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EFFECTS OF DIFFERENT SUGARS, ALCOHOLS, ANTIOXIDANTS ON GOAT MILK TABLETS CONTAINING *LACTOBACILLUS ACIDOPHILUS* LA-5

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Abstract: Probiotics were added to goat milk powder, which would not only improve the intestinal flora balance but also would promote human and animal health. In this paper, the effects of different sugars, alcohols, antioxidants on *Lactobacillus acidophilus* goat milk tablets were investigated by single factor tests based on viable count, suitable hardness, friability, and sensory evaluation. The optimal formulation of goat milk tablets with *Lactobacillus acidophilus* was obtained: sucrose 7 %, glucose 1.3 %, xylitol 5 %, acesulfame 0.04 %, D-sodium ascorbate 0.06 %, D-mannitol 0.3 %, tea polyphenols 0.018 %, microstalline cellulose 0.4 %. The application of this composition gives good sensory evaluation, high viable lactobacilli count, suitable hardness and friability of the resulting goat milk tablets of *Lactobacillus acidophilus*.

Keywords: *goat milk tablets, Lactobacillus acidophilus, viable count*

INTRODUCTION

Probiotics are a kind of living microbial agents that can change the ecological balance of intestinal flora and play a beneficial role to the host [1]. *Lactobacillus acidophilus* is an important microorganism in the gastrointestinal tract, which belongs to the genus Lactobacillus, gram positive, facultative anaerobic bacteria, anaerobic or low oxygen environment in the solid substrate surface growth [2]. *Lactobacillus acidophilus* has the antibacterial, anticancer, and lowering cholesterol properties [3, 4]. In addition, both probiotics and human gastrointestinal tract are found to have different strains of *Lactobacillus acidophilus* [5].

Functional food products including specific probiotic have already been developed in different formulations, such as fermented milk, chewing gum, beverages, cottage cheese, cheese, powdered milk, dairy desserts [6 – 8]. Modern yoghurt production is a well-controlled fermentation process that utilizes milk, milk powder, sugar, fruit, coloring agents, flavors, and specific pure cultures of lactic acid bacteria (*Streptococcus thermophilus* and *Lactobacillus bulgaricus*) to conduct fermentation process [9]. However, the high-water activity (aw) limited shelf-life stability of these products including probiotics. Therefore, the development of dry formulations that can protect probiotic bacteria from the harsh conditions in the stomach as well as during storage at room temperature is required. Probiotics are very sensitive to high aw and low *p*H level. Goat milk has been reported to have higher digestibility and lower allergenic properties than cow milk, and has certain therapeutic values, which makes him attract a lot of consumers [10]. The nutritional advantage of goat milk compared to cow milk has been attributed to the small size of fat globules [11]. Therefore, goat milk is more suitable as health care food for infants, kids and elderly. What's more, many researchers have focused their attention on milk tablets of probiotics [12], probiotic ice cream from goat milk and probiotic yogurt [13]. Especially, the advantages of goat milk tablet with high nutritive value are convenient, and suitable for the distribution to remote areas. Also, probiotic milk tablets have obvious advantages compared with liquid dairy products of probiotics during storage [12]. However, there are several problems that cause probiotics viable count decrease quickly and affect the viability of probiotic organisms during processing [5].

Biopolymers have been used as excipients for tablet development. Alginate is one of the most popular natural biopolymers used for probiotic encapsulation due to the fact that it is natural, safe and widely accepted in the food, drink and pharmaceutical industries [14]. Microcrystalline cellulose (MCC) can be used as a plastically deforming, directly compressible material to improve the quality of tablets [15]. In addition, the hydroxypropyl methylcellulose phthalate (HPMCP) is used as tablet forming matrix because of its insoluble property in gastric fluids (*p*H 1.5), but which can dissolve rapidly in the upper small intestine where the *p*H value is around 5.5 according to the manufacturer's instructions [14]. Also, Cruz [16] evaluated the stability of probiotic yoghurts supplemented with glucose oxidase and packaged in different plastic packaging systems with different oxygen permeability transfer rates ranging from 0.09 to 0.75 mL O_2 day^{-1}. Corcoran *et al* [17] found that the presence of 19.4 mM glucose resulted in up to 6 log enhanced survival following 90 min of exposure to simulated gastric juice at *p*H 2.0 as compared to the control. Therefore, the excipients are important to develop milk tablet formulation containing probiotics in diary industry.

The medicinal efficacy of probiotic food products depends upon the number of viable and active cells per gram or milliliter of the products at the moment of consumption [18]. It is therefore essential to ensure a high survival rate of the probiotics during production as well as over the product shelf life in order to maintain consumer confidence in probiotic products [19]. Many factors were found to influence the viability of probiotic microorganisms in food products during production, processing and storage. The identified factors include food parameters (*p*H, titratable acidity, molecular oxygen, water activity, presence of salt, sugar and chemicals like hydrogen peroxide, bacteriocins, artificial flavoring and coloring agents); processing parameters (heat treatment, incubation temperature, cooling rate of the product, packaging materials and storage methods, and scale of production); and microbiological parameters (strains of probiotics, rate and proportion of inoculation). Weinbreck *et al* [20] reported that a water activity of 0.7 resulted in 10 log cycle reduction in viable counts of *L. rhamnosus* GG within 2 weeks of storage. Hoobin *et al* [21] suggested that moisture uptake properties and molecular mobility of the matrix composition, as opposed to the relative humidity of the environment, are better determinants of probiotic viability during storage.
The main objective of the present study is to develop a goat milk tablet formulation for maximum protection of the viability of *Lactobacillus acidophilus* during storage.

MATERIALS AND METHODS

Materials

The Strain of *Lactobacillus acidophilus* (LA-5) was provided by School of Food and Biological Engineering Shaanxi University of Science and Technology.

Freeze-drying probiotic biomass

Stock culture of *Lactobacillus acidophilus* (LA-5) was stored at -80 °C in MRS broth (Hope Bio-Technogy Co., Ltd. Qingdao) supplied with 24 % (v/v) skim milk. *Lactobacillus acidophilus* (LA-5) was cultured for 22 h in MRS broth at 37 °C. Biomass was then harvested by centrifugation (4500×g, 10 min at 4 °C), washed and resuspended in 0.9 % (w/v) saline to obtain final concentration of 10^9–10^{10} CFU·mL^{-1}. Then, the sample was dried using a freeze-dryer (LGJ-15D, Sihuan Co., Ltd. Beijing City, China) to obtain dried powder. The concentration of *Lactobacillus acidophilus* (LA-5) in biomass powder was analyzed and found to be approximately 10^{11} CFU·g^{-1}.

Preparation of goat milk tablets

The goat milk tablets of *Lactobacillus acidophilus* were made based on excipients (sucrose, inulin, xylitol, acesulfame, sodium erythorbate, tea polyphenol, microcrystalline cellulose, mannitol), and obtained by direct compression of a homogeneous mixture of dry powders containing different concentrations excipients. The pressure should be controlled under 8 ± 0.5 KN, and goat milk tablets weights were set at 0.9 ± 0.1 g.

Viable count of goat milk tablets

In accordance with a slightly modified method [22], each table after compressing was immediately broken and dispersed in 100 mL of phosphate buffer (*p*H 6.8). A serial dilution of this suspension was made in 0.9 % sterile normal saline until an appropriate cell density was obtained and subsequently plated in triplicate in MRS agar plates. These plates were incubated at 37 °C for 48 h in an anaerobic environment. The colony forming unit (CFU) was finally enumerated and converted to log CFU. The viable count (Y1) of goat milk tablet containing *Lactobacillus acidophilus* was calculated by Equation (1).

$$Y1 = \frac{W1 \times W2}{W} \quad (1)$$

where: *W* is the weight of goat milk tablets, *W1* is viable count of per mL bacteria suspension, and *W2* the volume of bacterial suspension

Determination of hardness

The hardness was measured in triplicate and recorded by tablet hardness-testing device (YD-I, Tianjin Optical Instrument Co., Ltd. Tianjin) tablet hardness tester, and the standards of hardness for milk tablets generally control between 3.5-5.0 kgf [23].

Determination of fragmentation

The determination of fragmentation was measured by tablet friability tester (CS-II, Tianjin Optical Instrument Co., Ltd. Tianjin).

Sensory evaluation

Using the method of grading test, 15 trained food researchers were divided into three groups, the scores of different combinations were scored (out of 100) and the results were recorded. The scoring program includes 5 indicators: appearance, flavor, color, taste and texture, specific scoring rules as shown in Table 1 [24].

***Table 1**. Sensory evaluation of goat milk tablets*

Project	**Appearance (15 points)**	**Color (15 points)**	**Taste (25 points)**	**Flavor (25 points)**	**Texture (20 points)**
Good	12 - 15 Smooth appearance, uniform thickness, neat edges	12 - 15 yellow, uniform color	20 - 25 Smooth and non-stick teeth	20 - 25 moderate	15 - 20 Uniform structure, close organization
Common	9 - 11 a small amount of fragmentation	9 - 11 Yellow, uniform distribution of color	15 - 19 No rough, non-stick teeth	15 - 19 light or slightly thicker	11 - 14 Relatively uniform structure
Bad	<9 Severe surface crushing	<9 Dark yellow, color distribution	<15 Rough texture and sticky teeth	<15 Goat too strong or not	<11 loose structure with a soft cross section

RESULTS AND DISCUSSION

Effect of sucrose on *Lactobacillus acidophilus* goat milk tablets quality

4 %, 5 %, 6 %, 7 %, and 8 % of sucrose were added into that mixtures including 0.1 % of *Lactobacillus acidophilus* bacteria powder, 0.4 % of microcrystalline cellulose and goat milk powder, milk tablets were obtained by direct compression using a single punch tablet press. The evaluated indexes of viable count, hardness, friability and sensory evaluation are shown in Figure 1.

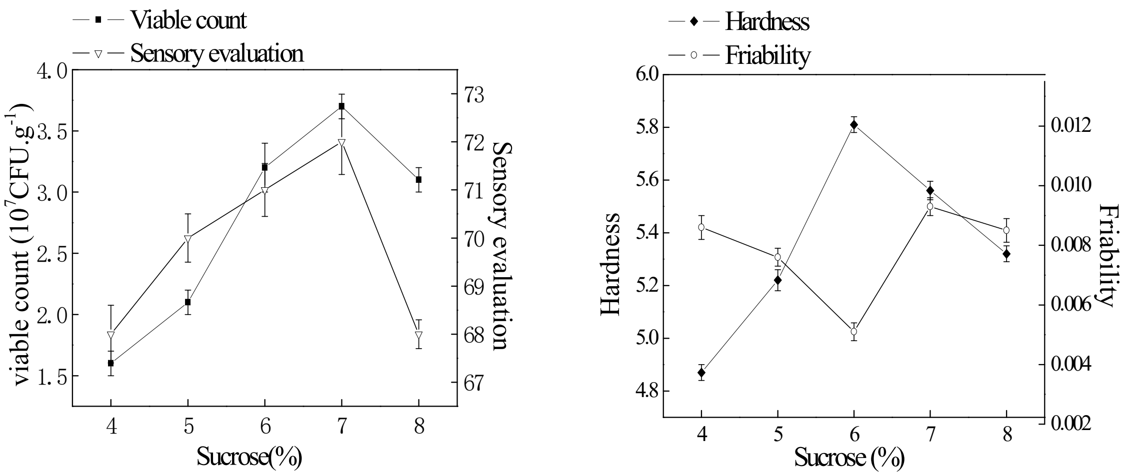

Figure 1*. Effect of sucrose on LA-5 goat milk tablet*

Figure 1 shows that when the added amount of sucrose gradually increased from 4 % to 8 %, the number of viable counts increased and the sensory evaluation improved at first and then decreased rapidly, while the hardness of the goat milk tablets increased with increasing the sucrose concentration. There was a clear inflection point when sucrose concentration was 7 %, the viable count got maximum value of 3.7×10^7 $CFU\cdot g^{-1}$, and the score of sensory evaluation was 73. However, when the concentration of sucrose was 6 %, the hardness reached the maximum value of 5.81 kgf and friability reached the minimum value of 0.51 %, because the viable count and sensory evaluation were main indexes for milk tablets, and when the sucrose content was 7 %, the hardness was 5.5 kgf, which meet consumer's requirement, therefore, 7 % sucrose was used as optimal concentration. From the Figure 1 it could be seen that the viable count will decrease when the concentration of sucrose exceeded 7 %.

Effect of glucose on *Lactobacillus acidophilus* goat milk tablet quality

0.7 %, 0.9 %, 1.1 %, 1.3 % and 1.5 % of glucose were added into that mixtures including 7 % sucrose, 0.1 % of *Lactobacillus acidophilus* bacteria powder, 0.4 % of microcrystalline cellulose and goat milk powder, respectively, and milk tablets were obtained by direct compression using a single punch tablet press. The evaluated indexes of viable count, hardness, friability and sensory evaluation are shown in Figure 2.

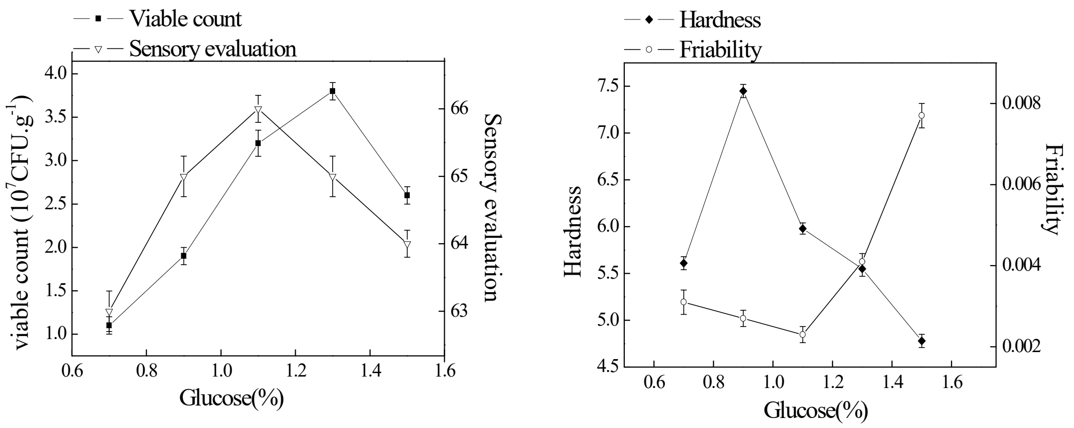

Figure 2. *Effect of glucose on LA-5 goat milk tablet*

Glucose not only can be used as a sweetener, giving milk tablet tasty, but also can increase the milk tablet crisp sense, avoid milk too hard to affect the taste. Figure 2 showed that when the concentration of glucose increased from 0.7 % to 1.5 % gradually, and the number of viable bacteria and hardness showed the trend of increased at first and then decreased rapidly. When the content of glucose was 1.3 %, the number of viable bacteria exhibited a maximum value of 3.8×10^7 CFU·g^{-1}. However, with the concentration increased of glucose, the friability decreased firstly and then increased. When the content of glucose was 1.1 %, the friability reached the minimum value of 0.23 % and sensory evaluation reached the maximum value of 66. However, considering the preparation of probiotic goat milk tablets, the number of live bacteria as the main index, therefore, the added amount of glucose was 1.3 %.

Effect of xylitol on *Lactobacillus acidophilus* goat milk tablet quality

3 %, 4 %, 5 %, 6 %, and 7 % of xylitol were added into that mixture including 7 % sucrose, 1.3 % glucose, and 0.1 % of *Lactobacillus acidophilus* bacteria powder, 0.4 % of microcrystalline cellulose and goat milk powder, respectively, and milk tablets were obtained by direct compression using a single punch tablet press. The evaluated indexes of viable lactobacilli count, hardness, friability and sensory evaluation are shown in Figure 3.

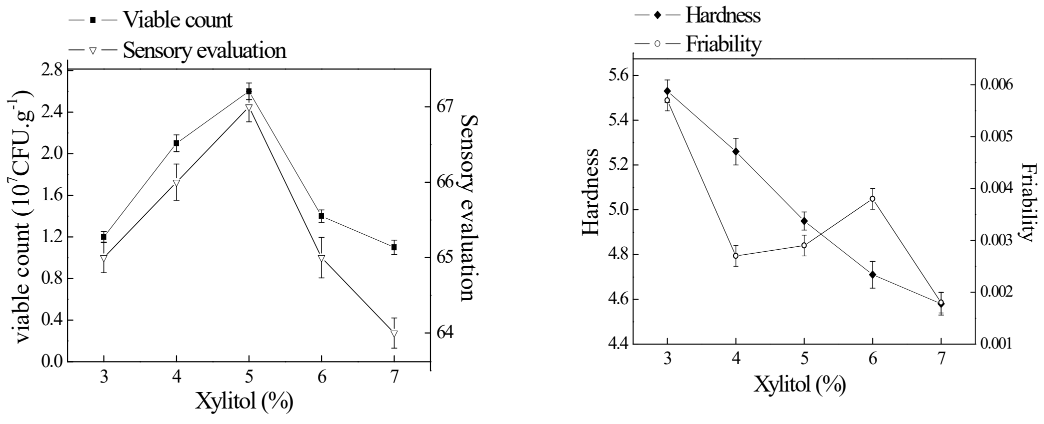

Figure 3. *Effect of xylitol on LA-5 goat milk tablet*

Xylitol can promote the growth of probiotics, antiquaries [25]. Figure 3 showed that when the adding amount of xylitol gradually increased from 3 % to 7 %, the number of viable lactobacilli cells increased and the sensory evaluation improved at first and then decreased rapidly. There was an obvious inflection point when adding 5 % of xylitol, the number of living bacteria and sensory evaluation reached the maximum values of 2.6×10^{7} CFU·g^{-1} and 68, respectively; but the hardness and friability were 5.0 kgf and 0.29 %, respectively. As the viable count and the sensory evaluation were the main indexes, the added amount of xylitol was determined to be 5 %.

Effect of acesulfame on *Lactobacillus acidophilus* goat milk tablet quality

The addition of acesulfame was 0.01 %, 0.02 %, 0.03 %, 0.04 %, 0.05 %, respectively, 7 % sucrose, 1.3 % glucose, 5 % xylitol, and 0.1 % of *Lactobacillus acidophilus* bacteria powder, 0.4 % of microcrystalline cellulose and goat milk powder mixed and compressed into tablets. The goat milk tablets of different formulation were used to measure viable count, hardness, friability and sensory evaluation. The results are shown in Figure 4.

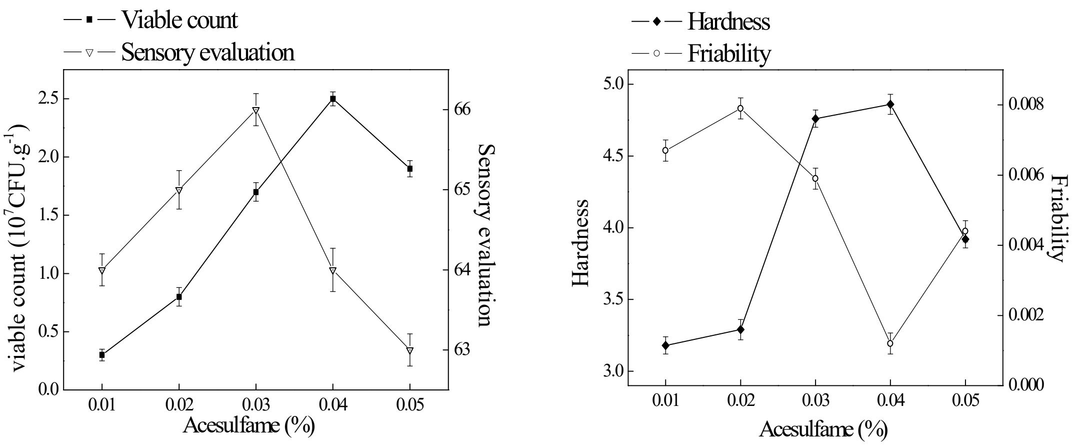

Figure 4*. Effect of acesulfame on LA-5 goat milk tablet*

Figure 4 shows that when the added amount of acesulfame increased gradually, the number of viable lactobacilli cells and the hardness increased at first and then decreased, the friability decreased at first and then increased when the dosage was slightly larger, which would cause goat milk tablets to be too sweet and to decrease their sensory evaluation. When the amount of acesulfame was 0.04 %, the viable lactobacilli count and the hardness were 2.5×10^{7} CFU·g^{-1} and 4.86 kgf, respectively, the friability reached its minimum value of 0.12 %, at good sensory evaluation. Therefore, the addition of 0.04 % of acesulfame was selected to provide some technical basis for further experiments.

Effect of D-sodium ascorbate on *Lactobacillus acidophilus* goat milk tablet quality

The addition of D-sodium ascorbate was 0.04 %, 0.05 %, 0.06 %, 0.07 % and 0.08 %, respectively, 7 % sucrose, 1.3 % glucose, 5 % xylitol, 0.04 % acesulfame and 0.1 % of *Lactobacillus acidophilus* bacteria powder, 0.4 % of microcrystalline cellulose and goat milk powder mixed and compressed into tablets. The parameters of goat milk tablet are shown in Figure 5.

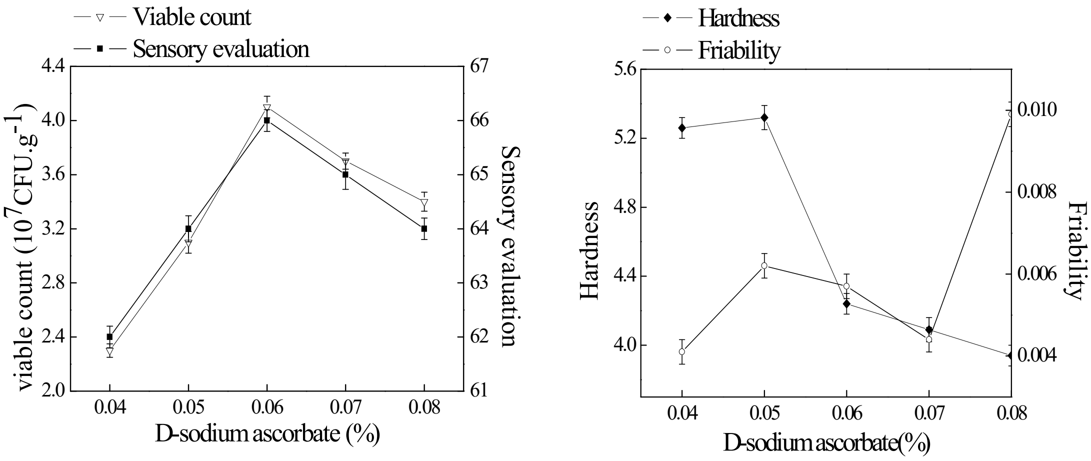

***Figure 5**. Effect of D-sodium ascorbate on LA-5 goat milk tablet*

D-sodium ascorbate as an antioxidant, has a certain role in promoting cell growth [26]. Figure 5 showed that when D-sodium ascorbate increased, the viable count, the sensory evaluation and the hardness of the goat milk tablets increased at first and then decreased, when the adding amount was 0.06 %, the number of viable bacteria, sensory evaluation and hardness were 4.1×10^7 CFU·g^{-1}, 65 and 4.3 kgf, respectively. While the friability was 0.57 % that maintained a trend decreased at first and then increased. Considering the index of viable count was prominent, therefore the content of 0.06 % D-sodium ascorbate was chose to conform the formulation of goat milk tablets.

Effect of tea polyphenols on *Lactobacillus acidophilus* goat milk tablet quality

The addition of tea polyphenols was 0.012 %, 0.014 %, 0.016 %, 0.018 %, and 0.02 %, respectively, 7 % sucrose, 1.3 % glucose, 5 % xylitol, 0.04 % acesulfame, 0.06 % D-sodium ascorbate, and 0.1 % of *Lactobacillus acidophilus* bacteria powder, 0.4 % of microcrystalline cellulose and goat milk powder were mixed and compressed into tablets. The results are shown in Figure 6.

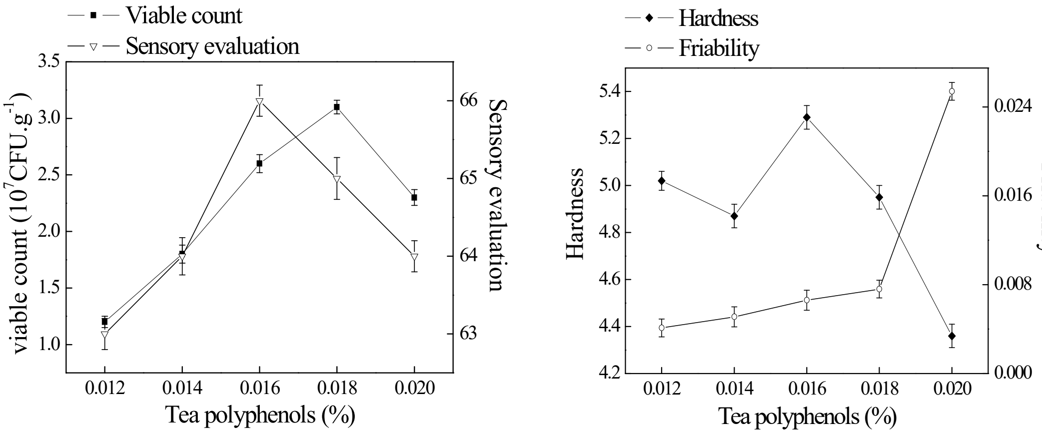

Figure 6*. Effect of tea polyphenol on LA-5 goat milk tablet*

Tea polyphenols not only as an antioxidant, but also gives special milk tea flavor [2, 23]. Figure 6 showed that when the adding amount of tea polyphenols was gradually increased, the number of live bacteria increased and hardness of goat milk tablets were improved at first and then decreased. When the addition of tea polyphenols was 0.018 %, the viable bacteria number reached maximum 3.1 × 10^7 CFU·g^{-1}, friability reached 0.46 % that showed an increasing trend with the concentration increased of tea polyphenols, and the sensory evaluation was also good. Yang *et al.* studied the important role of tea polyphenols in improving the intestinal environment and maintaining the balance of human body [23].

Effect of microcrystalline cellulose on *Lactobacillus acidophilus* goat milk tablet quality

The addition of microcrystalline cellulose was 0.2 %, 0.3 %, 0.4 %, 0.5 % and 0.6 %, respectively, 7 % sucrose, 1.3 % glucose, 5 % xylitol, 0.04 % acesulfame, 0.06 % D-sodium ascorbate, 0.018 % tea polyphenols, 0.1 % of *Lactobacillus acidophilus* bacteria powder, and goat milk powder were mixed and compressed into tablets. Taking goat milk tablets of different addition of microcrystalline cellulose to measure viable count, hardness, friability and sensory evaluation, the results are shown in Figure 7.
Microcrystalline cellulose as a binder, the right addition amount had good adhesive effect, but when the excessive addition will occur disintegration [27]. The Figure 7 showed that when the adding amount was gradually increased, the hardness of goat milk continued to drop, the number of viable bacteria increased first and then decreased, and friability showed an increasing trend. When the amount was 0.4 %, goat milk tablet hardness was 4.88 kgf, and the number of live bacteria and sensory evaluation reached maximum, 2.1 × 10^7 CFU·g^{-1} and 56, respectively, but friability was 0.51 %, which was the minimum, so the optimal concentration of microcrystalline was 0.4 %. In addition, Chan *et al* [28] reported mortality of 2 log for *Lactobacillus acidophilus* in hydroxypropyl cellulose and alginate tablets after 5 weeks of storage at 25 °C.

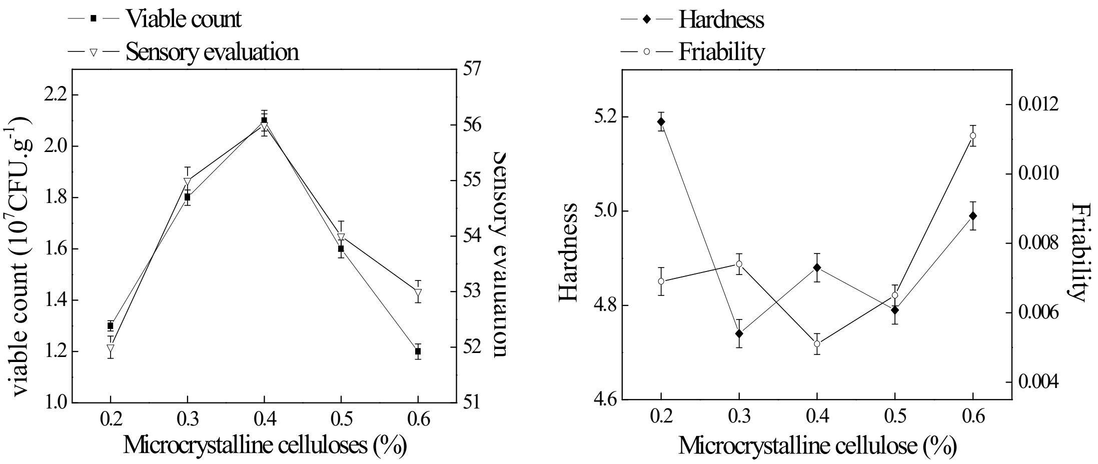

***Figure** 7. Effect of microcrystalline cellulose (MCC) on LA-5 goat milk tablet*

Effect of D-mannitol on *Lactobacillus acidophilus* goat milk tablet quality

The addition of D-mannitol was 0.1 %, 0.2 %, 0.3 %, 0.4 % and 0.5 %, respectively 7 % sucrose, 1.3 % glucose, 5 % xylitol, 0.04 % acesulfame, 0.06 % D-sodium ascorbate, 0.018 %, 0.4 % microcrystalline, and 0.1 % of *Lactobacillus acidophilus* bacteria powder, and goat milk powder were mixed and compressed into tablets. The parameters of goat milk tablet are shown in Figure 8.

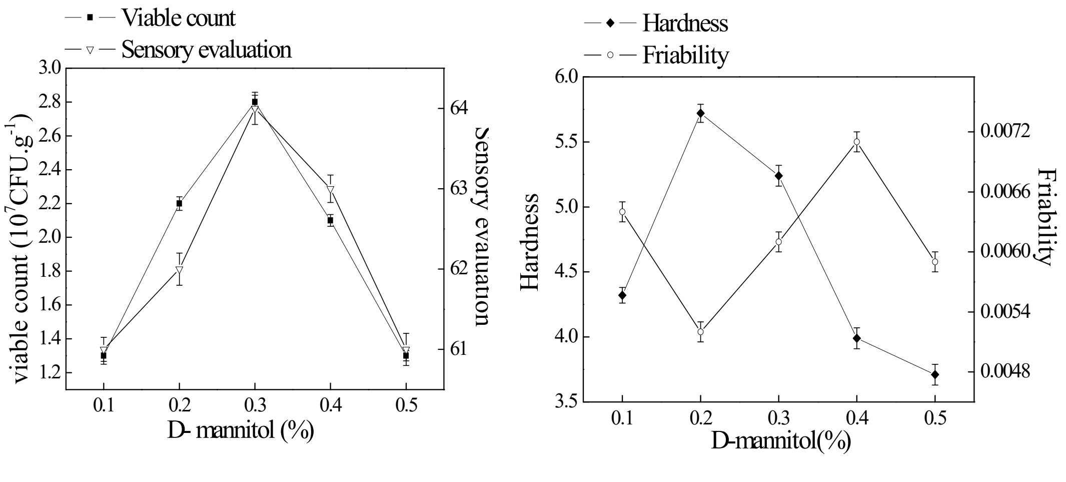

***Figure 8**. Effect of D-mannitol on LA-5 goat milk tablet*

Figure 8 showed that when the adding amount of D-mannitol increased gradually, the number of live bacteria goat milk tablets and sensory evaluation increased first and then decreased. the content of 0.3 % D-mannitol showed a significant turning point, the number of live bacteria and sensory evaluation reached the maximum value 2.8 × 10^7 CFU·g^{-1} and 64. However, when the content of D-mannitol was 0.2 %, hardness and friability showed a turning point, 5.74 kgf, 0.52 %, respectively, but the viable count and sensory evaluation were main indexes, therefore, the selection of 0.3 % D-mannitol as an experimental basis for PB screening.

CONCLUSIONS

In this study, the concentrations of different sugars (glucose, sucrose), alcohols (xylitol, D-mannitol) antioxidants (D-sodium ascorbate, tea polyphenols), food additives (acesulfame, microcrystalline cellulose) were optimized for the preparation of goat milk tablets with *Lactobacillus acidophilus* LA-5. The experimental results showed that the optimal tablet composition of the goat milk power included 7 % of sucrose, 1.3 % of glucose, 5 % of xylitol, 0.04 % of acesulfame, 0.06 % of D-sodium ascorbate, 0.018 % of tea polyphenols, 0.4 % of microcrystalline cellulose and 0.3 % of D-mannitol. The prepared goat milk tablets had higher viable lactobacilli count, suitable hardness and friability, at good sensory evaluation. This experiment also lays the theoretical foundation for the next experiments for the application of goat milk tablets in the industry. In addition, based on the formula, we can add some other food functional factors, such as Soybean isoflavone, Vitamin C, Alkaloid etc. to improve the health of the human body.

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