

## Optimizing some conditions for spray drying in synbiotic capsule from *Bacillus subtilis* natto strain

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**ABSTRACT :** The study is of determination of various conditions for spray drying in producing synbiotic in the form of capsule from *Bacillus subtilis* natto. The experiments were conducted to examine effects of various factors such as the resistant starch-to-matodextrin ratio before drying, the inlet gas temperature and the inlet flow. The optimization experiments are administered: a ratio of wall materials to the core material is 5% (w/v) in which the ratio of resistant starch and maltodextrin is 1:9, the inlet gas temperature is 110°C, the inlet flow is 5.60 ml/minute and the spray pressure is 2 bar. In the condition for spray drying as mentioned earlier, the initial step for trial production of synbiotic capsules was conducted with the cell density of *B.subtilis* natto being  $8.55 \pm 0.18 \log(\text{CFU/g})$ , the activating effect of nattokinase is 518.2 FU/g and the moisture is 9.11%. During 60 days' preservation of the product, the resulting indexes such as the cell density of *B.subtilis* natto in the capsule, the activating effect of nattokinase and the moisture are stable.

**KEYWORDS** –*Bacillus subtilis* natto, nattokinase, spray-drying, synbiotic, capsule.

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### I. INTRODUCTION

A long time ago, probiotic was known in products of functional foods and pharmaceuticals. To promote utility from the probiotic microbe, the densities of probiotic microbes present in the product and still alive through the human digesting process should be as high as expected. Many researches show that the products containing probiotic microbes under no control after going through the human digestive system give a very low cell density of probiotic microbes [1]. This is also one of the drawbacks of the products with probiotic addition in the nature going through a digesting process. Accordingly micro-encapsulation is defined as a method to improve the microbe's living conditions and conserve the probiotic activating effects, and protect microbial cells in the extreme conditions of the digestive system. The probiotic and prebiotic agents would be combined into the synbiotic one to improve possibilities to survive for the probiotic microbe under the condition of going through the medium of stomach and small intestine and to accelerate their effects in colon. Among various probiotic activating effects, it is necessary to take account of functional enzymes which can enhance functions of different organs in a human body, contributing to improving health for people.

In all over the world including Vietnam there have been researches on the biomass collection and the creating of the metabolic products and the probiotic preparations derived from a typical microbe, *B.subtilis*. First, the *B.subtilis* as a probiotic product in spores can be stored at room temperature in dry condition without affecting the survivability. The second advantage is that the spores can survive moving through the stomach's gastric juice with high pH acidity [2][3]. Creation of synbiotic capsules by means of the spray drying method from *B.subtilis* natto rich in activating effect of nattokinase is aimed at meeting the demand of health care is highly significant in terms of theoretical and practical aspects. The method to apply the capsule form to the production is needed as it helps in preserving the preparation for a long time, keeping the product's features stable and putting into mass production.

### II. MATERIALS AND METHODS

#### 2.1. Selected microbial species

*Bacillus subtilis* natto selected from a collection of HCMC University of Technology.

#### 2.2. Method

**Quantifying microbial cells:** Counting the number of bacteria by disk spreading on the jelly medium.

**Yield of microcapsules:** measuring by % of the total microbial density in the product compared to the density of microorganism contained in the inlet solution [4].

**Activating effect of nattokinase:** measured by fibrin disintegration of nattokinase contained in the solution after fermentation of *B.subtilis* natto (FU/ml) [6].

**Moisture:** drying the sample at 103°C and weighing until it reaches a constant mass.

### 2.3. Spray drying system

Miniature spray drying system (SD-06, Labplant, England) is used. The machine can operate with the minimal volume of 100ml/ 1 sample. The parameters that the machine can vary in a large range, viz. the inlet flow with peristaltic pump: 4.5 – 36.25 ml/minute, the spray pressure: 1 – 3 bar, and the inlet temperature: 0 – 250°C.

## III. RESULTS AND DISCUSSION

### 3.1. Examining materials used in the micro-encapsulation

For the purpose of examining proportions of material mixture servicing micro-encapsulation in finding out a proper proportion in keeping *B.subtilis* natto alive in the extreme medium in a human body and promoting probiotic activating effect of the microbe, *B.subtilis* natto was chosen to conduct the micro-encapsulation with a 5% (w/v) concentration of the carrier by using spray drying method [4]. Various materials in the micro-encapsulation include the resistant starch and the maltodextrin of different percentages. Specifically, 5% of (w/v) dry matter or 5g dry matter was used in 100ml solution after fermentation. Each spray drying process consumed a 200ml solution; therefore, it was necessary to add a 10g dry matter. As such, the ratio of the resistant starch ratio (g) and the maltodextrin (g) might vary under the condition of keeping a 10g of dry matter.

**Table I:** Relationship between the resistant starch-to-maltodextrin ratio in spray drying solution against the tracking criteria.

Tracking criteria	Resistant starch – to – maltodextrin ratio		
	1.5 : 8.5	<b>1 : 9</b>	0.5 : 9.5
Cell density log(CFU/g)	8.34 ± 0.05	<b>8.55 ± 0.07</b>	8.51 ± 0.03
Yield of microencapsules (%)	80.82	<b>81.20</b>	79.20
Nattokinase (FU/g)	515.0	<b>518.5</b>	510.8
Moisture (%)	16.63	<b>9.11</b>	9.01

The materials for the micro-encapsulation added to the solution before the spray drying might affect the cell density of *B.subtilis* natto, yield of microcapsules, the activating effect of nattokinase, the moisture. The extreme changes in the concentration might affect the drying process. If the concentration of the carrier's components is lower, part of the materials would be stuck to the equipment's wall, the drying process will be difficult to take place and the resulting performance would decrease. Conversely, if the concentration is increased the drying process would happen more easily and the results of production would be higher. However, if the concentration of the materials for the micro-encapsulation increase too much, the quality of the product will reduce after drying. The given results after such an examination showed that a 5% (w/v) concentration the carrier was suitable and the ideal resistant starch-to-maltodextrin ratio of 1:9 demonstrated the highest performance in making the preparation of *B.subtilis* natto - based micro-encapsulation by using the spray drying method.

### 3.2. Examining effect of the technical parameters on the spray drying process

#### 3.2.1. Inlet gas temperature

The resulting concentration of dry matter is 5% (w/v) while the resistant starch-to-maltodextrin ratio is 1:1 max. In this test, only the inlet gas temperature is examined, as outlet gas temperature depends on various factors. The test was conducted with inlet gas temperatures of 80, 90, 100, 110 and 120°C in turn. The remaining parameters of the drying device include the spray pressure of 2 bar, and the speed of inlet peristaltic pump was 6 rpm equivalent to the inlet flow of 5.60 ml/minute. The testing results are shown in Table II.

**Table II:** Correlation of the inlet gas temperature and the tracking criteria

Tracking criteria	Inlet temperature (°C)				
	80	90	100	<b>110</b>	120
Cell density log(CFU/g)	8.68 ± 0.1	8.65 ± 0.15	8.62 ± 0.07	<b>8.56 ± 0.22</b>	8.29 ± 0.08
Moisture (%)	17.91	10.88	10.09	<b>9.11</b>	9.09
Nattokinase (FU/g)	(*)	(*)	(*)	<b>515.8</b>	(*)

(\*): uncheck

The temperature is an important factor during the drying process [5]. The inlet gas temperature does not only affect performance of the drying process but also the quality of the collected product after drying, e.g. moisture, solubility, particle size and cell density. In the context of low inlet gas temperature and speed of water evaporation while high moisture of the used materials brought about phenomenal sticking on the collection utensil and difficulties in the prototype collection process and affected the preparation over preservation duration. When the inlet gas temperature increased, speed of water evaporation and particle size went up while moisture in the used materials decreased. However, if the temperature continued going up and the quality would be questionable. Basing on the results from the examination of effect of starting temperature on cell density and moisture, only the activating effect of nattokinase in optimal treatment was chosen. Through these experiments,

the inlet gas temperature of 110°C was chosen, corresponding to a  $8.56 \pm 0.22 \log(\text{CFU/g})$  cell density of *B.subtilis* natto, 9.11% moisture and 515.8 FU/g activating effect of nattokinase in the preparation.

### 3.2.2. Inlet flow

Speed of the inlet pump seriously affected the sprayed solution flow, machine capacity and even temperature of outlet gas. By using the peristaltic system, the speed of pumping varied among 5 rpm, 6rpm and 7rpm corresponding to the sprayed solution flows of 4.67 ml/minute, 5.60 ml/minute and 6.53ml/minute, respectively. In these experiments, the sprayed solution flows between 4.67 ml/minute and 6.53 ml/minute were used while the remaining factors of inlet temperature were optimized at 110°C, spray pressure of 2 bar and dry matter concentration of 5% (w/v), including the resistant starch-to- maltodextrin ratio 1:9. The results are shown in Table III.

**Table III:** Correlation between sprayed solution flows and the tracking criteria

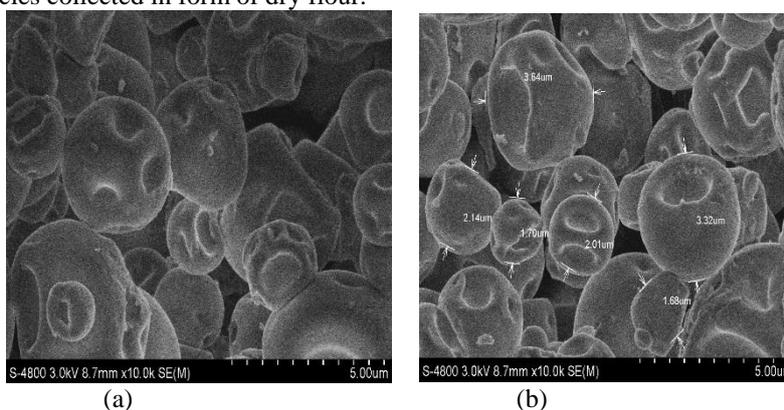
Tracking criteria	Sprayed solution flow (ml/minute)		
	4,67	<b>5,60</b>	6,53
Cell density log(CFU/g)	8,48 ± 0,05	<b>8,55 ± 0,03</b>	8,57 ± 0,1
Moisture (%)	8,07	<b>9,10</b>	12,82
Nattokinase (FU/g)	(*)	<b>518,2</b>	(*)

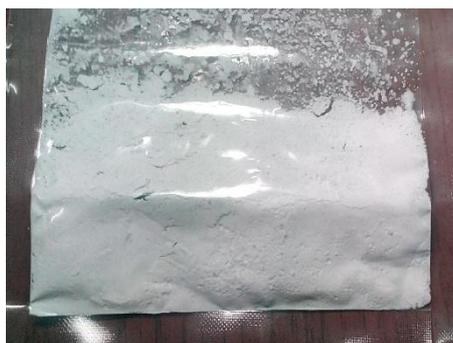
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In fact, the sprayed solution flow affects the product's features after drying such as moisture, solubility, particle size and cell density. In the case of low sprayed solution flow, duration of the materials staying in the drying chamber was kept higher for ensuring that all the constituents inside the particle were steadily exposed to the temperature; therefore, with greater loss of water the moisture of the materials became lower after drying. In case of low moisture the products collected would have be drier, which makes it difficult for any phenomenal sticking to the utensil walls to occur, and enables faster drying process and higher quality product collection. Conversely, in case of higher speed of inlet solution flow, lower lay time of the materials staying in the drying chamber, less water evaporation from the solution, the moisture of the materials would increase. Accordingly, in case of a high moisture, the phenomenal sticking to the utensil walls would occur and resulting performance of the product after the spray drying process would drop down. Basing on the results of the examination of effects of the initial temperature on cell density and moisture, only the activating effect of nattokinase in optimal treatment was chosen. Through these experiments, it was found that the optimal sprayed solution flow should be 5.60 ml/minute corresponding to  $8.55 \pm 0.03 \log(\text{CFU/g})$  cell density, 9.10% moisture and 518.2% active nattokinase.

### 3.3. Examining characteristics and particle-structure of *B.subtilis* natto after micro-encapsulation by the spray drying method

Micro-encapsulation by the spray drying method is commonly used in the industries of food, pharmaceutical, and biotechnology. The spray drying process includes the atomization of emulsions or suspensions containing the probiotic agent and the carrier, which results in fast water evaporation and the micro-encapsulation particles collected in form of dry flour.





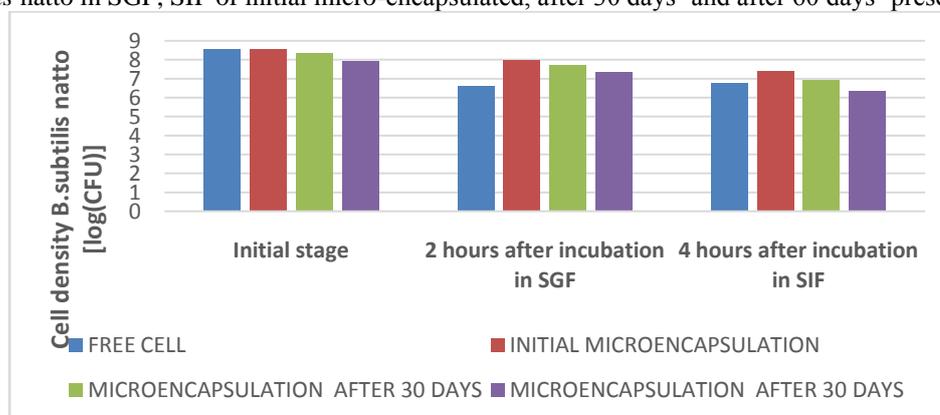
(c)

**Figure 1:** (a) photos of micro-encapsulation particles taken by SEM at 10000 times magnification, (b) size, (c) micro-encapsulation preparation

The drying process is controlled by the temperatures of inlet and outlet gas, flow of inlet solution and spray pressure. It is the technical parameters of the spray drying process which affect the characteristics and structure of micro-encapsulation particles such as solubility, particle size, etc. As a result, the size of micro-encapsulation particles is 3.64 $\mu$ m. Figure 1 (a), shows the photo of micro-encapsulation particles after spray drying and figure 1 (c) shows the preparation in fine white.

### 3.4. Recheck some characteristics and probiotic activating effects of *B.subtilis* natto in the micro-encapsulation preparation over preservation duration.

For the purpose of examining effects of the spray drying method and roles of the micro-encapsulation materials viz. resistant starch and maltodextrin used in the micro-encapsulation spray drying process on possibilities of improving quantity and conserving probiotic traits of *B.subtilis* natto within extreme medium and technical conditions of the spray drying process, Figure 2 shows the findings of possibility to survive of *B.subtilis* natto in SGF, SIF of initial micro-encapsulated, after 30 days' and after 60 days' preservation.



**Figure 2:** Possibility to survive of *B.subtilis* natto after incubation in SGF and SIF of the initial micro-encapsulated, after 30 days' and after 60 days' preservation.

In the micro-encapsulated cells, the possibility to survive of *B.subtilis* natto after micro-encapsulation process remains very high after 2 hours' incubation in SGF and after 4 hours' incubation in SIF. The micro-encapsulation process for making synbiotic preparation gives considerably higher effect on protection of *B.subtilis* natto compared to that in freeform. Such a combination has helped in enhancing percentage of *B.subtilis* natto alive in the conditions of the pH 2 and bile salt level of 0.3%.

In summary, the results given above show *B.subtilis* wrapped with micro-encapsulation by means of the spray drying method can considerably conserve and improve possibility of the probiotic microbe to overcome various extreme conditions in the digestive system ( low pH degree of gastric juice in the stomach and that of bile salts in the small intestines as well as preservation condition of the preparation. A combination of resistant starch and maltodextrin was selected in this research can help microbial cells overcome those extreme conditions.

### 3.5. Testing the production of synbiotic capsules

The capsule is a good way to protect *B.subtilis* natto cell in the preservatory process. The capsule sheath can prevent from absorption of oxygen into the product, and limit possible probiotic induction creating H<sub>2</sub>O<sub>2</sub> and therefore decreasing the product quality. The pelleting technology was carried out at Tipharco, a pharmaceutical company in Tien Giang province with pouring device compatible with the 500mg capsule. The synbiotic capsules were preserved at the cool temperature of 10<sup>0</sup>C. The results from observations of the product over preservation time are shown in the following table:

**Table IV:** Changes in the cell density of *B.subtilis* natto and active nattokinase, and the medium's moisture over preservation time

Length of time (days)	Cell density log(CFU/g)	Nattokinase (FU/g)	Moisture (%)
Initial micro-encapsulation	8.55 ± 0.18	518.2	9.11
Micro-encapsulation after 30 days	8.31 ± 0.04	518.5	9.14
Micro-encapsulation after 60 days	7.92 ± 0.09	515.5	9.18

The chart above indicates that the densities of *B.subtilis* natto in the preparation deteriorates over the periods of preservation while the activating effects on the preparation are rather stable during 60 days as observed fluctuating between 518.2 and 515.5 FU/g. As such, the figure for synbiotic capsule remains less than 10% in compliance with standards applicable to synbiotic products.

The synbiotic capsule examined after 60 days shows that the 92.63% density of *B.subtilis* natto meets the standards for probiotic products and allows for *B.subtilis* natto to survive, and the indication of activating effect of nattokinase is stable in the 515.5 FU/g preparation.

## IV. CONCLUSION

From different tests on producing synbiotic capsules from *Bacillus subtilis* natto, it is found that:

The parameters collected from micro-encapsulation by spray drying method: concentration of dry matter of the solution is 5% (w/v) while the resistant starch to maltodextrin ratio is 1:9, the inlet gas temperature is 110<sup>0</sup>C, the outlet gas temperature is 60<sup>0</sup>C, sprayed solution flow is 5.60 ml/minute (equivalent to speed peristaltic pump 6rpm) and spray pressure is 2 bar.

For the initial step of testing synbiotic capsules, the cell density of *B.subtilis* natto cells was 8.55 ± 0.18 log(CFU/g), the activating effect of nattokinase 518.2 FU/g and the moisture 9.11%.

After 60 days' preservation, it is observed that the cell density of *B.subtilis* natto in the capsule, activating nature of nattokinase and degree of moisture are stable.

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