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10.2478/aucft-2019-0009

EFFECT OF SODIUM TRIPOLYPHOSPHATE, GLYCEROL, VITAMIN B_2, MANNITOL AND SOLID MALT EXTRACT ON SURVIVAL OF *BIFIDOBACTERIUM BIFIDUM* DURING FREEZE-DRYING

– Short communication –

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Abstract: The effect of sodium tripolyphosphate, glycerol, vitamin B_2, mannitol and solid malt extract on the freeze-dried powder survival rate and the viable cells of the *B. bifidum* BB01 were studied by single-factor test in this study. The optimal concentration of protectant for *B. bifidum* during freeze-drying were 1.5% (W/V) sodium tripolyphosphate, 12% (W/V) glycerol, 6% (W/V) vitamin B_2, 6% (W/V) mannitol and 6% (W/V) solid malt extract, and the survival rate of bacteria was 18.63%, 22.98%, 24.13%, 24.19% and 39.77%, respectively.

Keywords: *Bifidobacterium bifidum*, freeze-drying, protective agents, probiotics.

INTRODUCTION

With the introduction of the concept of probiotics, probiotic food has potential market of functional food in the world, and its output value has exceeded 65% of the world's GDP (Holzapfel et al., 2006). As one of the most important probiotics, *B. bifidum* has been widely used in functional foods. Many physiological functions of *B. bifidum* have been reported: decrease serum cholesterol (Xiao et al., 2003; Beena et al., 1997); relieve lactose intolerance (Parracho et al., 2007; Shu et al., 2016); improve the digestive system (Guyonnet et al., 2009) and delay aging (Yamazaki et al., 2002). At present, the main way obtained by vacuum freeze-drying technology for the *B. bifidum* (Zhang et al., 2005). However, how to increase the survival rate of the bacteria in the freeze-drying process and improve the storage stability became a technical problem.

This research investigates five protective agents to reduce the mortality rate of the bacteria during the freeze-drying process. Single-factor tests are used to obtain the best concentration of sodium tripolyphosphate, glycerol, vitamin B_2, mannitol and solid malt extract, which provides certain reference for the optimization of the multiple cryoprotectant.

MATERIALS AND METHODS

Microorganism and media. *B. bifidum* BB01 was from School of Food and Biological Engineering, Shaanxi University of Science & Technology. MRS broth was used to activate and ferment the strain; MRS agar medium was used to determine the viable cells.

Certrifugal collection of B. bifidum broth. Activated *B. bifidum* was cultured in MRS broth at 37°C for 18h, and finally centrifuged at 6000rpm for 15min to obtain *B. bifidum* cells.

Freeze-drying process. Protectant of B. bifidum cells were firstly frozen at -45°C for 12-24h, and finally frozen at -55°C, 4-6pa for 24h using vacuum lyophilizer.

Viable counts. The dried powder was rehydrated with sterile saline and then measured viable bacteria counts when pre and post freeze-drying by standard plate count.

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Calculation of survival.

$$\text{Survival (\%)} = \frac{\text{viable counts after freeze} - \text{drying}}{\text{viable counts before freeze} - \text{drying}} \times 100\%$$

$$\text{The viable counts of power} = \frac{\text{total number of viable cells lyophilized}}{\text{power weight}} \times \text{bacterial suspension volume}$$

RESULTS AND DISCUSSIONS

Effect of sodium tripolyphosphate on viability of *B. bifidum* during freeze drying. Effects of various concentrations of sodium tripolyphosphate on viability of *B. bifidum* during freeze-drying were shown in Figure 1. With the concentrations of sodium tripolyphosphate increased from 0.5 to 1.5%, the viable counts and the survival rate of the bacteria rose rapidly, but between 1.5 to 2.5%, those both reduced. When add of sodium tripolyphosphate was 1.5%, the viable counts and the survival rate reached maximum which were 18.63% (the control group was 0.03%), 7.75×10^{10}cfu/g (the control group was 0.07×10^{10}cfu/g), respectively.

Sodium tripolyphosphate and sodium glutamate are of the same class of low molecular weight compounds. Wang et al (2013) found that when add of sodium glutamate was 7g/100mL as a protective agent, the survival rate of *C. tropical* was 27.92%. The same result was confirmed from our study. However, Zhao et al (2009) found that when add of sodium glutamate was 2.5% (W/V), the survival rate of *Oenococcus oeni* reach high at 72.4%. The reason may be due to individual differences of strains.

Effect of glycerol on viability of *B. bifidum* during freeze drying. Effects of various concentrations of glycerol on viability of *B. bifidum* during freeze-drying were shown in Figure 2. When add of glycerol was 12%, the survival rate and the viable counts of *B. bifidum* reached maximum 22.98%, 8.15×10^{10}cfu/g, respectively.

Zeng et al (2013) found that when the concentration of glycerol was 20g/L, the survival rate of *Lactobacillus casei* reached 60%, which was 53% higher than the control group. The results showed that the glycerol as protectant had an obvious effect on viability of bacteria during freeze-drying.

Effect of vitamin B_2 on viability of *B. bifidum* during freeze drying. Effects of various concentrations of vitamin B_2 on viability of *B. bifidum* during freeze-drying were shown in Figure 3.The viable counts and the survival rate of the bacteria increased slowly during 4%-6%, but when 6 to 10%, those both decreased rapidly. When the concentration of vitamin B_2 was 6.0%, the survival rate and the viable counts of bacteria were the highest (24.13%), 6.59×10^{10}cfu/g, respectively.

Zhang et al (2013) found that when add of vitamin B_2 was 1% (W/V), the survival rate and the viable counts of *Lactobacillus bulgaricus* were 16.3%, 1.26×10^{11} cfu/mL, respectively. This result showed that vitamin B_2 as a protectant, the influence was not very obvious compared with other protective agents.

Effect of mannitol on viability of *B. bifidum* during freeze drying. Effects of various concentrations of mannitol on viability of *B. bifidum* during freeze-drying were shown in Figure 4. During 2 to 6%, the survival rate and the viable cells of *B. bifidum* increased rapidly and reached maximum 24.19%, 8.99×10^{10}cfu/g, respectively.

However, Zhang et al (2010) found that when the concentration of mannitol was 50g/L, the survival rate of *Bifidobacterium bifidum* reached 88.34%, which was 85.72% higher than the control group.

Effect of solid malt extract on viability of *B. bifidum* during freeze drying. Effects of different concentrations of solid malt extract on survival of *B. bifidum* during freeze-drying were shown in Figure 5. With the concentrations of solid malt extract increased from 3 to 6%, the survival rate and the viable counts of bacteria increased progressively, while both of them decreased with the solid malt extract was 6%-15%. When the concentration of solid malt extract was 6.0%, the survival rate and the viable counts of bacteria reach their maximum value which were 39.77%, 1.75×10^{11}cfu/g, respectively.

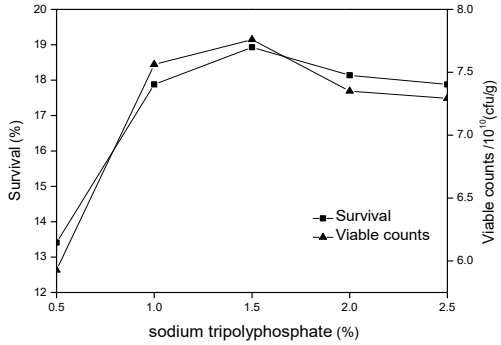

Figure 1. Effect of sodium tripolyphosphate on viability and survival of *B. bifidum*

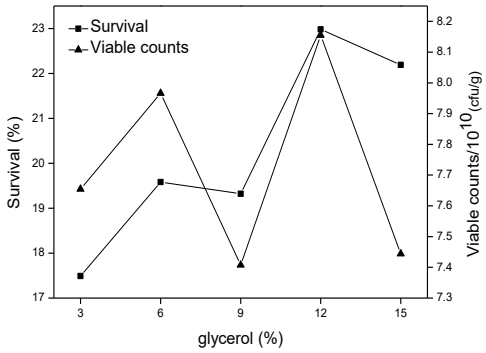

Figure 2. Effect of glycerol on viability and survival of *B. bifidum*

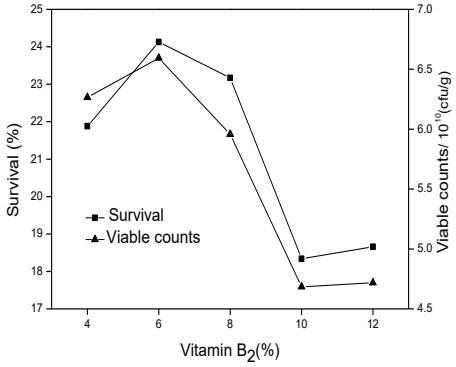

Figure 3. Effect of vitamin B_2 on viability and survival of *B. bifidum*

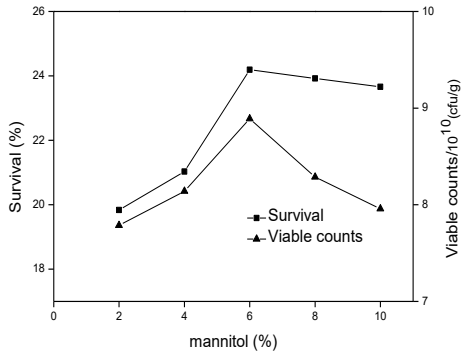

Figure 4. Effect of maniol on viability and survival of *B. bifidum*

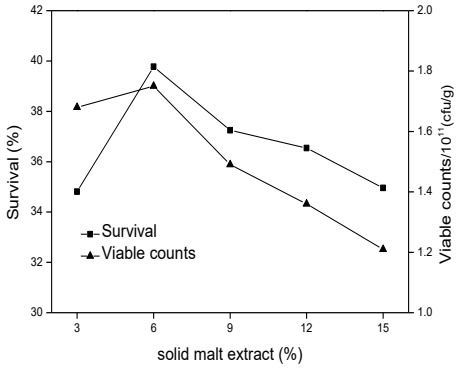

Figure 5. Effect of solid malt extract on viability and survival of *B.bifidum*

The research indicated that it was difficult to obtain a satisfactory protective effect for a single protective agent (Dong et al., 2007; Yuan et al., 2003). Meanwhile, it was well known that malt extract was rich in maltose, glucose, laevulose, peptide, vitamin and so on (Huang, 2012). Therefore, solid malt extract that was used as protective agent of *B. bifidum* was better than other kinds of single protective agent during freeze-drying.

CONCLUSIONS

Adding of sodium tripolyphosphate, vitamin B_2, glycerol, solid malt extract and mannitol as protective agents had an obvious effect on viability of *B. bifidum* BB01 during freeze-drying. In addition, adding of sodium tripolyphosphate had minimal effects while solid malt extract had imperative influence on survival rate of *B. bifidum*. When the concentration of solid malt extract was 6.0% (W/V), the survival rate and the viable cells of *B. bifidum* both reached maximum 39.77%, 1.75×10^{11}cfu/g.

ACKNOWLEDGEMENTS
The research was funded by the Key Research and Development Program of Shaanxi (Program No. 2019ZDLNY06-03) the Science and Technology Bureau of Weiyang District (No.201831) and the science and technology project of Weinan city (No.2017CXFC-27).

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