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Effect of Refrigeration on Viability of Immobilized Probiotic Bacteria in Alginate Coat of Strawberry

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Abstract: Immobilization of *Lactobacillus acidophilus* and *Bifidobacterium lactis* were carried out in calcium alginate coat of strawberries by using 2% (w/v) concentration of sodium alginate solution. Probiotic-coated samples were analyzed for titratable acidity, pH and viable cell counts of probiotics over 8 days of storage at refrigerator. The titratable acidity and pH of samples didn't demonstrate any significant changes during storage period ($P > 0.05$). The maximum reduction of the viability of *Lb. acidophilus* in the final day of storage was 0.31 log which evaluated nonsignificant ($P > 0.05$). But the significant reductions of the viable cell counts of *B. lactic* observed from second day ($P < 0.05$). The maximum fall rate of the viability of *B. lactic* reached 3.23 log at the last day of storage. Obtained results indicated that this probiotic immobilization technique more effectively protected the viability of *Lb.acidophilus* than of *B. lactis* against adverse effects of storage environment.

Key words: *Lb. acidophilus* · *B. lactis* · Viability · Immobilization · Coating · Alginate.

INTRODUCTION

Understanding of potential benefits of probiotics has led to the interesting use of these bacteria as a dietary adjunct. However, most of the probiotic products don't have a long shelf life, even when stored at low temperatures [1]. This remains as a big concern to both manufacturers and consumers, as the probiotic effects can only be exerted if a sufficient number of viable bacteria survive passage through the stomach and be delivered to the site of action [2]. It should be mentioned that recommended therapeutic minimum of probiotics per gram or milliliter of foods is 10^7cfu [1]. Encapsulation method is a good technique to solve the problem of poor viability of probiotics in food products and gastrointestinal tract [3-7].

The other widely used technique in food industries is coating of fresh products like fruits by suitable edible films [8-12]. Coating method can maintain the quality and extend shelf life of sensitive fruits like strawberry which is greatly used in preparation of desirable deserts [13].

A common point in two mentioned techniques is application of proper hydrocolloids like alginate [14, 10].

Alginate is the salt of alginic acid, polymer of D-mannuronic acid (M) and L- glucoronic acid (G), which is isolated from brown seaweed [15]. Calcium alginate not only delays the senescence process in fruits when applied as fruit edible coat [16, 10], but also well entraps bacterial cells in its fine pores when used in encapsulation technique [14, 17].

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The objective of the present study were to combine encapsulation and fruit coating techniques to investigate the possibility of immobilization of probiotics (*Lactobacillus acidophilus and Bifidobacterium lactis*) in alginate coat of strawberries. Then the vitality of these immobilized microorganisms on coated fruit were studied over 8 days of storage at refrigerator (5°C).

MATERIALS AND METHODS

Strawberry (*Fragaria* × *ananassa*) cv. Camarosa, were purchased from local market and coating processes were carried out on the same day.

Food grade sodium alginate (Sigma-Aldrich Pte Ltd, Singapore), calcium chloride (Merck Co. Germany), sodium citrate (Merck Co. Germany), *Lactobacillus acidophilus* La-5 and *Bifidobacterium lactis* Bb- 12 (Chr. Hansen, Denmark), MRS (de Man Rogosa, Sharpe) broth and agar (Merck Co. Germany) were the other materials used in present study.

Cultivation and Preparation of Starter Culture: Exactly 3 g of La-5 and Bb- 12 starter cultures were individually inoculated into 50 mL MRS broth, then both incubated at 37 °C for 24 h under aerobic and anaerobic conditions respectively. Activated cells were harvested by centrifuging at 3000 g for 5 min at 25°C and washed twice with sterile Ringer solution [7].

Preparation of Solutions: Sodium alginate solution (2 %, w/v), was prepared by stepwise addition of sodium alginate salt in distilled water, followed by heating at 70°C, while stirring until the solution became clear [10].

Calcium chloride solution (2 %, w/v) was prepared to cross-link the used carbohydrate polymer (sodium alginate) because divalent cations such as Ca^{+2} preferentially bind to polymer of L-glucronic acid of alginate and result in building of calcium alginate gel [5].

Sodium citrate solution (1%, w/v) used as chelating agent which shares affinity for calcium thus destabilizing the alginate gel resulting in release of entrapped cells [18].

The solutions were sterilized in 121°C for 15 min then cooled to environment temperature [18]. Before the onset of the coating process, washed activated probiotic cells were mixed with sterile sodium alginate solutions.

Preparation of Samples: Fresh strawberries without any signs of mechanical damage or fungal decay were selected and sanitized by immersion in 10 mg/L sodium hydrochlorite solution for 4 min, rinsed and dried by natural convection at 25°C prior to cutting them in small cube pieces. Coating process included (a) dipping the fruit pieces into sodium alginate solution containing probiotic, (b) allowing 1 min for dipping off the residual solution and (c) submerging them for 2 min in the solution of calcium chloride. All above steps were performed under perfectly sanitary conditions [10].

Coated fruits after drying on sterile filter papers, stored in a dry and clean plastic container at the refrigerator (5 °C) for 8 days. All of following chemical and microbiological analysis were carried out on samples in triplicate at 0, 2, 4, 6 and 8 days of refrigerated storage period.

Chemical Analysis

PH and Titratable Acidity: Samples were ground using an Ultraturrax at 8500 rpm for 1 min. The pH was determined using a pH-meter (Hanna Instruments 8521) using 10 g aliquot of homogenates made up to 100 ml with distilled water [10].

The titratable acidity of samples (control and coated, individually) was determined according to the AOAC 942.15 method, using a 10 g aliquot of homogenates made up to 100 mL with distilled water. This was titrated with 0.1 N NaOH to an end point of pH= 8.1 [19]. Titratable acidity was expressed as percentage of citric acid.

Microbiological Analysis: Precisely 5 g of coated fruit was liquefied in 45 mL of sterile sodium citrate solution at pH 6.0. Then a quarter strength Ringer's solution (Oxoid, Basingstoke, UK) was used in the preparation of serial dilutions. A pour plate method was employed for the determination of microbial groups. Enumeration of *Lb. acidophilus* and *B. lactis* were conducted on MRS agar at 37 °C under aerobic and anaerobic (GasPakPlus system, Merck, Germany) conditions, respectively, for 72 h [18]. Survival of cells has expressed in colony forming units per gram of samples (cfu/g).

Statistical Analysis: Data were recorded as mean ± S.D of three replications. Analysis of t-student was carried out at significant level of 0.05 by using SPSS software (Version 12.0, SPSS Inc. US).

RESULTS AND DISCUSSION

PH and Titratable Acidity: Determination of pH and titratable acidity of samples were performed to show that if these fruit attributed parameters could have effective impact on the viability of immobilized probiotics in strawberry coat. The pH value of samples varied from 3.84 ± 0.31 at day 0 to 3.79 ± 0.34 at day 8. At day 0

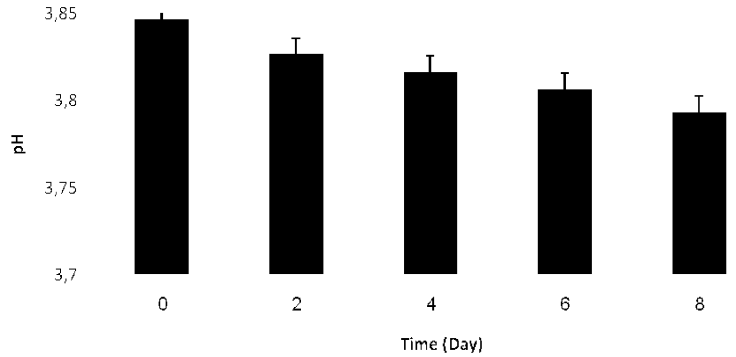

Fig. 1: pH variations of probiotic coated strawberry over 8 day storage at refrigerator

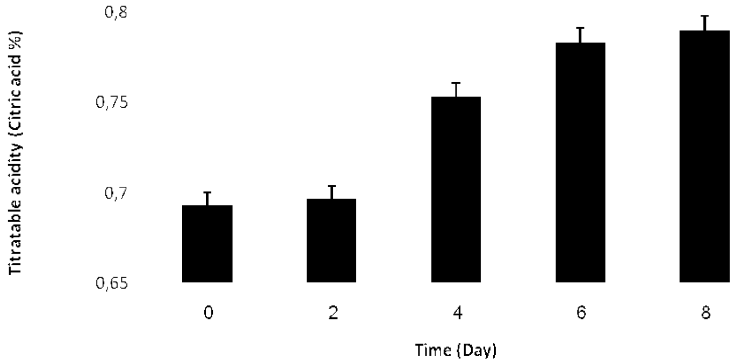

Fig. 2: Titratable acidity variations of probiotic coated strawberry over 8 day storage at refrigerator

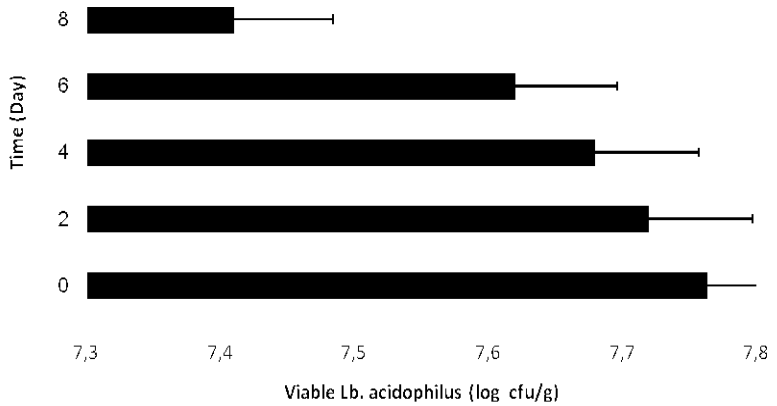

Fig. 3: Variations of viable cell counts (log cfu/g) of immobilized *Lb. acidophilus* in alginate coat of strawberry over 8 day refrigerator storage

the titratable acidity of coated fruits was 0.69 ± 0.21 which reached 0.79 ± 0.14 at the last day of storage. The pH and titratable acidity values of samples didn't significantly change ($P > 0.05$) during 8 days at refrigerator (Figures 1 and 2). These results agreed with those by Han *et al.* [9] reported the alginate coat of strawberry slowed effectively the changes on pH and acidity during cold storage. It was probably because the coat of fruit delayed fruit senescence [11] by modifying the internal atmosphere i.e., the endogenous CO_2 and O_2 concentration of the fruit, thus retarding ripening [20]. As the changes of pH and titratable acidity are sings of metabolic activity of encapsulated probiotics [21], it can conclude that the activity of immobilized probiotic bacteria in alginate coat of strawberry was intangible. On the other hand, the pH and titratable acidity of fruit couldn't have any impact on the viability of immobilized probiotics.

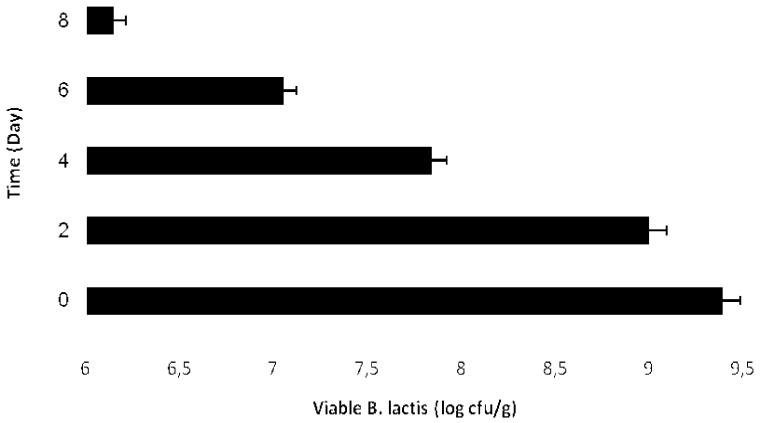

Fig. 4: Variations of viable cell counts (log cfu/g) of immobilized *B. lactis* in alginate coat of strawberry over 8 day refrigerator storage

Viable Cell Counts of Immobilized Probiotics : Variations in counts of immobilized *Lb. acidophilus* La-5 and *B. lactis* Bb- 12, in alginate coat of strawberries, during the 8 days of storage period at refrigerator are shown in Figures 3 and 4, respectively.

The viable cell count of *Lb. acidophilus* was 7.76 log cfu/mL at day 0. After 2, 4, 6 and 8 days of refrigerator storage the number of this bacterium reached 7.72, 7.67, 7.62 and 7.41 log cfu/g respectively. All of mentioned changes showed non-significant slight decline ($P< 0.05$) so that the difference between the days 0 and 8 was only 0.31 log cfu/g. However, the remained viable *Lb. acidophilus* on the fruit surface was $\times 10^7$cfu/g at the end of storage period. This finding was in good agreement with studies carried out on encapsulated *Lb. acidophilus* with calcium alginate indicating high viability of immobilized *Lb. acidophilus* in harsh environmental conditions [3, 22]. On the other hands, Kim *et al.* [4] reported a good efficiency of calcium alginate micro encapsulation on viability of *Lb. acidophilus* ATCC 43121 in acidic media.

The viability of *B. lactis* decreased from 9.39 at day 0 to 9.00 log cfu/g at day 2. This non-significant reduction was 0.38 log. In the next days the reduction of the viable cell count of this bacterium were significant even respect to their last evaluation days. The significant decline of *B. lactis* between days 4, 6, 8 and 0 were 1.54, 2.34 and 3.23 log respectively. Mentioned results obviously indicated the significant falling of the viability of immobilized *B. lactis* in alginate coat of strawberry. Despite the protection provided by alginate coating, cell mortality occurred at high rate. The high mortality rate of *B. lactis* can be attributed to the diffusion of environmental oxygen through the porous of calcium alginate matrix as this bacterium is strictly anaerobic microorganism [3]. At the final day of storage period the remained count of *B. lactis* per gram of coated strawberry was $\times 10^6$ cfu. It can demonstrate this fact that the alginate gel creates, to some extent, anoxic region in some pore-less parts of gel [23].

CONCLUSION

The potential of calcium alginate coat of strawberry in protection of *Lb. acidophilus* viability was significantly higher than of *B. lactis* in storage period at refrigerator temperature. The pH and titratable acidity of probiotic-coated strawberry remained unchanged under our storage conditions so they couldn't have any effect on the viability of imbedded probiotics. The viable *Lb. acidophilus* and *B. lactis* loads of coated fruits were $\times 10^6$ and $\times 10^7$ cfu/g respectively at the last day of storage. In the case of *Lb. acidophilus*, the viable cells were maintained above the recommended therapeutic minimum (10^7cfu/g), throughout storage. However, our findings indicate that alginate coated strawberry is a well carrier of *Lb. acidophilus*. It can be a novel method for protection of used *Lb. acidophilus* in strawberry deserts.

REFERENCES

1. Lee, Y.K. and S. Salminen , 1995. The coming of age of probiotics. Trends Food Science and Technol., 6: 241-5.
2. Chan, E.S. and Z. Zhang, 2005. Bioencapsulation by compression coating of probiotic bacteria for their Protection in an Acidic Medium. Process Biochemis., 40: 3346-3351.

3. Anal, A.K. and H. Singh, 2007. Recent advances in microencapsulation of probiotics for industrial applications and targeted delivery. Trends in Food Science and Technol., 18: 240-251.
4. Kim, S. J., S.Y. Cho, S.H. Kim, O.J. Song, II-S. Shin, D.S. Cha and H.J. Park, 2008. Effect of microencapsulation on viability and other characteristics in Lactobacillus acidophilus ATCC 43121. LWT, 41: 493-500.
5. Krasaekoopt, W., B. Bhandari and H. Deeth, 2003. Evaluation of encapsulation techniques for probiotics for yogurt. International Dairy J., 13: 3-13.
6. Lakkis, J.M., 2007. Encapsulation and controlled released-Technologies in food systems: Application of probiotic encapsulation in dairy products. Blackwell Publishing.
7. Òzer, B., H.A. Kirmaci, E. Senel, M. Atamer and A. Hayaloglu, 2008. Improving the viability of Bifidobacterium bifidum BB-12 and Lactobacillus acidophilus LA-5 in white-brined cheese by microencapsulation. International Dairy J., pp: 1-8.
8. Greener, I.K. and O. Fennema, 1989. Barrier properties and surface characteristics of edible, Bilayer Films. J. Food Sci., 54: 1393-1399.
9. Han, C., Y. Zhao, S.W. Leonar and M.G. Traber, 2004. Edible coatings to improve storability and enhance nutritional value of fresh and frozen strawberries (Fragaria × ananassa) and raspberries (*Rubus ideaus*). Postharvest Biology and Technol., 33: 67-78.
10. Olivas, G.I., D.S. Mattinson and G.V. Barbosa-C´anovas, 2007. Alginate coatings for preservation of minimally processed 'Gala' apples. Postharvest Biology and Technology, 45: 89-96.
11. Tanada-Palmu, P.S. and C.R.F. Grosso, 2005. Effect of edible wheat gluten-based films and coatings on refrigerated strawberry (*Fragaria ananassa*) quality. Postharvest Biology and Technol., 36: 199-208.
12.. Vargas, M., A. Albors, A. Chiralt and C. Gonz´alez-Mart´ınez, 2006. Quality of cold-stored strawberries as affected by chitosan-oleic acid edible coatings. Postharvest Biology and Technol., 41: 164-171.
13. Duxbury, D.D., 1992. Strawberries: source of natural flavor and nutrition. Food Process., 53: 7- 97.
14. Mandal, S., A.K. Puniya and K. Singh, 2006. Effect of alginate concentrations on survival of microencapsulated *Lactobacillus casei* NCDC-298. International Dairy J., 16: 1190-1195.
15. Harris, P., 1990. Food gels: Alginates. Elsevier Applied Science: London.
16. Moayednia, N., M.R. Ehsani, Z. Emamdjomeh, M. Mazaheri Asadi, M. Mizani and A.F. Mazaheri, 2010. A note on the effect of calcium alginate coating on quality of refrigerated strawberry. Irish J. Agricultural and Food Research, In press.
17. Moayednia, N., M.R. Ehsani, Z. Emamdjomeh, M. Mazaheri Asadi, M. Mizani and A.F. Mazaheri, 2009. The Effect of Sodium Alginate Concentrations on Viability of Immobilized Lactobacillus Acidophilus in Fruit Alginate Coating During Refrigerator Storage. Australian Journal of Basic and Applied Sci., 3(4): 3213-3226.
18. Krasaekoopt, W., B. Bhandari and H. Deeth, 2004. The influence of coating materials on some properties of alginate beads and survivability of microencapsulated probiotic bacteria. International Dairy J., 14: 737-743.
19. AOAC, 1995. Official Methods of Analysis, 17th (ed). Association of Analytical Chemists International, Washington, DC.
20. Bai, R.K., M.Y. Huang and Y.Y. Jiang, 1988. Selective permeabilities of chitosan-acetica acid complex membrane and chitosan-polymer complex membrane for oxygen and carbon dioxide. Polymer Bulletin, pp: 20- 83.
21. Sultana, K., G. Godward, N. Reynolds, R. Arumugaswamy, P. Peiris and K. Kailasapathy, 2000. Encapsulation of probiotic bacteria with alginate-starch and evaluation of survival in simulated gastrointestinal conditions and in yoghurt. International Journal of Food Microbiol., 62: 47-55.
22. Chandramouli, V., K. Kailasapathy, P. Peiris and M. Jones, 2004. An improved method of microencapsulation and its evaluation to protect *Lactobacillus* spp. in simulated gastric conditions. J. Microbiological. Methods, 56: 27-35.
23. Talwalkar, A. and K. Kailasapathy, 2003. Effect of microencapsulation on oxygen toxicity in probiotic bacteria. Australian J. Dairy Technol., 58: 36-39.