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THE EFFECT OF EIGHT THERMAL PROTECTANTS ON THE SURVIVAL RATE AND THE VIABLE COUNTS OF *LACTOBACILLUS CASEI* AFTER HEAT TREATMENT IN FERMENTED GOAT MILK

– Research paper –

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Abstract: In order to improve the survival rate of probiotics and produce probiotic goat milk from fermented goat milk of *Lactobacillus casei* L61 by spray drying. Spray drying has been applied to large-scale industrial production of milk powder due to its high efficiency and low cost. However, high temperatures in spray drying can result in the loss of large numbers of probiotic.The purpose of this paper is to study the effects of eight thermal protectants including skim milk, sucrose, glucose, β-cyclodextrin, gelatin, maltodextrin, glycerol, trehalose on the survival rate and viable counts of *L.casei* L61 after heat treatment by the single factor experiment. All protective agents have a positive effect on increasing the survival rate of *L.casei* L61 (p<0.05). The results indicated that the survival rates of *L.casei* L61 were up to the maximum of 10.94%, 1.13%, 3.04%, 0.21%, 6.97%, 0.075, 4.71% and 0.29%, while the additions of skim milk, sucrose, glucose, β-cyclodextrin, gelatin, maltodextrin, glycerol, trehalose were 20mg/L, 10%, 7%, 15%, 1.5%, 3%, 8mL/L, 10%, respectively; the viable counts after heat treatment are 19.69, 0.81, 1.78, 0.455, 12.2, 0.12, 2.75, 0.435(×10^6CFU/mL), respectively. This paper provides technical a reference for the development of probiotic goat milk powder.

Keywords: *Lactobacillus casei*; thermal protectants; spray drying; goat milk; heat resistance.

INTRODUCTION

Goat milk and its products are favored by consumers because of its composition close to human milk and have high levels of free amino acids (Haenlein, 2004). Probiotic microorganisms play an important role in the fermented food industry as starter culture (Chen et al., 2017). Commercial starter cultures have been widely used in fermented foods. The starter culture can contribute to the attributes of foods, such as texture, amora, and also inhibit some pathogenic microorganisms (Johansen，2018). The lactase enzymes from the starter culture in the dairy product ensure that the lactose in the human gut reaches a level that does not cause adverse reactions (Posecion et al., 2005).

The large-scale lactic acid bacteria drying products have attracted more and more attention in recent years with increasing market demand (Su et al., 2018). Spray drying and freezing drying are most common processes to obtain dry productions (Yonekura et al., 2014). Freeze-drying is a time-consuming and high cost process (Her et al., 2015; Agudelo et al., 2017). By contrast, spray drying is a faster, higher productivity technique that effectively transforms microorganisms into solids to increase shelf life in powder production (Gharsallaoui et al., 2007). However, probiotic microorganisms are usually sensitive to heat. High temperatures in spray drying can cause microbial cell damage (Nunes et al., 2017).

Thus, in order to prevent these damages, the thermal protectants were required to protect starter culture. Several types of protection for probiotics have been studied, trehalose has the best thermal protective potential (Nunes et al., 2017).

Mandal et al (2006) embedded *Lactobacillus casei* NCDC-29 in different concentrations of

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sodium alginate, and treated for a period of time under low pH, high bile salt concentration and heat treatment conditions. The test results showed that the Sodium alginate has a certain bacterial protection effect. The effect of different trehalose concentrations on the viable counts of spray-dried *rhamnose lactobacillus* GG and *Lactobacillus rhamnosus* E-97800 powder were studied (Sunny-Roberts et al., 2009; Ying et al., 2012). Ananta et al (2005) concluded that skim milk, oligofructose or polydextrose have a good protective effect on *Lactobacillus rhamnosus* GG. Chávez et al (2007) demonstrated that the sugars have protective effects by using soy protein and maltodextrin or skim milk and gum arabic as heat-resistant protective agents.

The *Lactobacillus casei*, a kind of gram-positive bacteria, can help to improve aberrant bowel movements and is effective against respiratory infections (Isolauri et al., 1991). *Lactobacillus casei* has been widely used in fermented foods because of its good cholesterol-lowering and anti-tumor effects (Her et al., 2015).

In the previous work, four strains with good antioxidant capacity and DPPH clearance rate have been screened, among which the most heat resistant is *L.casei* L61 (Chen et al., 2012). The optimal nutritional formula for antioxidant peptide production by *Lactobacillus casei* have been explored (Shu et al., 2018). In this study, the effects of eight thermal protectants (skim milk, sucrose, glucose, β-cyclodextrin, gelatin, maltodextrin, glycerol, trehalose) on the heat resistance of *Lactobacillus casei* L61 in fermented goat milk were investigated by single factor experiments. Subsequently, the optimal protection conditions of each thermal protectants were obtained and the protective mechanism of protective agents was preliminarily explored to provide technical support for the production of antioxidant peptide probiotic goat milk powder.

MATERIAL AND METHODS

Microorganism: *L.casei* L61, obtained from school of Food and Biological Engineering, shaanxi University of Science and Technology, was inoculated into the sterile MRS broth medium（MRS, Hopebio, Qingdao, China）, mixed well, and then cultured in a 37 ° C incubator for 24 hours to obtain a generation of activation solution, and then the above activation solution was taken as 5% (v/v) was inoculated in MRS broth medium and cultured at 37 ° C for 24 h to obtain a second-generation activating solution, and the activation was repeated for three generations, and the third-generation culture time was 18 h. The third-generation bacterial solution obtained above was inoculated into the reconstituted goat milk medium at a 5% (v/v) inoculum, and the mixture was thoroughly mixed, and then fermented to a complete curd in a 37 ° C constant temperature incubator, taken out and stored in the refrigerator at 4 ° C.

Preparation of Fermented Goat Milk: The defatted goat milk powder was formulated into a reconstituted milk with a concentration of 11% and pasteurized at 105 ° C for 15 min. After cooling, the activated strain of *L.casei* L61 was added according to the inoculation amount of 5%, and the fermentation was carried out in a constant temperature water bath at 41 ° C. After fermenting for 16 hours, the fermented goat milk was taken out.

Determination of cell counts: Before the heat treatment, the sample of fermented goat milk by *L.casei* L61 was diluted with MRS medium to determine the number of viable cells. The plates were carried out at 37℃ for 48 h. The viable counts of *L.casei* L61 (CFU/mL) were obtained in triplicates by plating on the plate and the results obtained were considered as “before heat treatment” data. After a 75 ℃ constant temperature water bath for 10 minutes, select the appropriate gradient plate coating to calculate the viable counts (Shu et al., 2015). These results mean after “heat treatment” data. The survival rate was calculated according to following equation:

Survival rates % = (CFU/mL after heat treatment / CFU/mL before heat treatment) ×100%.

RESULTS AND DISCUSSION

Effect of skim milk on viable counts and survival rate of *L.casei* L61

The skim milk powder is formulated into different concentration (0, 10, 15, 20, 25, 30 g/L (w/v)) and sterilized in a water bath at 95 ° C for 15 min. After cooling to room temperature. Add to the fermented milk and mix. After heat treatment in a constant temperature water bath at 75 ° C for 10 min, immediately take out and cool, dilute by gradient, and select two suitable gradients for plate coating, three parallels for each gradient, colony count and calculate cell viability. The result was shown in Figure 1. Adding a certain amount of skim milk to the fermented goat milk can enhance the heat resistance of the bacteria and increase the number of viable counts and the survival rate of the cells in the fermented milk per unit volume. In addition, with the increase of skim milk addition, the viable count and survival rate of the bacteria increased rapidly. When the amount of skim milk added was 20g/L, the viable count and survival rate reached the maximum, respectively, 19.69×10^6 CFU/mL. Compared with the control group, the viable count and survival rate were significantly improved ($p<0.01$). When the amount of skim milk added exceeded 20%, the viable count and survival rate of the cells decreased sharply. It can be seen that skim milk can have a good heat protection effect.

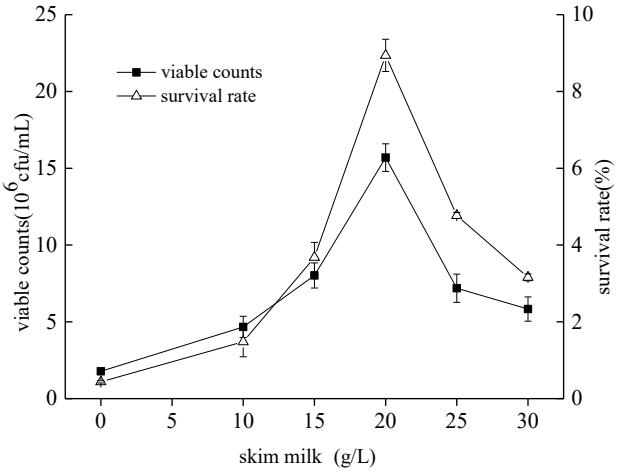

Figure 1. Effect of skim milk on the viable counts and survival rate of *L.casei* L61

Effect of sucrose on viable counts and survival rate of *L.casei* L61

A sterilized sucrose solution was added to the *Lactobacillus casei* L61 fermented milk to add 0, 2, 4, 6, 8, 10% (w/v), respectively, and the number of viable cells was determined after heat treatment to obtain cell survival rate, the result was shown in Figure 2.

Adding a certain amount of sucrose to the fermented goat's milk is conducive to the survival of the cells under high temperature conditions; as shown in the figure, after the high-temperature water bath is added to the fermented goat milk with sucrose, the viable cell count and cell viability are obvious. Higher than the control group without added sucrose. The results showed that the viable count and survival rate increased with the increase of sucrose addition in the range of sucrose addition ($p<0.05$). When the sucrose addition amount was 10%, the viable count and the cell viability reached the maximum, which were 0.81×106 CFU/mL, 1.13%, respectively.

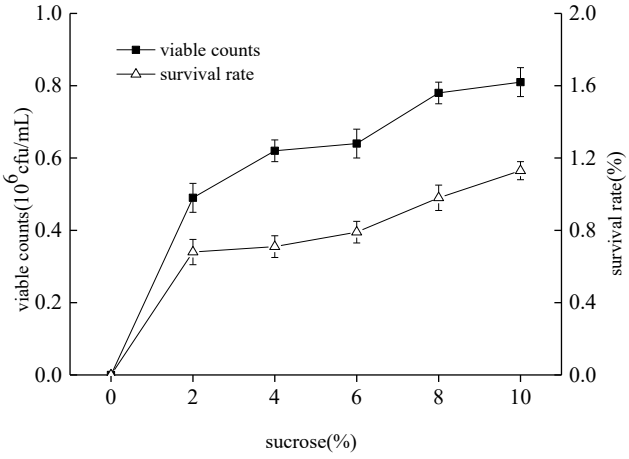

Figure 2. Effect of sucrose on the viable counts and survival rate of *L.casei* L61

Effect of glucose on viable counts and survival rate of *L.casei* L61

A sterilized glucose solution (0, 1, 3, 5, 7, 9% (w/v)) was added to the *L. casei* L61 fermented milk, and the survival rate was determined by the number of viable cells after heat treatment, the result was shown in Figure 3. When a certain amount of glucose is added, the number of viable counts and the survival rate after the high temperature water bath can be effectively improved compared with the fermented milk without added glucose ($p<0.05$). When the amount of glucose added was 7%, the viable cell count and cell viability reached the maximum, meaning 1.78×10^6CFU/mL and 3.04%, respectively.

Effect of β-cyclodextrin on viable counts and survival rate of *L.casei* L61

Sterilized beta-cyclodextrin solution was added to fermented milk of *Lactobacillus casei* L61, and the dosage was 0, 5, 10, 15, 20 and 25% (w/v), respectively. The viability of *Lactobacillus casei* L61 was calculated by counting the number of viable bacteria after heat treatment. And the results were various as shown in Figure 4. With the increase of dosage, the number of viable bacteria per unit volume and the survival rate of bacteria in the control group increased. When the dosage was 15%, the number of viable bacteria and the survival rate reached the maximum, which were 0.455×10^6 CFU/mL and 0.21%, respectively. The results showed that β-cyclodextrin had a certain effect on enhancing the heat resistance of *Lactobacillus casei* L61, but the heat protective effect was not particularly significant (p<0.05).

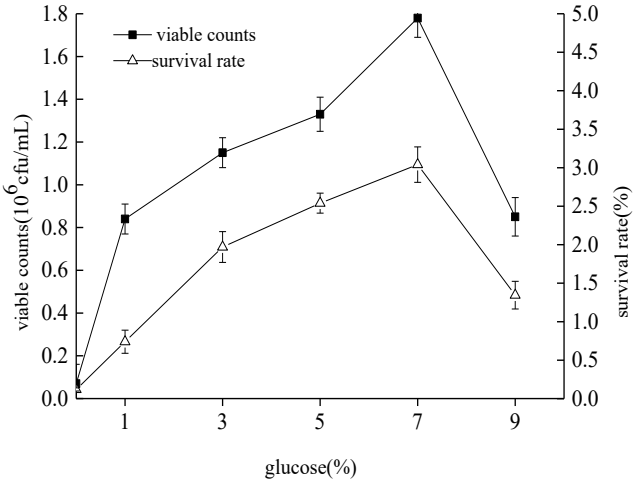

Figure 3. Effect of glucose on the viable counts and survival rate of *L.casei* L61

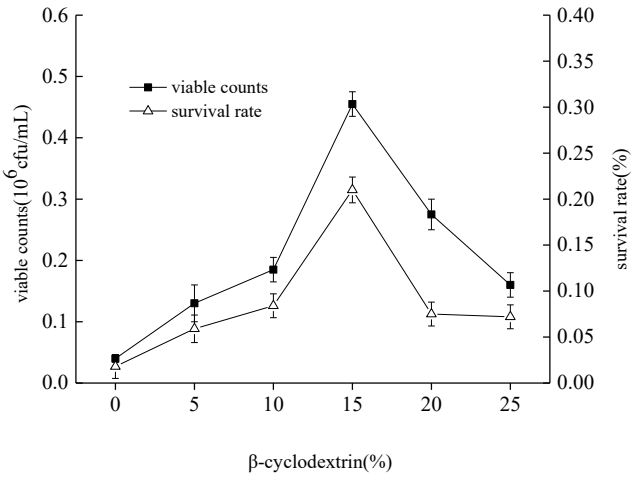

Figure 4. Effect of β-cyclodextrin on viable counts and survival rate of *L.casei* L61

Effect of gelatin on viable counts and survival rate of *L.casei* L61

The gelatin solution after sterilization and cooling was added to goat milk by fermented *Lactobacillus casei* L61. The additions of gelatin solution were 0, 0.5, 1.0, 1.5, 2.0 and 2.5% (w/v), respectively. The viable bacteria were counted after heat treatment to obtain the survival rate of the goat milk. The results were different as shown in Figure 5.

Figure 5 shows that when gelatin has a good heat-resistant protective effect on *Lactobacillus casei* in fermented goat milk, and different dosages of the same protective agent show different protective effects. From the broken line trend in the Figure 5, with the increase of gelatin content, the number of viable bacteria per unit volume and the viability of bacteria in fermented milk increased first and then decreased (p<0.05). When gelatin content was 1.5%, the number of viable bacteria and viability of bacteria was the highest, which were 12.2×10^6 CFU/mL and 6.97%, respectively.

Effect of maltodextrin on viable counts and survival rate of *L.casei* L61

The addition of sterilized and cooled maltodextrin solution to goat milk fermented by *L. casei* L61 was 0, 1, 3, 5, 7 and 9% (w/v). The viability of the bacteria was calculated by counting the number of viable bacteria after heat treatment. The results were shown in Figure 6. At first, the number of viable counts and survival rate showed a significant difference with different dosage of the same protective agent. When no protective agent was added, *L. casei* L61 was basically inactivated. With the increase of maltodextrin dosage, the number of viable counts and survival rate of fermented milk per unit volume increased sharply. When the dosage was 3%, the number of live bacteria and survival rate reached the maximum, which were 0.12×10^6 CFU/mL and 0.075%, respectively. When the addition of maltodextrin was more than 3%, it showed a slow downward trend. However, it can be seen that maltodextrin has poor thermal protective effect on *Lactobacillus casei*, and the solubility of maltodextrin is poor, so the follow-up is not a further research object (p<0.05).

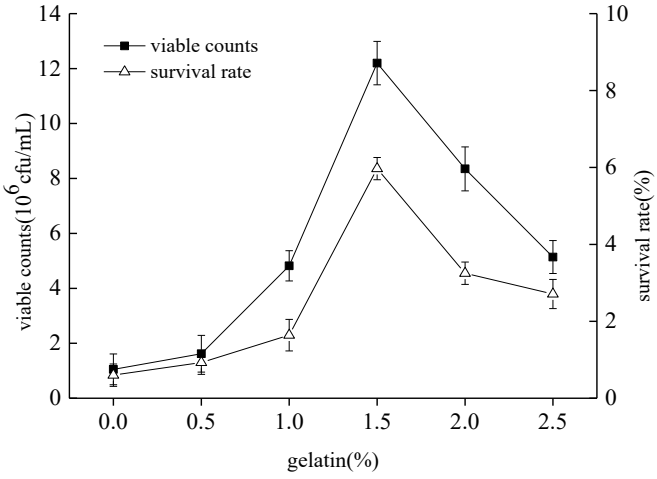

Figure 5. Effect of gelatin on the viable counts and survival rate of *L.casei* L61

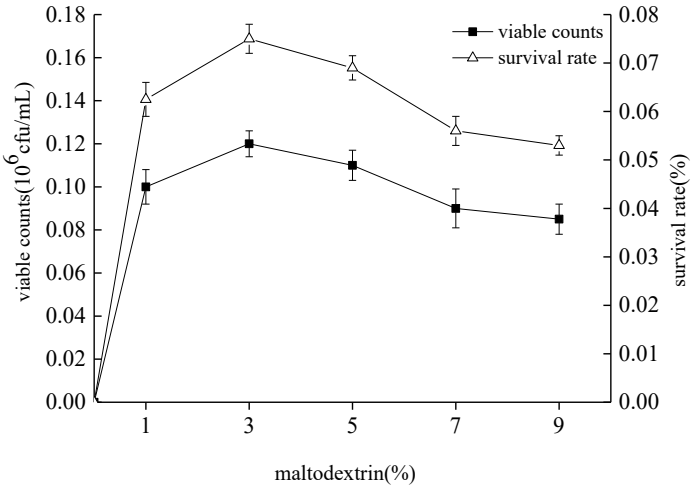

Figure 6. Effect of maltodextrin on viable counts and survival rate of *L.casei* L61

Effect of glycerol on viable counts and survival rate of *L.casei* L61

Adding sterilized and cooled glycerol solution to fermented goat milk by *Lactobacillus casei* L61 to make it add 0, 4, 6, 8, 10 and 12 mL/L (v/v) respectively. After heat treatment, the viable counts and the survival rate of the bacteria were calculated. The results were shown in Figure 7.

According to the Figure 7, within the range of glycerol addition, with the increase of glycerol addition, the number of viable bacteria and the survival rate reached the maximum at 8 mL/L of glycerol, which were 2.75 $\times 10^6$ CFU/mL and 4.71% respectively. With the continuous increase of glycerol content, the number of viable bacteria and the survival rate of bacteria in fermented milk decreased sharply. The results showed that the heat resistance of *Lactobacillus casei* L61 could be significantly enhanced by adding a certain amount of glycerol to fermented goat milk (p<0.01).

Pan et al. (2005) studied that when glycerol content increased from 1% to 5%, the survival rate of bacteria showed a gradual upward trend, which was consistent with the trend of this experiment.

Effect of trehalose on viable counts and survival rate of *L.casei* L61

The trehalose solution after sterilization and cooling was added to the fermented milk of *Lactobacillus casei* L61, and the amount of trehalose added was 0, 5, 10, 15, 20 and 25% (w/v), respectively. The colonies were counted after heat treatment and the survival rate of the bacteria was calculated. The results were shown in Figure 8.

A certain amount of trehalose was added to fermented goat milk as heat-resistant protector of *Lactobacillus casei* L61. As can be seen from the Figure 8, the number of viable bacteria and viability of fermented milk increased rapidly in unit volume. With the increase of trehalose content, the number of viable bacteria and viability increased first and then decreased (p<0.05). When the dosage increased to 10%, the number of viable bacteria and the survival rate of bacteria reached the maximum, 0.435×10^6 CFU/mL and 0.29%, respectively. With the increase of the dosage, the number of viable bacteria and the survival rate began to decrease.

The results of this study were consistent with those of *Lactobacillus acidophilus* and *Bifidobacterium* (Fan et al., 2011).

Studies have shown that the protective mechanisms of most protective agents can be roughly divided into three types: one is to enhance the tolerance of probiotic cells to adverse environments, the other is to provide some physical shield to protect the cells, and the third is that the protective agents have good drying kinetics (Agudelo et al., 2017; Su et al., 2018). Anekella,et,(2013) proved that maltodextrin ensured the high survival rate of probiotics in spray drying.

De Giulio et al. (2005): Some low molecular

weight sugars (maltose, glucose, trehalose and sucrose) were proved that these sugars as protectants enhanced the survival rate of LAB. These sugars can protect microorganisms by acting on the polar groups of phospholipids in cell membranes through hydrogen bonds (Su et al., 2018). Jae-young et al. (2014) study showed that the survival rate of Lactobacillus casei reached the highest when 1% sucrose solution was added in the process of SFD (Her et al., 2015) . Microencapsulation with trehalose has the best protective effect on Lactobacillus acidophilus (Nunes et al., 2017; Su et al., 2018). Gelatin is a denatured protein, which is easy to agglutinate when dissolved in water and has good water holding capacity. It can improve the heat resistance of bacteria by preventing cell deformation (Sultan et al.2015). Skim milk has a good thermal protective effect. The reason may be that whey protein can form a layer of protein membrane outside the cell wall of bacteria, which can reduce the damage of cell wall and cause the exudation of intracellular substances.

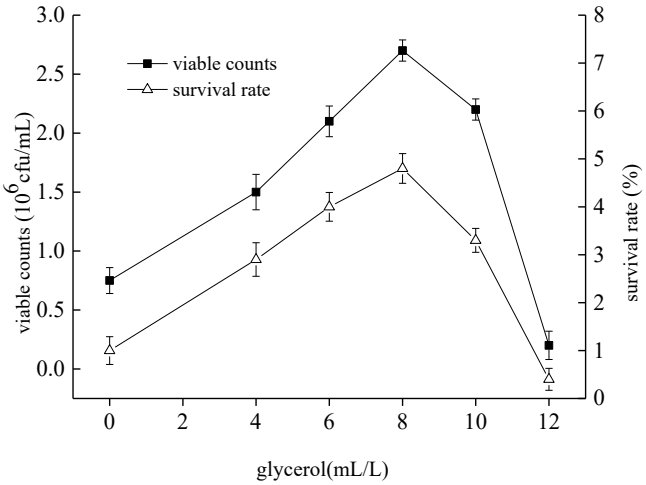

Figure 7. Effect of glicerol on the viable counts and survival rate of *L.casei* L61

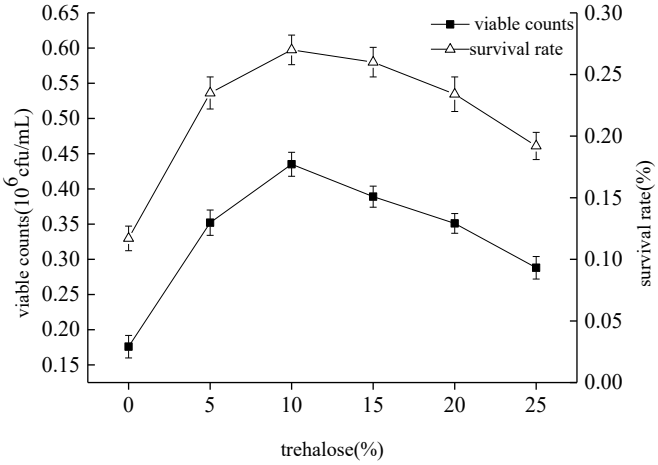

Figure 8. Effect of trehalose on viable counts and survival rate of *L.casei* L61

CONCLUSION

In this study, the effects of thermal protectants on the spray drying of goat milk fermented by *Lactobacillus casei* L61 had been verified. The survival rate of *Lactobacillus casei* was enhanced in varying extent by adding appropriate concentration of heat-resistant protectant in fermented goat milk. Among all the thermal protectants, skim milk, sucrose, glucose, gelatin, glycerol, trehalose exhibited significant promotion on the survival rate of *Lactobacillus casei* in heat treatment (p<0.05). The optimal of skim milk, sucrose, glucose, gelatin, glycerol, trehalose to goat milk was 20mg/L, 10%, 7%, 1.5%, 8mL/L, 10%, respectively. To the extent, the use of such heat-resistant protectants in spray drying offers a potential tool to increase the survival rate of *Lactobacillus casei*. The results of this experiment provide a reference for the development of composite heat-resistant protectant, and provide technical support for the development of antioxidant peptide probiotic goat milk powder.

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