total viable counts in goat LC-yogurt, then the enriching effect decreased with time until 4.5 hours, and at this time, the viable counts were respectively 2.50×10^9 cfu/mL, 2.55×10^9 cfu/mL and 2.50×10^9 cfu/mL, both of which were higher than the control group (1.82×10^9 cfu/mL) at the end of fermentation. However, there was not significant difference between the viable counts at 0.4% stachyose concentration and the control group. The above data indicated that a higher concentration of stachyose had a better enriching effect. The stachyose concentration is appropriate to be between 0.6%~1.0%.

From Figure 3(c) and (d), the effects of stachyose at all concentrations on acidity (pH) are corresponded with total number of bacteria in goat LC-yogurt. Among them, the corresponding pH and acidity at 4.5h were respectively 4.53 and 93°T, 4.54 and 90°T, 4.54 and 89°T, and of control group were 4.59 and 88.4°T when the concentration of stachyose was 0.6%, 0.8% and 1.0%.

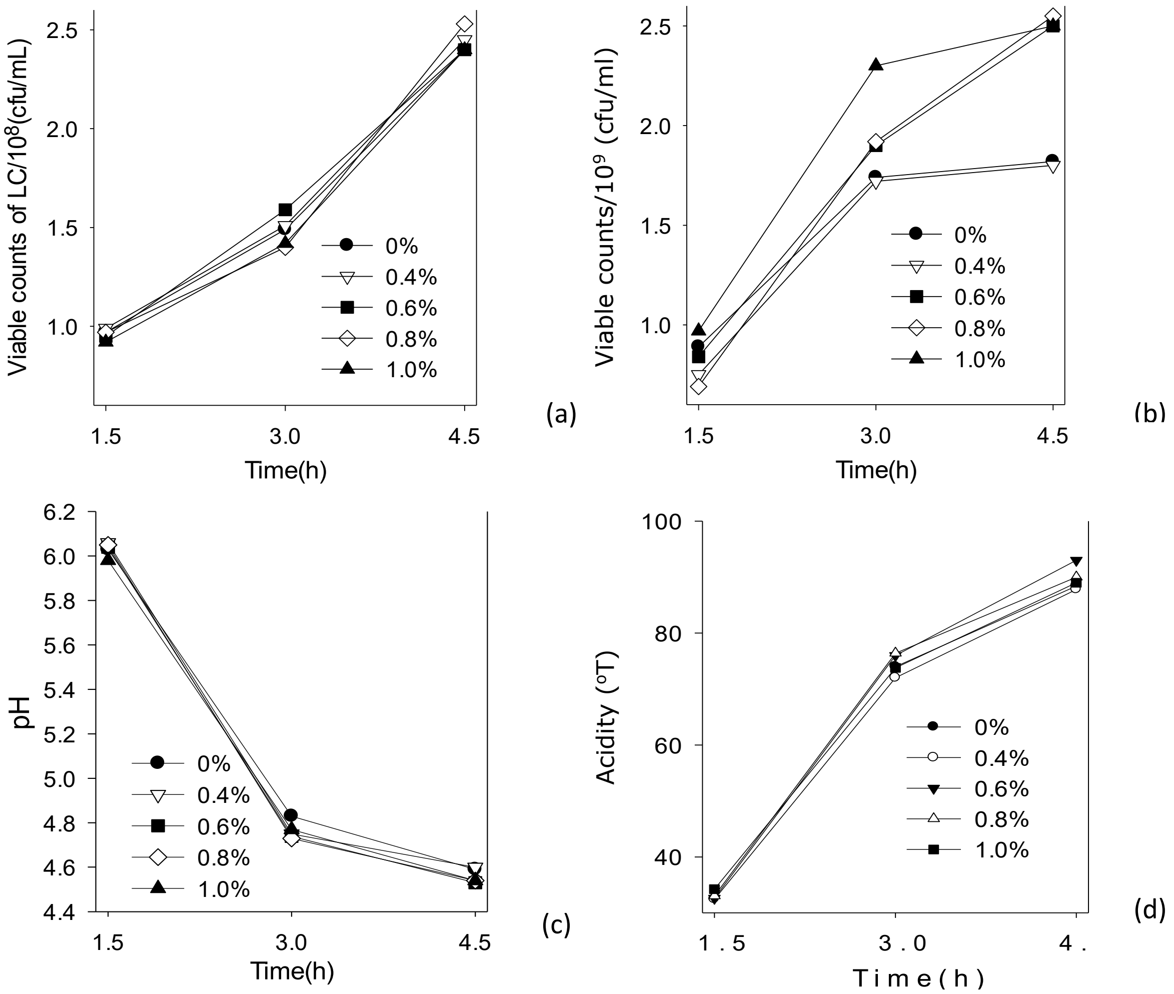

Figure 3 Effect of stachyose on viable counts of *L. casei*, viable counts, pH and acidity in goat LC-yogurt

As it is shown in Figure 4, the consumption of stachyose is larger in goat LC-yogurt. When the stachyose was 0.4%, the consumption of stachyose was minimal (1.22 mg/mL), and then decreased to 1.74 mg/mL at 0.8%.

It is clear from that above that the optimum additive amount of stachyose for goat LC-yogurt was 0.8%. At this time, the total viable bacteria and viable counts of LC were highest, the pH and acidity were 4.54 and 90 °T, and stachyose consumed less at the same time.

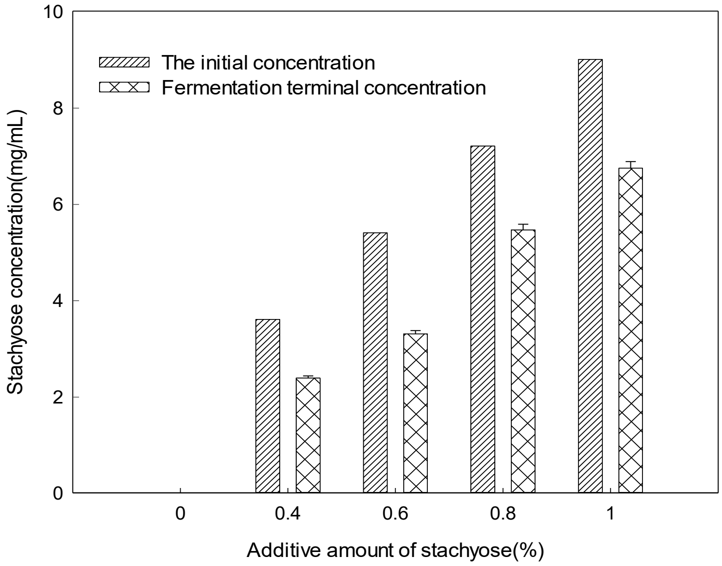

Figure 4. Change in stachyose content of goat LC-yogurt

DISCUSSION

On the basis of the goat yogurt fermented by probiotics including *L. acidophilus* or *L. casei* besides *S. thermophilus* and *L. bulgaricus*, stachyose was added to develop several new synbiotic goat yogurt products. The experimental results show that the proper amount of stachyose can effectively increase the number of LA in goat LA-yogurt and total viable counts in goat LC-yogurt throughout the fermentation period.

In goat LA-yogurt, almost all concentrations of stachyose can effectively increased the amount of LA, but the enriching effect of LA is reduced with the increase of stachyose concentration. At this time, the viable counts of LA reached the maximum (3.00×10^7 cfu/mL) at stachyose 0.4%. Besides, the total viable counts at the higher concentration of stachyose were lower than that of the control group, compared with the lower concentration of stachyose. It may be related to the high osmotic pressure formed by the high concentration of stachyose in the culture environment. Moreover, our study finds that the inhibition of the growth rate of bacteria in goat LC-yogurt was weak with the higher concentration of stachyose at the end of fermentation. The possible reason was that stachyose raised the activity of α-galactosidase, lactate dehydrogenase, thereby relieving a part of inhibition of lactose on the growth of the cells (Wei et al., 2013).

The study found that the optimal additive amount of stachyose were 0.4% and 0.8% when goat LA (LC)-yogurt was fermented with a ratio of LA (LC):LB:ST=1:2:1, which was found to be different from other research (Wang et al., 2015; Zhang et al., 2014). Wang et al found that adding 6% sucrose and 1% stachyose to ferment acidophilic goat milk, and the total number of bacteria reached the maximum (1.39×10^9 cfu/mL) at 8h. This result is quite different from the total number of bacteria we have optimized and the possible reason could be the differences between the total inoculum size and bacteria proportion.

However, further research is needed on the effect of stachyose on the viable count and physicochemical properties of goat yogurt, as well as the number of bacteria of each yogurt during storage.

CONCLUSIONS

Additive amount of stachyose were optimized by single factor experiments. The results are as follow: In goat LA-yogurt, the optimum amount of stachyose was 0.4%, the number of LA reached the maximum (3.00×10^7 cfu/mL), the acidity and pH were 111.6°T and 4.59. In goat LC-yogurt, the optimal additive amount of stachyose is 0.8%. Under this amount, the pH, acidity, the viable counts of LC, the total viable counts were 4.53, 93°T, 2.53×10^8 cfu/mL, 2.55×10^9 cfu/mL. In both types of yogurt, stachyose is consumed less and has the health effect of synbiotics. This provides a reference for the subsequent production of fermented goat milk with synbiotics.

ACKNOWLEDGMENTS

The work was partially supported by the key project of Science and Technology Department of ShaanxiProvince (No. 2018ZDXM-NY-085) and the science and technology project of Xi'an city 201806118YF06NC14(2).

REFERENCES

1. Chen, H., Ji, L. & Shu, G., Wang, Z. (2011). Effect of Lithium Chloride and Sodium Propionate on Growth of Selected Probiotics. *Key Engineering Materials*, 480-481, 66-69.
2. Chen, H., Wang, C. & Shu, G. (2010). Technological optimization of set-style goat yogurt fermentation. *Science and technology of food industry*, 35(12), 71-74.
3. Chen, H., Zhang, Q. & Wan, H. (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.
4. Chen, H., Ma, D. & Li, Y. (2016). Optimization the Process of Microencapsulation of Bifidobacterium bifidum BB01 by Box-Behnken Design, *Acta Universitatis Cibiniensis. Series E: Food Technology*, 20, 2, 17–28. DOI: 10.1515/aucft-2016-0012.
5. Duan, S. (2016). Properties and Applications of Stachyose. *Beverage Industry,* 19(5), 74-78.
6. García-Rebollar P, Cámara L & Lázaro RP. (2016). Influence of the origin of the beans on the chemical composition and nutritive value of commercial soybean meals. *Anim Feed Sci Technol*, 221, 245-261.
7. Gustaw, W., Kordowska, W. & Koziol, J. (2011). The influence of selected prebiotics on the growth of lactic acid bacteria for bio-yoghurt production. *Acta Sci Pol Technol Aliment*, 10 (4), 455-466.
8. Huang, J., Xin, X., Pang, M. & Yang, H. (2010). Application progress of prebiotics in global dairy products. *Shandong Food Ferment*, 1(156), 40-43.
9. Hu, X. (2005). Prebiotics:the bifidus stimulater. *Industrial Microbiology*, 35(2), 50-60.
10. Kailasapathy, K. & Chin, J. (2000). Survival and therapeutic potential of probiotic organisms with reference to *Lactobacillus acidophilus* and *Bifidobacterium spp*. *Immunol Cell Biol,* 78, 80–88. DOI:10.1046/j.1440-1711.2000.00886.x
11. Li, L. & Gui, J. (2006). Study of the stachyose, an extract of Chinese herbal medicine, on pasm lipoplusaccharide endotoxin（LPS）and intestinal microflora in experimental liver cirrhosis rats. *Chinese Journal of Microecology,* 18(2), 107-109.
12. Li, W., Li, Z. & Han, X., et al. (2016). Enhancing the hepatic protective effect of genistein by oral administration with stachyose in mice with chronic high fructose diet consumption. *Food Function*, 7(5), 2420-2430.
13. Liang, Y. & Pang, Z. (2009). Application Status and Safety of Sweeteners in Sugar-free Foods. *Modern Food Science and Technology*, 25(8), 964-966.
14. Lu, J. (1999). Studies on the new special colouring method for smear of lactic acid bacteria in yogurt. *Microbiology*, 26(4), 281-282.
15. Marion, P. C., Beatriz, S. F. & Adriana, S. (2015). Cupuassu (Theobroma grandiflorum) pulp, probiotic, and prebiotic:Influence on color, apparent viscosity, and texture of goat milk yogurts. *Journal of Dairy Science*, 98(9), 5995-6003.
16. Pan, Q. & Zeng, X. (2017). The Proliferation Mechanism of *Lactobacillus plantarum* RB1 Stimulated by Stachyose. *Current microbiology*,74(6), 732-738.
17. Roberfroid, M., Gibson, G. R. & Hoyles, L. (2010). Prebiotic effects:metabolic and health benefits. *British Journal of Nutrition*, 104, S1-S63.
18. Shu, G., & Chen, H., et al. (2011). Effect of bile and nalidixic acid on growth of selected probiotics. *Advanced Materials Research,* 322, 248-251.
19. Shu, G. & Chen, H. (2013). Effects of inoculum on fermentation of goat milk by *L.acidophilus* and *L. casei. Food Science and Technology*, 38(11), 49-52.
20. Shu G., Wang S. & Chen Z. (2015). Effect of bacteria proportion on the fermentation of goat yogurt with probiotic culture. *Acta Scientiarum Polonorum, Technologia Alimentaria*, 14, 4, 407-414.
21. Wang, X. & Wang, Y. (2012). Effects of Oligo Saccharides and Chlorella on the Growth of Probiotics and Quality of Chlorella Yogurt, *Food Science,* 33(07), 158-162.
22. Wei, Y. & Zeng X. (2013). Study on the Effect of stachyose on the Proliferation of *Lactobacillus Plantarum, Journal of Chinese Institute Of Food Science and Technology*, 13(12), 103-108.

23. Wang, T. & Fang X. (2015). Effects of stachyose and xylitol on lactic acid bacteria proliferation, *Journal of Hebei University (Natural Science Edition)*, 35(5), 503-507.
24. Zhao, J., Meng, Z., Li, G. & Wang, Y. (2009). Study on processing of coagulation Schizandra chinensis yogurt. *Food Science and Technology,* 34(8), 49-51.
25. Zhang, R. & Zhang, F. (2014). Effect of Prebiotics on the Number of *Lactobacillus* in yogurt. *The processing of agricultural products*, 17, 18-22.