Fermentation of Tomato Juice by Cell Immobilized *Lactobacillus acidophilus*

V. An-Erl King, Hui-Ying Huang[1], Jen-Horng Tsen[1]

Department of Food Science and Biotechnology, National Chung-Hsing University; [1]Department of Nutrition,

China Medical University, Taichung, Taiwan.

Purpose. Ca-alginate entrapped *Lactobacillus acidophilus* was used to ferment tomato juice and enhance the survival of the bacteria in the product.
Methods. *L. acidophilus* was immobilized in gel beads with diameters of about 2.6 mm. Tomato juice was made from fresh raw tomatoes, followed by fermentation for 80 h with free and immobilized cells.
Results. Immobilized cells leaked from the gel beads and proliferated in the juice during the fermentation; the final viable cell number reached 10^7 CFU/mL in the juice and above 10^{10} CFU/mL-gel in gel beads. Free cells reached about 10^9 CFU/mL during fermentation. Immobilized cells endured the adverse conditions in tomato juice; furthermore, viable cell numbers and sensory score results were higher compared with free cells. The viable cell counts of immobilized *L. acidophilus* were maintained at 10^7 CFU/mL-gel in the fermented tomato juice after 10 weeks of cold storage at 4 °C, compared with 10^4 CFU/mL of free cells.
Conclusions. Ca-alginate immobilized *L. acidophilus* enhanced the viable cell number and improved the sensory quality of fermented tomato juice. Our findings could be applied to the development of probiotic tomato juice. **(Mid Taiwan J Med 2007;12:1-7)**

Key words

calcium alginate, fermentation, immobilized cell, *Lactobacillus acidophilus*, tomato juice

INTRODUCTION

Probiotics are beneficial microorganisms. When ingested on a regular basis, probiotics can improve overall health, balance the microflora of the intestinal tract, enhance immune system function, and assist in the absorption of vitamins and minerals [1]. The most commonly consumed probiotics are lactic acid bacteria (LAB) including *Lactobacillus acidophilus, Lactobacillus plantarum* and *Streptococcus lactis*. Probiotic foods are usually produced by fermentation. However, for a probiotic product to be beneficial, it must contain at least 10^6 cfu/g of viable bacteria [2].

Probiotic products are commonly available in the form of fermented milk and yogurt. However, many individuals cannot consume these products because of their high cholesterol and lactose content. Fruit and vegetable juices have been suggested as media for cultivating probiotics because of their beneficial nutrients [3,4]; however probiotic stability in non-dairy products is difficult to maintain during storage. Probiotic encapsulation might solve this problem [5]. Encapsulation is one of the methods of cell immobilization. Advantages of immobilized cell technology include prevention of interfacial inactivation, stimulation of production and excretion of secondary metabolites, continuous utilization, and protection against disadvantageous and unfavorable environments [6-8]. In addition, immobilized cell

---

Received : 19 June 2006. Revised : 7 August 2006.
Accepted : 16 August 2006.
Address reprint requests to : Jen-Horng Tsen, Department of Nutrition, China Medical University, 91 Hsueh-Shih Road, Taichung 404, Taiwan.

fermentation has been shown to increase the cell concentration as well as cell survival and operating efficiency [9,10].

Tomatoes are widely consumed worldwide. Most tomatoes are eaten as processed products such as tomato juice, puree, ketchup, sauce, and canned tomatoes. Tomato juice has been used in the manufacture of probiotic juice by lactic acid fermentation in order to improve its health effects [11]. Researchers used tomato juice as a raw material for the production of probiotic juice by four lactic acid bacteria (*L. acidophilus, L. plantarum, L. casei, and L. delbrueckii*) [12]. It was found that tomato juice was a proper medium for lactic acid fermentation, and the probiotic tomato juice obtained could serve as a health beverage for vegetarians or consumers who are allergic to dairy products. Immobilized *L. acidophilus* has been used in banana puree fermentation [13,14], and it was found that viable cell numbers were significantly raised owing to the protective effect provided by the cell immobilization matrix against the unfavorable circumstances in banana media. The purpose of this study was to increase the fermentation efficiency of *L. acidophilus* in tomato juice by Ca-alginate entrapment of the bacteria. This study will contribute to the development of an appropriate probiotic juice.

MATERIALS AND METHODS

Materials and culture

L. acidophilus BCRC 10695 was obtained from the Biosource Collection and Research Center of the Food Industry Research and Development Institute in Hsinchu, Taiwan, kept on MRS agar (Difco Laboratories, Detroit, MI, USA) and stored at −20°C. Na-alginate and calcium chloride ($CaCl_2$) (Sigma, St. Louis, MO, USA) were used to form the immobilizing matrix for the entrapment of cells.

Preparation of tomato juice

Fresh raw tomatoes *(Lycopersicon esculentum)* were purchased from a local market and washed in a soak tank. The fruit was then chopped in a Waring blender for 45 sec. A 25 cm (length) × 12 cm (width) × 0.5 cm (inside height) thin stainless steel trough was constructed to simulate a plate-type heat exchanger. The sample was held in the trough at 85°C for 6 sec, cooled in an ice bath, and then filtered through a 30-mesh screen. The resulting tomato juice was held at 92°C for 90 sec and then cooled to 25°C [15]. Thermally-treated juice was poured aseptically into flasks for fermentation.

Culture preparation

L. acidophilus was cultured statically in MRS broth. A 1% inoculum was added, incubated at 37°C for 12-14 h, collected by centrifugation at 8,000xg for 15 min at 0°C and washed twice with 0.1% peptone water [16].

Cell immobilization

A concentrated cell suspension was obtained by suspending cell cultures from the previous procedures in 0.1% peptone water. The concentrated cell suspension was then mixed at a 1:1 volume ratio with 2% (w/v) sterile Na-alginate solution, and the resulting 1% alginate-bacteria mixture was extruded as droplets through a needle using a peristaltic pump (Eyela MP-3, Japan). The droplets were then dropped into a gently stirred (70 rpm) sterilized 0.1 M calcium chloride solution at room temperature and stored in the solution for 30 min to allow for the complete gelation of the cell immobilized Ca-alginate gel beads before use [17]. The finished beads had a diameter of about 2.6 mm.

Fermentation of tomato juice

A 2% (v/v) inoculum of *L. acidophilus* was added to tomato juice for free cell fermentation and a 4% (v/v) inoculum was added to tomato juice for immobilized cell fermentation. The cell concentration in the immobilized gel beads had been half-diluted during cell immobilization; therefore the concentration of immobilized bacteria was doubled, so that equal initial cell concentrations could be obtained. Inoculated-juice was incubated at 37°C for 80 h. The variations of viable cell number, pH, and sugar content were measured periodically during incubation.

Colony counting

Free cells were cultured in MRS agar medium after a series of dilutions, and the number of viable cells was determined by a standard plate count method using Lactobacilli MRS medium at 37℃ for 48 h. For immobilized cells, Ca-alginate gel beads containing cells were depolymerized in sterile 1% (w/v) sodium citrate solution with gentle shaking for 20 min at room temperature to produce a cell suspension. The cells were then serially diluted and cultured as free cells for colony counting [18,19].

Chemical analyses

Sugar content was analyzed by HPLC [20]. The pH was measured directly using a pH meter (HI 9021, Hanna Instruments, Taiwan). All of these determinations were carried out in six replications.

Cold storage

The fermented samples were stored at 4℃ for 10 weeks, and colonies were counted at weekly intervals [21].

Sensory evaluation

Overall acceptance sensory tests were performed on the free cell and immobilized cell fermented products during cold storage as previously described [22]. Panelists were asked to taste each sample and to rate its overall acceptance using a 9-point hedonic scale: the scores ranged from 1 = dislike very much, to 5 = neither like nor dislike, to 9 = like extremely well, i.e. higher sensory score indicated better overall acceptance. Samples were tested by 40 panelists and the average of the scores was calculated.

RESULTS

Growth of immobilized and free cells

Figure 1 shows the growth of both free and immobilized *L. acidophilus* in tomato juice during fermentation. The initial viable cell numbers of free cells and Ca-alginate immobilized cells were 3.24×10^5 (CFU/mL) and 4.17×10^5 (CFU/mL-gel), respectively. The viable cell number of immobilized cells was found to be higher than that of free cells; the immobilized cells which leaked during fermentation grew in the medium as free cells. Fermentation reached a steady-state after 80 h.

Variations of sugar content and pH during fermentation

The variations of sugar content and pH in tomato juice for free and immobilized *L. acidophilus* fermentation are shown in Figs. 2 and 3, respectively. It was found that the immobilized cells consumed more sugar compared with free cells during fermentation. On the other hand, the pH value of the immobilized cell fermentation was found to be lower than that of the free cell.

Cell viability and sensory result during cold storage

The cell viability of *L. acidophilus* in fermented tomato juice during cold storage is shown in Table 1. The viable cell count of immobilized cells was higher than 10^7 CFU/mL-gel even after 10 weeks of cold storage at 4℃; however, that of free cells was only 10^4/mL. Sensory evaluation revealed that the overall acceptance of immobilized cell fermentation was better than free cells during storage (Table 2). The sensory score of free cell fermented product reached the critical point of like and dislike, i.e. 5, at the stage of 10 weeks. This indicated that the product became unacceptable after 10 weeks of storage.

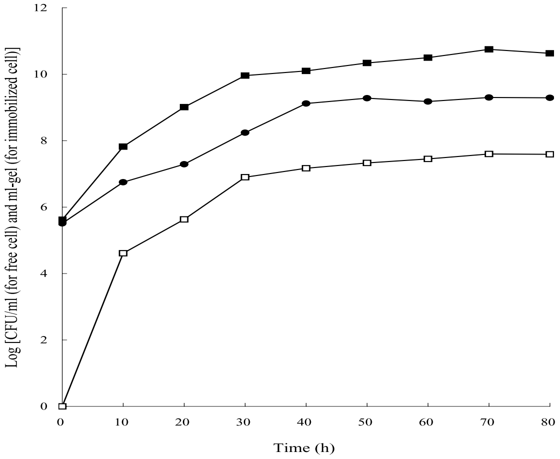

Fig. 1. Growth curves for free and Ca-alginate immobilized *L. acidophilus* in tomato juice. □:immobilized cell (suspension). ■: immobilized cell (gel bead). ●: free cell.

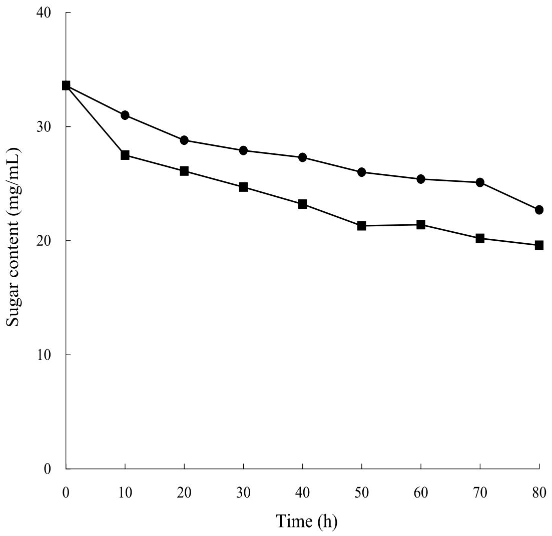

Fig. 2. Changes in sugar content of tomato juice for free and Ca-alginate immobilized *L. acidophilus* fermentation. ■: immobilized cell. ●: free cell.

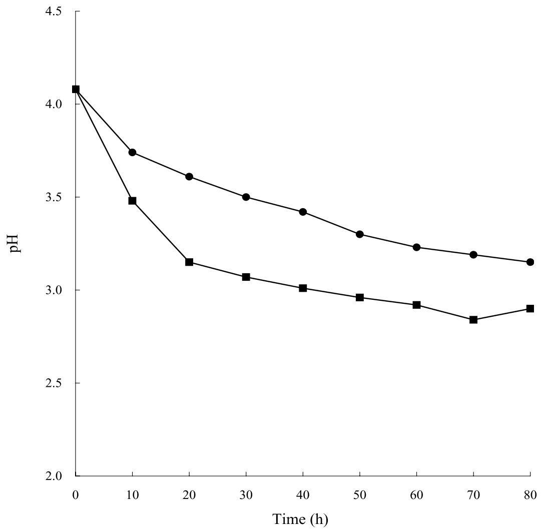

Fig. 3. Changes in pH during fermentation of tomato juice by free and Ca-alginate immobilized *L. acidophilus* fermentation. ■: immobilized cell. ●: free cell.

Table 1. Effect of cold storage (4℃) on the viable cell number of free and immobilized *L. acidophilus* in fermented tomato juice

Time (wk)	Free (CFU/mL)	Immobilized (CFU/mL-gel)
0	$1.6 \pm 0.5 \times 10^{9a*}$	$9.2 \pm 0.4 \times 10^{10a}$
1	$1.4 \pm 0.2 \times 10^{9a}$	$8.5 \pm 0.8 \times 10^{10a}$
2	$1.1 \pm 0.4 \times 10^{9a}$	$6.7 \pm 0.1 \times 10^{10a}$
3	$8.8 \pm 0.2 \times 10^{8b}$	$4.8 \pm 0.5 \times 10^{10a}$
4	$7.3 \pm 0.6 \times 10^{8b}$	$2.1 \pm 1.5 \times 10^{10a}$
5	$5.9 \pm 1.0 \times 10^{7c}$	$7.2 \pm 0.6 \times 10^{9b}$
6	$4.3 \pm 1.1 \times 10^{7c}$	$5.4 \pm 1.3 \times 10^{9b}$
7	$2.7 \pm 0.5 \times 10^{6d}$	$4.1 \pm 0.5 \times 10^{9b}$
8	$1.8 \pm 0.7 \times 10^{6d}$	$9.6 \pm 2.2 \times 10^{8c}$
9	$8.6 \pm 1.2 \times 10^{5e}$	$5.4 \pm 1.5 \times 10^{8c}$
10	$3.9 \pm 1.0 \times 10^{4f}$	$8.6 \pm 0.5 \times 10^{7d}$

$^{*a\text{-}f}$The experimental values (means and standard deviations for n = 6) without the same superscript are significantly different ($p < 0.05$) in the same column.

Table 2. Sensory score (overall acceptance) of fermented tomato juice containing free and immobilized *L. acidophilus* during cold storage (4℃)

Time (wk)	Free cells	Immobilized cells
0	7.5	7.9
1	7.4	7.7
2	7.1	7.6
3	6.7	7.3
4	6.5	7.0
5	6.4	6.9
6	6.2	6.7
7	6.0	6.6
8	5.8	6.3
9	5.5	6.1
10	5.0	5.9

9-point hedonic scale.

DISCUSSION

Effect of immobilization on the growth of cells

In immobilized cell fermentation, some diffusion of cells from the entrapped gel beads occurred because of the growth of bacteria in the immobilized gel beads; these cells grew in the medium which initially had no cells [23]. Bacteria proliferated in the medium suspension around 6 h after the initiation of the fermentation of immobilized cells, and the final cell concentration in the suspension approached the level of 10^7 CFU/mL (Fig. 1). The ultimate viable cell number of the free cell fermentation was around 10^9 CFU/mL, and the cell concentration in the gel beads of the immobilized cell fermentation was higher than 10^{10} CFU/mL-gel (Fig. 1). These results indicate that higher viable cell numbers are obtained during immobilized cell fermentation than during free cell fermentation [24]. This might be because immobilized cells were protected from oxygen and high concentrations of substrates and products [10,25,26] and unfavorable conditions, such as low pH; therefore better results were achieved by immobilized cell fermentation.

Effect of immobilization on sugar content and pH during fermentation

Figures 1, 2, and 3 reveal that possible relationships existed between variations of cell growth, pH, and sugar. Better cell growth might have been due to the better utilization of the tomato juice medium, resulting in higher sugar consumption and higher acid production [27]. This led to a lower pH and lower residual sugar content in the immobilized cell fermentation compared to free cells.

Effect of cold storage on cell viability and sensory property

Table 1 shows that *L. acidophilus* immobilized in Ca-alginate has a higher survival rate than free cells during cold storage at 4°C. The results of this study suggest that cell immobilization could be used not only to enhance the fermentation efficiency but also to increase the survival of *L. acidophilus* in tomato juice (Fig. 1, Table 1). The sensory scores of fermented tomato juice containing immobilized *L. acidophilus* are better than free cells (Table 2). This might be because of the higher viable cell number of immobilized cells and because unfavorable deterioration reactions were inhibited during storage.

In conclusion, Ca-alginate immobilized *L. acidophilus* raises the number of viable cell during fermentation and cold storage (4°C), and ameliorate the sensory property of fermented tomato juice compared with free cells during storage. Results obtained in this study will be helpful for developing an appropriate probiotic juice with more health benefits.

ACKNOWLEDGMENT

The authors want to thank the China Medical University for the financial support of this research (grant number CMU94-066).

REFERENCES

1. Lee YK, Salminen S. The coming of age of probiotics. *Trends Food Sci Technol* 1995;6:241-5.
2. Shah NP. Functional foods from probiotics to prebiotics. *Food Technol* 2001;55:46-53.
3. Luckow T, Delahunty C. Which juice is 'healthier'? A consumer study of probiotic non-dairy juice drinks. *Food Qual Preference* 2004;15:751-9.
4. Luckow T, Delahunty C. Consumer acceptance of orange juice containing functional ingredients. *Food Res Int* 2004;37:805-14.
5. Mattila-Sandholm T, Myllärinen P, Crittenden R, et al. Technological challenges for future probiotic foods. *Int Dairy J* 2002;12:173-82.
6. de Backer L, Devleminck S, Willaert R, et al. Reaction and diffusion in a gel membrane reactor containing immobilized cells. *Biotechnol Bioeng* 1992;40:322-8.
7. Melzoch K, Rychtera M, Habova V. Effect of immobilization upon the production and behavior of *Saccharomyces cerevisiae*. *J Biotechnol* 1994;32:59-65.
8. Nath S, Chand S. Mass transfer and biochemical reaction in immobilized cell packed bed reactors: correlation of experiment and theory. *J Chem Technol Biotechnol* 1996;66:286-92.
9. Kearney L, Upton M, McLoughlin A. Enhancing the

viability of *Lactobacillus plantarum* inoculums by immobilizing the cells in calcium-alginate beads incorporating cryoprotectants. *Appl Environ Microbiol* 1990;56:3112-6.

10. Champagne CP, Lacroix C, Sodini-Gallot I. Immobilized cell technologies for the dairy industry. [Review] *Crit Rev Biotechnol* 1994;14:109-34.
11. Thakur BR, Singh RK, Nelson PE. Quality attributes of processed tomato products: a review. *Food Rev Int* 1996;12:375-401.
12. Yoon KY, Woodams EE, Hang YD. Probiotication of tomato juice by lactic acid bacteria. *J Microbiol* 2004; 42:315-8.
13. Tsen JH, Lin YP, King VAE. Banana puree fermentation by *Lactobacillus acidophilus* immobilized in Ca-alginate. *J Gen Appl Microbiol* 2003;49:357-61.
14. Tsen JH, Lin YP, King VAE. Fermentation of banana media by using kappa-carrageenan immobilized *Lactobacillus acidophilus*. *Int J Food Microbiol* 2004;91:215-20.
15. Min S, Jin T, Zhang QH. Commercial scale pulsed electric field processing of tomato juice. *J Agric Food Chem* 2003;51:3338-44.
16. King VAE, Su JT. Dehydration of *Lactobacillus acidophilus*. *Process Biochem* 1993;28:47-52.
17. King VAE, Zall RR. Ethanol fermentation of whey using calcium alginate entrapped yeasts. *Process Biochem* 1983;18:17-20.
18. Champagne CP, Girard F, Rodrigue N. Production of concentrated suspensions of thermophilic lactic acid bacteria in calcium-alginate beads. *Int Dairy J* 1993; 3:257-75.
19. Champagne CP, Mondou F, Raymond Y, et al. Effect of immobilization in alginate on the stability of freeze-dried *Bifidobacterium longum*. *Biosci Microflora* 1996;15:9-12.
20. Villanueva-Suárez MJ, Redondo-Cuenca A, Rodriguez-Sevilla MD, et al. Characterization of nonstarch polysaccharides content from different edible organs of some vegetables, determined by GC and HPLC: comparative study. *J Agric Food Chem* 2003;51:5950-5.
21. Salji JP, Ismail AA. Effect of initial acidity of plain yogurt on acidity changes during refrigerated storage. *J Food Sci* 1983;48:258-9.
22. Roberts JS, Kidd DR. Lactic acid fermentation of onions. *Lebensm Wiss Technol* 2005;38:185-90.
23. Lebeau T, Jouenne T, Junter GA. Diffusion of sugars and alcohols through composite membrane structures immobilizing viable yeast cells. *Enzyme Microb Technol* 1998;22:434-8.
24. Pilkington H, Margaritis A, Mensour N, et al. Kappa-carrageenan gel immobilization of lager brewing yeast. *J Inst Brew* 1999;105:398-404.
25. Williams D, Munnecke DM. The production of ethanol by immobilized yeast cells. *Biotechnol Bioeng* 1981; 23:1813-25.
26. Talwalkar A, Kailasapathy K. Effect of microencapsulation on oxygen toxicity in probiotic bacteria. *Aust J Dairy Technol* 2003;58:36-9.
27. de Porres E, de Arriola MC, Garcia R, et al. Lactic acid fermentation of banana puree. *Lebensm Wiss Technol* 1985;18:379-82.

番茄汁的細胞固定化嗜酸乳酸桿菌發酵

金安兒　黃惠煐[1]　曾政鴻[1]

國立中興大學　食品暨應用生物科技學系

中國醫藥大學　營養學系[1]

目的　嗜酸乳酸桿菌經褐藻酸鈣固定化後，應用於番茄汁中進行發酵，以提升菌株在發酵產品中之存活率。

方法　發酵原料採用由新鮮番茄製得之番茄汁，內中包含菌體之細胞固定化膠球之直徑約2.6 mm，發酵時間爲80小時，並另以游離態菌體進行發酵以作爲對照組。

結果　在固定化細胞的發酵過程中，細胞生長會導致細胞由膠球漏出，並持續在番茄汁中生長。在固定化細胞發酵組中，番茄汁內的最終活菌數達10^7 CFU/mL，而膠球中則高於10^{10} CFU/mL-gel，在游離態細胞發酵組中之最終活菌數則約爲10^9 CFU/mL。固定化細胞能克服番茄汁中的不利條件，因此獲得優於游離態細胞的結果。在隨後的4℃ 10週貯藏過程中，發酵番茄汁中的固定化嗜酸乳酸桿菌之活菌數仍能維持10^7 CFU/mL-gel的濃度，而游離態活菌數只有10^4 CFU/mL。貯藏過程中官能品評的結果，亦顯示內中包含固定化嗜酸乳酸桿菌者要優於包含游離態者。

結論　本研究結果顯示利用褐藻酸鈣固定化嗜酸乳酸桿菌來發酵番茄汁，能有效提升發酵成品的活菌數及感官品質。（**中台灣醫誌 2007;12:1-7**）

關鍵詞

褐藻酸鈣，發酵，固定化細胞，嗜酸乳酸桿菌，番茄汁

聯絡作者：曾政鴻
地　　址：404台中市北區學士路91號
　　　　　中國醫藥大學　營養學系
收文日期：2006年6月19日　　　　修改日期：2006年8月7日
接受日期：2006年8月16日