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Effect of Cell Suspension-Alginate Ratio, Tween 80 and Oil-Water Ratio on Microcapsulation of *B. bifidum BB01* and *BB28*

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There are many reports on the study of microencapsulated bifidobacteria, the effect of concentration of sodium alginate, emulsifying time and immobilized time on microcapsulation of *B. bifidum BB28* and *BB01* have been invesgated. This study reported the viable counts and encapsulation yield (EY) of *B. bifidum BB01* and *BB28* encapsulated in different cell suspension-alginate ratios (1:5, 1:10, 1:15 and 1:20), in different Tween 80 content(0.2%, 0.4%, 0.6% and 0.8%) and in different oil-water ratios (1:4, 1:5, 1:6 and 1:7). It was studied by single factor experiment method, the results showed that this several factors impacted the viable counts and encapsulation yield (EY) of *B. bifidum BB01*and *BB28* significantly, and the optimum cell suspension-alginate ratios for *B. bifidum BB01 and BB28* were 1:5 and 1:10 respectively; the optimum Tween 80 concentration for *B. bifidum BB01 and BB28* were 0.8% and 0.6% respectively; the optimum oil-water ratios were 1:6 and 1:4 respectively.

Key words: Alginate, Bifidobacterium *bifidum*, Microencapsulation, Emulsion.

Probiotics are living microorganisms which are beneficial to human health when administered in adequate amounts (FAO/WHO, 2002; Guarner & Schaafsma, 1998). Bifidobacteria species have shown beneficial effects on immunomodulation and on the prevention of various intestinal diseases (Servin and Coconnier, 2003; Shah, 2007). However, in order to exert these beneficial effects for probiotics, they must be able to tolerate the acidic conditions of the stomach environment and the bile in the small intestine (Doleyres *et al*., 2004; Gardiner *et al*., 2000). The acidic environment of the stomach and the bile salts secreted into the duodenum are the main obstacles for the survival of the ingested bacteria. The tolerance of bifidobacteria to the pH values of the gastric juice is generally considered low (Matsumoto *et al*., 2004; Takahashi *et al*., 2004; Collado and Sanz, 2006; Charteris *et al*., 1998).What's more, the survival of probiotics during processing and storage of food is also essential for the development of products that have an adequate amount of viable cells (Champagne *et al*., 2005; Mattila-Sandholm *et al*., 2002; Stanton *et al*., 2005).

Microencapsulation is a packaging technology that using thin polymer coatings applied to solid or liquid droplets or gaseous material. To a certain extent, it can be used to protect the living bacteria (Anal & Stevens, 2005; Kailasapathy & Masondole, 2005). Protection of bifidobacteria by microencapsulation has been investigated (Lu-E. Shi *et al*., 2013; Capela *et al*., 2006 ; Chen *et al*., 2006 ; Heidebach *et al*., 2010). Many researchers have committed to study the microencapsulation of *Bifidobacterium*, for example, Stephanie S and co–workers (2012) have investigated the properties of microencapsulated *Bifidobacterium BB12*. However, there are little information about microencapsulated *Bifidobacterium BB0I* and *BB28*.

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In previous work, the effect of alginate and cell suspension on microcapsulation of *B. bifidum BB28* have been study (Chen *et al*., 2012). At the same time, the effect of concentration of sodium alginate,emulsifying time and immobilized time on microcapsulation of *B. bifidum BB28* and *BB01* also have been invesgated. The aims of this paper were to study some factors, which affect the process of microencapsulated *Bifidobacterium BB0I* and *BB28*, such as cell suspension-alginate ratios, Tween 80 concentration, oil-water ratios. The optimum conditions of microencapsulated *Bifidobacterium BB0I* and *BB28* will be observed. The results will be helpful to further optimize the process of *Bifidobacterium* microencapsulation, and provide reference for obtaining higher viable counts and entrapped yield of *Bifidobacterium* microcapsules.

MATERIALS AND METHODS

Materials

Bifidobacterium BB0I and *BB28* were used at active material for the microcapsules, they were obtained from College of Life Science & Engineering, Shaanxi University of Science & Technology. Alginate (Luo Senbo Technology Co., Ltd. Xi'an)was used as carrier agents. MRS broth (Hope Bio-Technogy Co., Ltd.Qingdao). Tween 80 (Chemical Reagent Factory, Dongli,Tianjin). Soybean oil (Fu Oil Co. Ltd. Shaanxi). All the chemicals used were of analytical grade. Centrifuge (LG10-2.4) was used to obtain bacteria suspension.

Microorganism

Bifidobacterium BB0I and *BB28* were cultured in MRS medium at 37°C for 24h, respectively. The cells were harvested by centrifugation at 4000g for 10 min at 4°C and washed twice before resuspending them in 5mL normal saline. The final cell concentration was adjusted to 1.0×10^{11} cfu/mL.

Microencapsulation.

Bifidobacterium BB0I and *BB28* were encapsulated in sodium alginate matrix. Sodium alginate solutions were prepared, sterilized by autoclaving (120°C for 15 min) and cooled to 38–40°C. Sodium alginate solutions (5mL, 10mL, 15mL or 20mL) and 1mLof cell suspension were transferred into a centrifuge tube and the content was vortexed to homogeneity. Soybean oil (36mL,66mL,96mL,126mL) containing Tween 80 0.2%,0.4%,0.6%,0.8% was taken in a beaker (300mL) and to this the alginate–cell mixture was added dropwise while stirring magnetically. After 15 min, a uniformly turbid emulsion was obtained to which 2% calcium chloride was quickly added for hardening of microcapsules and breaking the emulsion. The capsules were harvested by centrifuging at 350g for 10 min.

Viable count

The sample to be tested with sterile saline solution into the bacterial suspension, then it was diluted at 10 times, and taking the dilution of 10^{-7} to 10^{-8} of the suspension inoculation of 1mL to the top agar medium. After the bacterias were cultured for 48h at 37°C, we can observe and count the average values, and investigate the various factors on the microencapsulation of Bifidobacterium viable counts. The viable counts of microcapsules were weight through a formula according to Eq. (1)

$$VC=N\times T\times 10 \quad ..(1)$$

Where VC is viable counts of the original suspension on a per milliliter (cfu/mL).N is average colony number of 3 repeat solid culture in the same dilution (cfu) .T is times of dilution.

Encapsulation yield (EY)

Encapsulation yield (EY), which is a combined measurement of the efficiency of entrapment and survival of viable cells during the microencapsulation procedure, was calculated according to Eq. (2)

$$EY=N/N_0\times 100\% \quad ...(2)$$

Where N is the number of viable entrapped cells released from the microspheres, and N_0 is the number of free cells added to the biopolymer mix during the production of the microspheres.

RESULTS AND DISCUSSIONS

Effect of cell suspension-alginate ratio on encapsulation of *B. bifidum BB28* and *BB28*

According to the initial preparation conditions of microcapsulation, the difference proportion of prepared bacteria suspension volume (mL) and sodium alginate solution volume (mL)

were investigated, such as 1:5, 1:10, 1:15, and 1:20. The effect of various cell suspension-alginate ratios on encapsulation of *B. bifidum BB28* and *BB28* are shown in Fig. 1 and Fig. 2.

According to Fig.1 and Fig.2, with increasing of the proportion of sodium alginate and bacteria suspension, the viable counts and entrapped yield of *B. bifidum BB01* microcapsules continually decreased, this phenomenon may be due to the high proportion of sodium alginate and bacteria suspension .While the viable counts and entrapped yield of *B. bifidum BB28* microcapsules increased at first, and then decreased. When the cell suspension-alginate ratio was 1:10, the viable counts and entrapped yield of *B. bifidum BB28* of microcapsules up to 2.3×10^{9}cfu/mL and 74%, respectively. Although the more volume of bacterial suspension lead to the more number of core material of the microcapsule contained and the viable counts and entrapped yield should be very high, the bacterial suspension volume increased, sodium alginate solution volume will be reduced. As a result, the phenomenon of incomplete embedded will emerge, and most of the cells were not embedded strongly. Howerve, with increasing volume of sodium alginate, the entrapped yield and viable counts contained in microcapsules could gradually increase, but when the sodium alginate solution volume was too much, the microcapsule membrane will be thick, and inclusion of microcapsule was few, so the entrapped yield and viable counts contained in microcapsules declined again.

As a result, there is a preliminary

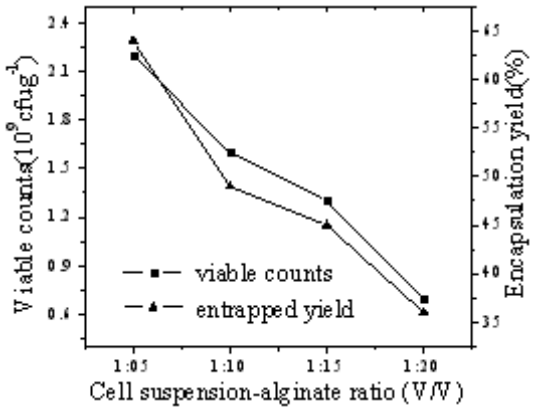

Fig. 1. Effect of cell suspension-alginate ratio on viable counts and entrapped yield of *B. bifidum BB01* of microcapsules

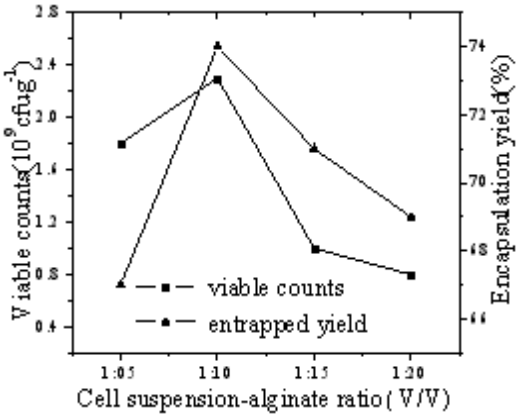

Fig.2. Effect of cell suspension-alginate ratio on viable counts and entrapped yield of *B. bifidum BB28* of microcapsules

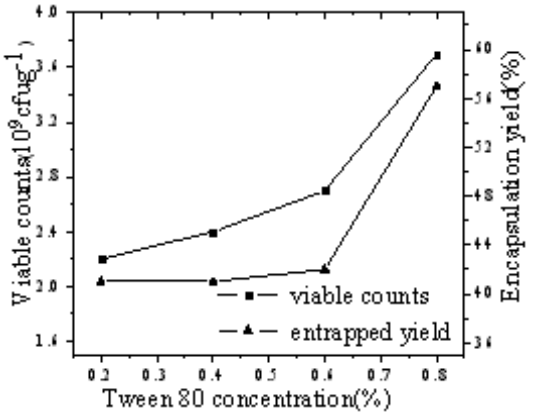

Fig. 3. Effect of Tween 80 concentration on viable counts and entrapped yield of *B. bifidum BB01* of microcapsules

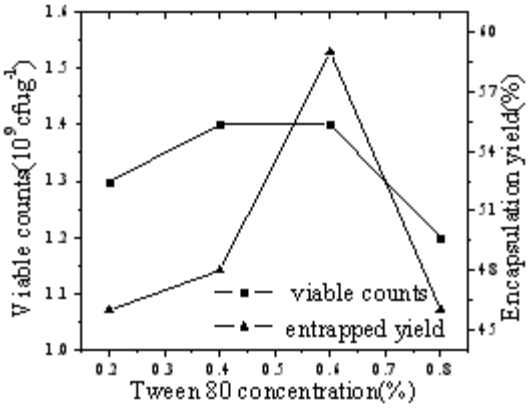

Fig. 4. Effect of Tween 80 concentration on viable counts and entrapped yield of *B. bifidum BB28* of microcapsules

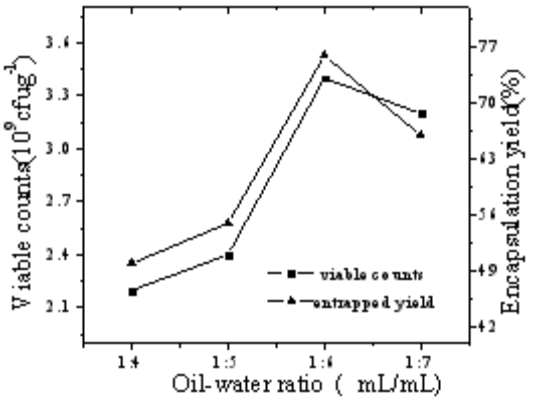

Fig. 5. Effect of oil-water ratio on viable counts and entrapped yield of *B. bifidum BB01* of microcapsules

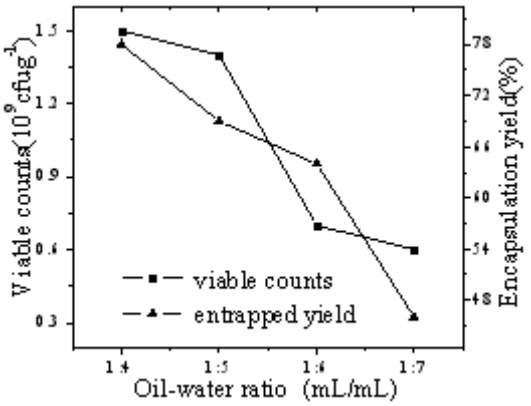

Fig.6. Effect of oil-water ratio on viable counts and entrapped yield of B. bifidum BB28 of microcapsules

determination about the cell suspension-alginate ratio for *B. bifidum BB01* and *BB28* microencapsulated. For *B. bifidum BB01*, the optimum cell suspension-alginate ratio was 1:5, which corresponds to viable counts and entrapped yield were 2.2×10^9 cfu/mL and 64%, respectively. For *B. bifidum BB28*, the optimum cell suspension-alginate ratio was1:10, which corresponds to viable counts and entrapped yield were 2.3×10^9 cfu/mL and 74%, respectively.

Effect of Tween 80 concentration on encapsulation of *B. bifidum BB28* and *BB28*

According to the initial preparation conditions of microcapsulation, Tween 80 concentration was adjusted to 0.2%, 0.4%, 0.6% and 0.8%, the results as shown in Figure 3 and Figure 4.

According to Fig.3 and Fig.4, with increasing of Tween 80 concentration, the viable counts and entrapped yield of *B. bifidum BB01* microcapsules continually increased. While the viable counts and entrapped yield of *B. bifidum BB28* microcapsules increased at first, and then decreased. When the Tween 80 concentration was 0.6%, the viable counts and entrapped yield of *B. bifidum BB28* microcapsules up to 1.4×10^9cfu/mL and 59%, respectively.The role of emulsifier is that reduce the interfacial tension of oil-water two-phase flow, and make the system stable, but the amount of emulsifier must be appropriate. If the amount is too little, emulsifier cannot make each droplet package completely in a continuous phase, it loosely arranged in small droplet surface and oil phase, so it does not allow the interfacial tension of oil-water two-phase flow down to the lowest, however, when the amount is too large, emulsifier will make the small droplet radius becomes too small.

As a result, there was a preliminary determination about the Tween 80 concentration for *B. bifidum BB01* and *BB28* microencapsulated. For *B. bifidum BB01*, the optimum Tween 80 concentration was 0.8%, which corresponds to viable counts and entrapped yield were 3.7×10^9cfu/mL and 57%, respectively. For *B. bifidum BB28*, the optimum Tween 80 concentration was 0.6%, which corresponds to viable counts and entrapped yield were1.4×10^9cfu/mL and 59%, respectively.

Effect of oil-water ratio on encapsulation of *B. bifidum BB28* and *BB28*

According to the initial preparation conditions of microcapsulation, the different proportion of mixed bacteria liquid volume (mL) and oil volume (mL)were adjusted to 1:4,1:5,1:6,1:7, the results as shown in Figure 5 and Figure 6.

According to Fig.5 and Fig.6, with increasing of the oil-water ratio, the viable counts and entrapped yield of *B. bifidum BB01* microcapsules increased at first, and then decreased. While the viable counts and entrapped yield of *B. bifidum BB28* microcapsules continually decreased. When the oil-water ratio was 1:6, the viable counts and entrapped yield of *B. bifidum BB01* microcapsules up to3.4×10^9cfu/mL and 76%, respectively. The reason of this tendency on figure was that the value about proportion of water and oil was too large. When the proportion of water and oil was low, small droplets which oil layer was

very thin, were wrapped in emulsion system of W/O type, the droplets colliding with each other in the continuous phase of water caused phenomenon of adhesion, when adding curing agent, the large particles and poor stability microcapsule was formed. But with the increase of the oil volume, the layer of wrapped small droplets became thicker, so water droplets were separated by thick oil layer, and the adhesive will be greatly reduced.

As a result, there is a preliminary determination about the oil-water ratio for *B. bifidum BB01* and *BB28* microencapsulated. For *B. bifidum BB01*, the optimum oil-water ratio was1:6, which corresponds to viable counts and entrapped yield were 3.4×10^9 cfu/mL and 76%, respectively. For *B. bifidum BB28*, the optimum oil-water ratio was 1:4, which corresponds to viable counts and entrapped yield were1.5×10^9cfu/mL and 78%, respectively.

CONCLUSIONS

This present work showed that several factors, including cell suspension-alginate ratio, Tween 80 and oil-water ratio, have an important influence on microcapsulation of *B. bifidum BB01* and *BB28*. The optimum cell suspension-alginate ratios for *B. bifidum BB01 and BB28* were 1:5 and 1:10 respectively; the optimum Tween 80 concentration for *B. bifidum BB01 and BB28* were 0.8% and 0.6% respectively; the optimum oil-water ratios were 1:6 and 1:4 respectively.

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