Research Article
Screening of Emulsification Conditions on Microcapsulation of *B. bifidum BB*01 and *BB*28 by Plackett-burman Design

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Abstract: This present study is based on the single factor test and the significant factors of emulsification conditions, which influence the process of microencapsulation of *B. bifidum BB*28 and *BB*01, will be screen out by Plackett-Burman experimental. Bifidobacterium *BB*0I and *BB*28 were encapsulated by using emulsion method respectively. Several factors, such as alginate concentration, cell suspension-alginate ratios, $CaCl_2$ concentration, Tween 80 concentration, emulsification time, encapsulating time and oil-water ratios have been investigated, the results showed that sodium alginate concentration, cell suspension-alginate ratios, encapsulating time and emulsification time have a significant effect for *Bifidobacterium BB*01 microcapsules; $CaCl_2$ concentration, cell suspension-alginate ratios, sodium alginate concentration and emulsification time have a significant effect for *Bifidobacterium BB*28 microcapsules.

Keywords: Bifidobacterium *bifidum*, emulsion, microencapsulation, plackett-burman design

INTRODUCTION

Probiotics are viable microorganisms which are beneficial to the host when administered in adequate amounts (Xiao *et al*., 2011). They are responsible for the protection of the human body from infections, especially along the colonized mucosal surfaces of the gastrointestinal tract (Fritzen-Freire *et al*., 2010; Sanders, 2003). Bifidobacterium is the most common genera of bacteria used as probiotics for the production of dairy products (Stephanie *et al*., 2012; Mohammadi *et al*., 2011). In order to exert 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. However, 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) and the bifidobacteria are vulnerable to oxygen. As a result, the viable counts of bifidobacteria in probiotic dairy products often have a exponential curve downward trend, which lead to those products have a difficult to achieve the healthy effect (Thomas *et al*., 2009).

Microencapsulation is a technology that can be used to render physical protection and improve the stability of probiotic organisms in functional food products (Kailasapathy, 2006; Patel and Patel, 2010; Brinques and Ayub, 2011). The main purpose of this technology is to protect probiotic organisms against an unfavorable environment and to allow their release in a viable and metabolically active state in the intestine (Filomena *et al*., 2012). In the process of microencapsulation of Probiotics, emulsion which is one of different techniques, should be used commonly (Gouin, 2004; Ribeiro *et al*., 1999).

When using the emulsion technique, many factors will influence the process of microencapsulation. The previous work aimed to study the effect of emulsification conditions on microcapsulation of *B. bifidum BB*28 and *BB*01 by using single factor test, such as cell suspension-alginate ratios, oil-water ratios, Tween 80 content and so on (He *et al*., 2012). However, the significant factors have not been screened out. This present study is based on the single factor test and the significant factors of emulsification conditions, which influence the process of microencapsulation of *B. bifidum BB*28 and *BB*01, will be screen out by Plackett-Burman experimental.

MATERIALS AND METHODS

Materials: *Bifidobacterium BB*0I and *BB*28 were used at active material for the microcapsules; they were

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obtained from College of Life Science and Engineering, Shaanxi University of Science and 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). Calcium chloride (Zhiyuan Chemical Reagent Co., Ltd. Tianjin). All the chemicals used were of analytical grade. Centrifuge (LG10-2.4) was used to obtain bacteria suspension.

Microorganism: *Bifidobacterium BB*0I and *BB*28 were cultured in MRS medium at 37°C for 24 h, respectively. The cells were harvested by centrifugation at 4000 g for 10 min at 4°C and washed twice before re-suspending them in 5 mL normal saline. The final cell concentration was adjusted to 1.0×10^{11} CFU/mL.

Microencapsulation: The sodium alginate solution was prepared, sterilized by autoclaving (120°C for 15 min) and cooled to 38-40°C. The bacteria suspension of *Bifidobacterium BB*0I and *BB*28 were encapsulated in sodium alginate matrix, respectively, then mixture was added to the vegetable oil containing Twain 80. After several minutes, the emulsion will be taken shape and injected into the CaCl₂ solution with sterile pressure nozzle. Continuing to stir the solution after a period of time, the microcapsule will be obtained. Finally, the microcapsules were filtered with gauze and washed with physiological saline for 3 times. The cell suspension-alginate ratios for *B. bifidum BB*01 and *BB*28 were 1:5 and 1:10, respectively; the Tween 80 concentration for *B. bifidum BB*01 and *BB*28 were 0.8 and 0.6%, respectively; the oil-water ratios were 1:6 and 1:4, respectively; the sodium alginate concentration for *B. bifidum BB*01 and *BB*28 were 2 and 2.5%, respectively; the encapsulating time for *B. bifidum BB*01 and *BB*28 were 13 and 15 min, respectively; the

emulsification time for *B. bifidum BB*01 and *BB*28 were 15 and 15 min, respectively; the CaCl₂ concentration for *B. bifidum BB*01 and *BB*28 were 1 and 1%, respectively.

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 1 mL to the top agar medium. After the bacterias were cultured for 48 h 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):

$$V_C = N \times T \times 10 \tag{1}$$

where,
V_C = Viable counts of the original suspension on a per milliliter (CFU/mL)
N = Average colony number of 3 repeat solid Culture in the same dilution (CFU)
T = 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):

$$EY = N/N_0 \times 100\% \tag{2}$$

where,
N = The number of viable entrapped cells released from the microspheres
N_0 = The number of free cells added to the biopolymer mix during the production of the microspheres

Table 1: The factors levels for emulsification conditions of plackett-burman of monolayer *B. bifidum BB*01 and *BB*28 microcapsules

	Factor level	X1 (%) (sodium alginate)	X3 (cell suspension-alginate ratios)	X5 (%) (CaCl₂)	X7 (%) (tween 80)	X9 (min) (emulsification time)	X10 (min) (encapsulating time)	X11 (oil-water ratios)
*B. bifidum BB*01	1	2	1:5	1	1	15	13	6:1
	-1	1.6	1:4	0.8	0.8	12	10	4.8:1
*B. bifidum BB*28	1	2.5	1:10	1	0.6	15	15	4:1
	-1	2	1:8	0.8	0.5	12	12	3.2:1

Table 2: The experimental design and results for emulsification conditions of plackett-burman of monolayer *B. bifidum BB*01 and *BB*28 microcapsules

Run	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11	Y1 (%)	Y2 (%)
1	1	-1	1	-1	-1	-1	1	1	1	-1	1	36.85	31.79
2	1	1	-1	1	-1	-1	-1	1	1	1	-1	40.39	35.84
3	-1	1	1	-1	1	-1	-1	-1	1	1	1	39.11	63.74
4	1	-1	1	1	-1	1	-1	-1	-1	1	1	36.42	31.90
5	1	1	-1	1	1	-1	1	-1	-1	-1	1	37.74	34.88
6	1	1	1	-1	1	1	-1	1	-1	-1	-1	33.90	65.15
7	-1	1	1	1	-1	1	1	-1	1	-1	-1	39.51	35.50
8	-1	-1	1	1	1	-1	1	1	-1	1	-1	39.88	67.51
9	-1	-1	-1	1	1	1	-1	1	1	-1	1	40.28	39.10
10	1	-1	-1	-1	1	1	1	-1	1	1	-1	39.85	30.83
11	-1	1	-1	-1	-1	1	1	1	-1	1	1	40.59	44.56
12	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	39.91	44.33

Plackett-burman design: In order to evaluate the effects of different emulsification conditions on microcapsulation of *B. bifidum BB*28 and *BB*01, a Plackett-Burman screening design were employed (Chakravarti and Sahai, 2002). Range of variable was determined according to previous studies (He *et al.*, 2012). Independent variables were sodium alginate concentration (X1), cell suspension-alginate ratios (X3), CaCl$_2$ concentration (X5), Tween 80 concentration (X7), emulsification time (X9), encapsulating time (X10) and oil-water ratios (X11). Each factor was tested at two levels, high (+1) and low (-1) and ranges of the dependent variable are listed in Table 1. The Plackett-Burman design suggested various experiment conditions in 12 blocks and averages of Encapsulation yield are presented in Table 2.

RESULTS AND DISCUSSION

Screening of the main emulsification conditions using Plackett-Burman design, each factor at two levels was carried on the analysis. The experimental results were determined by statistical software and the significant factor in several main influencing factors could be screened out on the response values. The Plackett-Burman design, which comprised 11 factors spanning over 12 runs with each factor fixed at two levels, was based on 7 single factors which effect embedding efficiency of *B. bifidum BB*0I and *BB*28. Plackett-Burman test design and code level of each factor were shown in Table 1.

Test design and results of Plackett-Burman were shown in Table 2, where X2, X4, X6, X8 were virtual items. The response values of Y1 and Y2 were embedding efficiency of *B. bifidum BB*0I and *BB*28 microcapsules, respectively.

*Bifidobacterium BB*01 and *BB*28 microencapsulation yield, which were obtained from experiment have been analysis by SAS9.1. The significant factors and its influence were shown in Fig. 1 to 4.

According to the Fig. 1, X4, X7, X9 and X10 showed a positive correlation with the embedded yield of *Bifidobacterium BB*01 microcapsules (Y1), namely when the value of four variable factors increased, the response value (encapsulation yield Y1) also increased, X4 was virtual items, which used to estimate the error set and it was removed. For the rest of the several factors X1, X2, X3, X5, X6, X8 and X11 showed a negative correlation with the embedded yield of *Bifidobacterium BB*01 microcapsules, namely when the value of 7 variable factors increased, the response value (encapsulation yield Y1) decreased. For these 7 factors, X2, X6 and X8 were used to estimate the error of virtual set, can be removed. As shown in Fig. 2, for these 7 single factors which effected *Bifidobacterium BB*01 microencapsulation yield, X1 (sodium alginate concentration), X3 (cell suspension-alginate ratios), X10 (encapsulating time) and X9 (emulsification time) have a significant effect and they can be found as follows: X1>X3>X10>X9. The effect of X1was the most obvious and its proportion reached more than 32%.

According to the Fig. 3, X2, X3, X5, X8 and X10 showed a positive correlation with the embedded yield of *Bifidobacterium BB*28 microcapsules (Y2), namely when the value of five variable factors increased, the response value (encapsulation yield Y2) also increased, X2 and X8 were virtual items which set to estimate error set and they can be removed. While the rest of the 6 factors X1, X4, X6, X7, X9 and X11 showed a negative correlation with the embedded yield of *Bifidobacterium BB*28 microcapsules, namely when the value of 7 variable factors increased, the response value (encapsulation yield Y2) decreased. For these 6 factors, X4 and X6 were used to estimate the error of virtual set, can be removed. As shown in Fig. 4, for these 7 single factors which effected *Bifidobacterium BB*28 microencapsulation yield, X5 (CaCl$_2$ concentration), X3 (cell suspension-alginate ratios), X1 (sodium alginate concentration) and X9 (emulsification time)
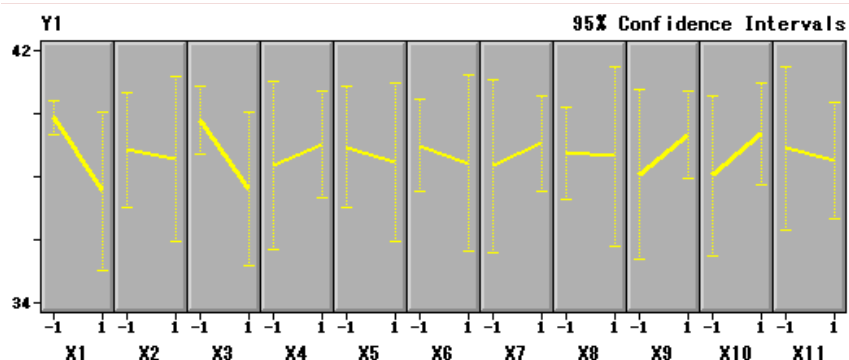

Fig. 1: The 95% confidence interval of the variable factor to microencapsulated yield Y1
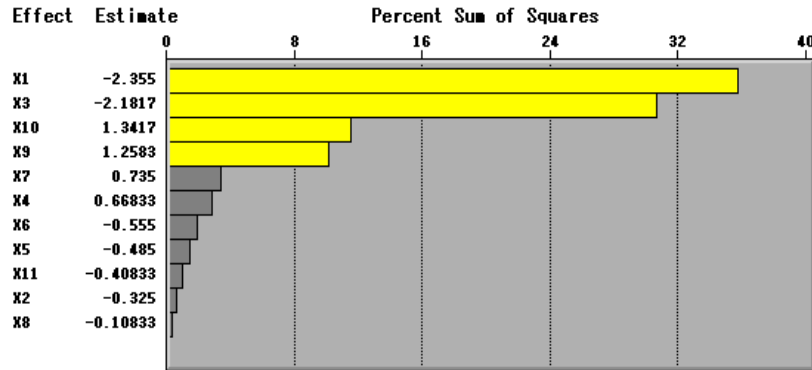
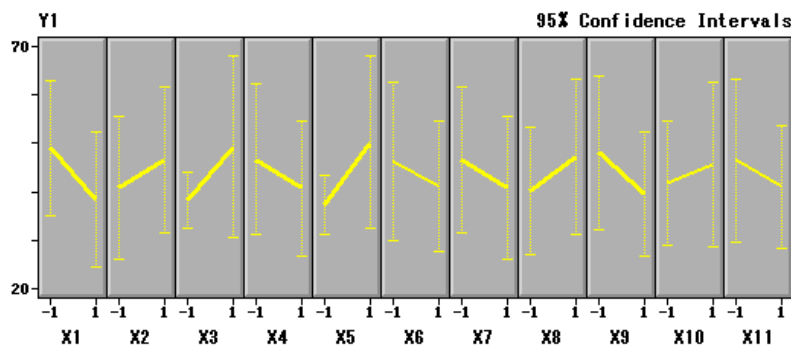Fig. 2: The effect of variable factor to microencapsulated yield Y1

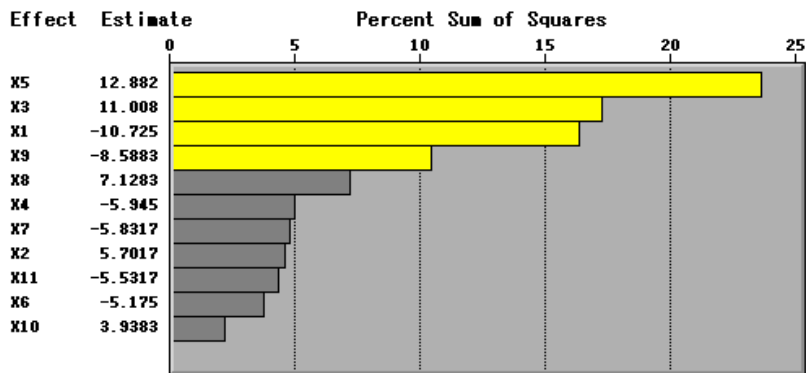Fig. 3: The 95% confidence interval of the variable factor to microencapsulated yield Y2

Fig. 4: The effect of variable factor to microencapsulated yield Y2

have a significant effect and they can be found as follows: X5>X3>X1>X9. The effect of X5was the most obvious and its proportion reached more than 20%.

CONCLUSION

In this study, *B. bifidum BB*28 and *BB*01 were encapsulated by using emulsion method respectively. Seven processing factors were investigated by Plackett-Burman design (PB). Test results showed that sodium alginate concentration, cell suspension-alginate ratios, encapsulating time and emulsification time have a significant effect for *B. bifidum BB*01 microcapsules; CaCl$_2$ concentration, cell suspension-alginate ratios, sodium alginate concentration and emulsification time have a significant effect for *B. bifidum BB*28 microcapsules.

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