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UTILIZATION OF ENCAPSULATED $CACO_3$ IN LIQUID CORE CAPSULES FOR IMPROVING LACTIC ACID FERMENTATION

(Penggunaan Kapsul Berteras Cecair yang Mengandungi $CaCO_3$ untuk Menambah Baik Penapaian Bakteria Asid Laktik)

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Abstract

Lactic acid bacteria (LAB) have been used for food fermentation due to its fermentative ability to improve and enhance the quality of the end food products. However, the performance of LAB is affected as fermentation time elapsed because the microbial growth is inhibited by its end product, i.e. lactic acid. In this study, a new approach was introduced to reduce the product inhibition effect using $CaCO_3$ which is encapsulated in spherical liquid core capsules of diameter 3.5mm and 3.6 mm produced through extrusion dripping method. The results showed that the pH and lactic acid concentration of LAB fermentation was well maintained by the capsules. The results of the fermentation conducted to control pH and lactic acid concentration using the capsules were better than those of the control set and comparable with that of the free $CaCO_3$ set. In addition, the viable cell concentration of *L. casei shirota* was high at the end of fermentation when the fermentation was conducted using the capsules. The results of this study suggested that the capsules have high potential to be applied for pH and lactic acid level control in LAB fermentation for various productions.

Keywords: calcium carbonate, lactic acid bacteria fermentation, liquid core capsule, pH

Abstrak

Bakteria asid laktik (LAB) telah banyak digunakan untuk penapaian makanan disebabkan oleh keupayaannya untuk meningkatkan dan memperkayakan kualiti produk makanan yang dihasilkan melalui penapaian. Namun, prestasi LAB adalah dipengaruhi oleh hasil penapaian iaitu asid laktik kerana pertumbahan mikrob terencat. Dalam kajian ini, satu kaedah baru diperkenalkan untuk mengurangkan kesan perencatan dengan mengapsulkan $CaCO_3$ di dalam kapsul berteras cecair yang berbentuk sfera dan berdiameter 3.5 mm dan 3.6 mm yang dihasilkan dengan menggunakan kaedah penitisan-penyemperitan. Hasil kajian menunjukkan pH dan kepekatan asid laktik semasa penapaian LAB telah dikawal dengan baik oleh kapsul – kapsul yang dihasilkan. Keputusan kajian yang dijalankan dengan menggunakan kapsul – kapsul untuk mengawal pH dan kepekatan asid laktik adalah lebih baik daripada keputusan kajian yang dijalankan dengan set kawalan, dan setanding dengan set yang menggunakan tanpa $CaCO_3$. Tambahan pula, kepekatan sel hidup bagi *L. casei shirota* adalah tinggi pada akhir penapaian apabila penapaian dijalankan dengan menggunakan kapsul – kapsul. Keputusan daripada kajian ini mencadangkan bahawa kapsul – kapsul yang dihasilkan menpunyai potensi yang tinggi untuk digunakan sebagai kawalan pH dan kepekatan asid laktik dalam pelbagai jenis pengeluaran.

Kata kunci: kalsium karbonat, penapaian bakteria asid laktik, kapsul berteras cecair, pH

Introduction

Lactic acid bacteria (LAB) are one of the microbes used in traditional food fermentation. LAB has been used for the fermentation of dairy and non-dairy products [1, 2]. For example, yogurt, cultured milk, cheese are dairy products that produced from LAB fermentation [2]. LAB is reported to play an important role in a number of Asian fermented non-dairy products such as '*tempoyak*' (acid fermented condiment) [3], "*tapai*" (rice wine) [4], '*kimchi'* (fermented vegetables) [1], "*idli*" in India, "*puto*" in Philippines (steamed bread) [1], "*sikhae*" in Korea and "*burong isda/dalag*" in Philippine (fermented mixture of salted fish and cereals) [1].

LAB has been recognised for their fermentative ability to enhance food safety and nutrients, improve organoleptic quality, as well as increase health related benefits [1, 4]. However, the role of LAB in food fermentation is diminished by the production of its end products, i.e. lactic acid [5, 6]. As the fermentation time elapsed, the concentration of lactic acid in the fermentation broth increased and hence the pH of the broth is consequently increased [5].

In common practice, calcium carbonate or advance instrument like pH controller is used to control the concentration of lactic acid and pH in the fermentation broth. However, the direct addition of calcium carbonate in the fermentation broth is not favorable for the subsequent purification process, and the use of pH controller is limited due to high cost. Therefore, in this research, it is proposed to develop a new approach to control pH during LAB fermentation using liquid core capsules. Liquid core capsules are easy to form at low cost.

Materials and Methods

Liquid core solution and gelation bath preparation

The inner liquid-core solution was prepared by adding 15 g/L of calcium chloride (CaCl2) (R&M Chemical, United Kingdom), 400 g/L of calcium carbonate ($CaCO_3$) (HmbG Chemical, France) and 15 g/L of carboxylmethylcellulose (CMC) (Calbiochem, Germany). The mixture of 15 g/L of alginate (Kimitsu-Kimica, Japan) and 1.5 g/L of Tween-80 (Merck, Germany) was prepared as the gelation bath.

Liquid core capsules formation

Liquid-core capsules were prepared using the extrusion-dripping method. The liquid-core solution was extruded through a hypodermic needle (Terumo, Japan) into the gelation bath. In this study, the outer diameter of the needles used was 0.6 mm and 1.1 mm. After the capsules were formed, the capsules were cured in 5 g/L alginate solution for 1 hour to strengthen the capsules. Then the capsules were washed with distilled water and stored in 15 g/L $CaCl_2$ solution.

Capsule properties characterization

A digital camera (Coolpix S2600, Nikon, Japan) was used to capture the image of 30 capsules. The image was analysed using Sigma ScanPro5 software (SPSS, USA) to determine the diameter, maximum length (Dmax) and minimum length (Dmin) of capsules. The sphericity of the capsules was quantified in term of sphericity factor (SF) using the following equation 1 [7]:

$$SF = (Dmax-Dmin)/(Dmax + Dmin) \quad (1)$$

Lactic acid bacteria fermentation

Lactobacillus casei shirota was selected as the model strain for this study. For each fermentation run, four sets of fermentation flask were prepared. The flask was filled with the de Man, Rogosa and Sharpe (MRS) broth. In which, Flask I was labeled for blank broth as control; Flask II was labeled for the broth with addition of free $CaCO_3$; Flask III and IV was labeled for the broth with addition of two sizes of liquid core capsules that encapsulated $CaCO_3$.

All the experiments were conducted at temperature of about 37 ^oC. The pH of the fermentation broth was measured using a pH meter (Mettler Toledo, Switzerland) at an interval of 4 hours. The lactic acid and glucose content were measured by a reflectometer (Merck, Germany), as per described in the standard procedure [8].

Viable cell concentration determination

The total number of viable cells was determined by the plate counting on the de Man, Rogosa and Sharpe (MRS) agar (Oxoid, UK) incubated at 37^{o}C for 48 hours.

Results and Discussion

Diameter and sphericity of capsules

Table 1 shows the diameter and SF of the liquid core capsules formed from needles with the diameter of 0.6 and 1.1 mm. The diameter of the capsules formed from 0.6 mm needle was smaller than that of capsules formed from 1.1 mm needle. The diameter of the capsule was proportional to the needle diameter because the diameter of the pendant droplet detached from the diameter was also proportional to the needle diameter [7, 9].

Table 1. Diameter and SF of the capsules

Needle diameter, mm	Diameter, mm	Sphericity factor (SF)
0.6	3.53 (±0.09)	0.0261 (±0.002)
1.1	3.62 (±0.04)	0.0648 (±0.006)

Note: The standard error of the measurements was shown in parentheses

The results showed that the capsules formed from the needles were generally spherical as the SF was less than 0.07. Although the capsules were formed using different diameter of needle, the capsules formed were spherical. This is because the capsules were formed from liquid core solution of sufficient viscosity and $CaCl_2$ concentration. The past studies showed that the addition of CMC in the liquid core solution increased the viscosity of the solution and consequently improved the sphericity of the capsules [10, 11]. In general, the concentration of $CaCl_2$ greater than 10 g/L is sufficient to form spherical ca-alginate particles [9]. In this study, the concentration of $CaCl_2$ of 15g/L was used, which is above the suggested critical concentration.

Effect of using encapsulated $CaCO_3$ in monitoring pH of fermentation process

Figure 1 shows the pH of the LAB fermentation broth for different experiment conditions. The pH of the broth in Flask I dropped from pH 5.4 to 3.5 after 40 hours of LAB fermentation, which was the greatest drop among the tested conditions. The results of the capsules (Flask III and Flask IV) were comparable to that of free $CaCO_3$ (Flask II). The pH of the broth was maintained above pH 5.5. Previous study has been reported that $CaCO_3$ has the ability to maintain LAB fermentation broth in the similar range [12]. Even so, LAB fermentation with free $CaCO_3$ (Flask II) showed slightly better results in controlling the pH of the broth. This is because the $CaCO_3$ was free in contact with the lactic acid formed in the medium without any obstacles. Therefore, the $CaCO_3$ can neutralize the acid in more effective way as compared to the $CaCO_3$ in the capsules. The $CaCO_3$ which confined inside the capsules faced diffusion resistance problem as it needed to diffuse through the alginate membrane [11]. As a result, it reacts with the acid at a lesser rate to maintain the pH of the fermentation broth. As shown in Figure 1, there was no apparent difference in the pH control ability between the capsule of 3.5 mm and 3.6 mm.

Lactic acid concentration of fermentation broth

The concentration of lactic acid produced by LAB was monitored and the results are shown in Figure 2. Throughout the 40 hours of fermentation, the concentration of lactic acid in the fermentation broth was increased. The lactic acid concentration in Flask I (control) increased continuously until the end of fermentation at 16.0 g/L. However, the lactic acid concentration in Flask II, III, and IV was maintained by $CaCO_3$ at the level of 10 – 12 g/L. There is no apparent difference was observed between the results of free $CaCO_3$ and encapsulated $CaCO_3$. The free lactic acid in the fermentation broth reacted with $CaCO_3$ to form calcium lactate and hence reduced the concentration of lactic acid in the broth as well as neutralized the pH of the broth (see Figure 1) [13].

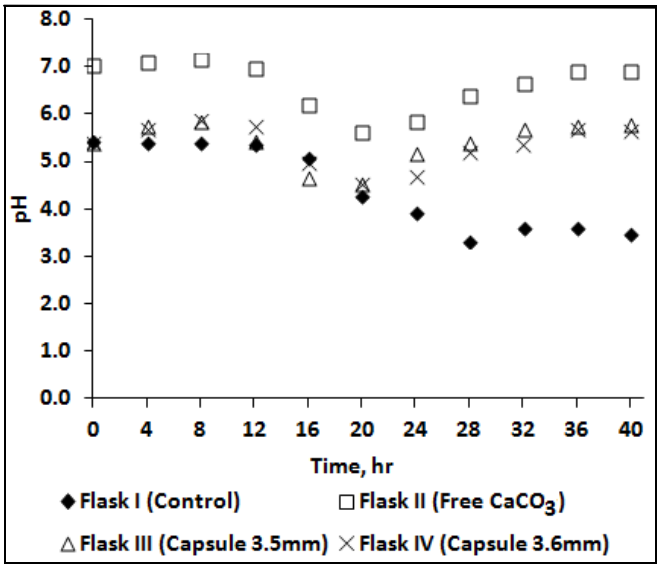

Figure 1. pH of LAB fermentation broth

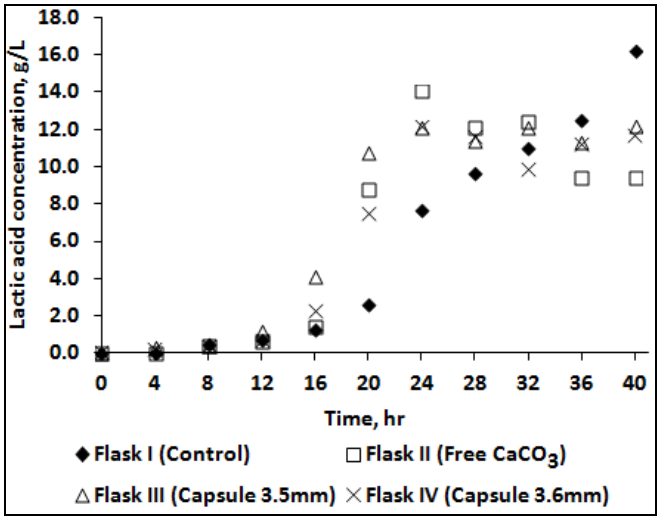

Figure 2. Lactic acid concentration of LAB fermentation broth

Viable cell concentration of LAB

The viable cell concentration of LAB (i.e. *L. casei shirota*) fermentation was at initial and at the end of the fermentation was determined and the results of different experimental conditions were compared, as shown in Table 2. The viable cell concentration of *L. casei shirota* at the end of fermentation was reduced in the case of Flask I (Control) and Flask II (Free $CaCO_3$). The results indicated that the viable cell concentration in LAB fermentation without $CaCO_3$ was reduced because the microbial growth is inhibited by its end-product, lactic acid [5, 6]. In the case of free $CaCO_3$ in Flask II, lower viable cell concentration was obtained at the end of fermentation could be due to the presence of $CaCO_3$ in free form created a less favorable environment for microbial growth. On the other hand, the viable cell concentration of *L. casei shirota* in Flask III and IV was high at the end of fermentation. The results suggested that the liquid core capsules provide a good condition for microbial growth as the pH and lactic acid

concentration were maintained at the desired level and the microbial cell was not in direct contact with the $CaCO_3$.

Table 2. Viable cell concentration of LAB fermentation

Flask number	Initial (0 hr), cfu/ml	End (40 hr), cfu/ml
I	12×10^9	1×10^8
II	18×10^9	5×10^8
III	1×10^9	1×10^9
IV	2×10^9	1×10^9

Note: The results were given based on the average of duplicate sampling

Conclusion

A new approach has been developed to maintain the pH and lactic acid concentration of LAB fermentation at the desired level. Spherical liquid core capsules of diameter of 3.5 mm and 3.6 mm were produced to encapsulate $CaCO_3$ from 0.6 mm and 0.8 mm hypodermic needle, respectively. The results showed that the pH of LAB fermentation was well maintained by the capsules at the desired level of above pH 5.5. Furthermore, the lactic acid concentration of LAB fermentation was averagely 11 g/L during the whole period of fermentation with the use of the liquid core capsules. In general, the results of the fermentation conducted using the capsules were better than that of control and comparable with that of free $CaCO_3$ in maintain the pH and lactic acid level during fermentation. In addition, the viable cell concentration of *L. casei shirota* was high ($> 10^9$ cfu/ml) at the end of fermentation when the fermentation was conducted using the capsules.

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