

The Effect of Sodium Alginate Concentrations on Viability of Immobilized *Lactobacillus Acidophilus* in Fruit Alginate Coating During Refrigerator Storage

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Abstract: Immobilization of *Lactobacillus acidophilus* was performed in the alginate coating of strawberry surface by using two different concentrations of sodium alginate solutions (2 and 3 % v/w). Scanning electron microscopy (SEM) of formed calcium alginate film on the fruit surface clearly showed entrapped *L. acidophilus* in gel nets. Obtained micrographs along with the microbiological analysis of two samples revealed the higher load of probiotic bacteria in fruits with higher concentration of sodium alginate in their coating formulations over 8-day study period at 5 °C. The viable count of entrapped *L. acidophilus* in fruit coating materials didn't change significantly throughout the storage period at 5 °C. So it can be said that immobilization of *L. acidophilus* in alginate matrix of strawberry coating effectively protected bacteria against the low temperature of refrigerator.

Key words: Immobilization, *Lactobacillus acidophilus*, Alginate coating.

INTRODUCTION

The study of edible coatings to extend the shelf life of fresh fruits is receiving great attention these days. Edible coatings can control gas and moisture transmission, producing a modified atmosphere in the food and also can serve as carriers of additives such as antimicrobials, antioxidants, color, flavors and nutraceuticals (El Gaouth *et al.*, 1991). Edible films and coatings are generally produced using biological materials like proteins, lipids and polysaccharides. Recently alginate is widely used as one of polysaccharide-based edible coatings to maintain the quality and extend shelf life of fresh products in the food industries (Mancin and Mchugh, 2000). Alginate is the salt of alginic acid, polymer of d-mannuronic acid (M) and l-gulucoronic acid (G), which is isolated from brown seaweed (Sime, 1990). This natural polymer has unique ability to bind multivalent cations, which is the basis of its gelling property, yielding insoluble hydrogels (Vennat *et al.*, 1998). On the other hand, alginate has been found as one of the best hydrocolloidal materials for cell entrapment, especially for probiotic bacteria, by encapsulation technique (Anil and Harjinder, 2007; Chan and Zhang, 2005). Bacteria (1-3 µm size) are well retained in alginate gel matrix which is estimated to have a pore size of less than 17 nm (Klenin *et al.*, 1983). The stabilization of probiotics using a carrier may improve survival of these microbes in product during processing (e.g. dairy products) and gastro-intestinal trace transmission (Goderska *et al.*, 2003).

The objectives of the present study were to investigate the possibility of immobilization of a model probiotic microorganism (*Lactobacillus acidophilus*) in different alginate concentrations used for coating the strawberry and to study the viability of the microorganism after the fruit coating steps during 8-day cold storage at 5 °C.

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MATERIALS AND METHODS

Fresh strawberries were bought in a local market on the same day of carrying out coating experiments. Food grade sodium alginate (Sigma-Aldrich Pte Ltd, Singapore), calcium chloride (Merck Co. Germany), sodium citrate (Merck Co. Germany), *Lactobacillus acidophilus* (La-5, 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:

3 g of starter culture (La-5) was inoculated into 50 mL MRS broth and incubated at 37 °C for 24 h under aerobic conditions. Activated cells were harvested by centrifuging at 3000 g for 5 min at 25 °C and washed twice with sterile Ringer solution.

Preparation of Solutions:

Sodium alginate solutions (2 % and 3 %, 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 (Olivas *et al.*, 2007).

Calcium chloride solution (2 % w/v) was prepared to cross-link the used carbohydrate polymer (sodium alginate) because divalent cations such as Ca²⁺ preferentially bind to polymer of L-glucuronic acid of alginate (Krasaekoopt *et al.*, 2003) and result in building of calcium alginate gel.

Sodium citrate solution (1%, w/v) used as chelating agents (Krasaekoopt *et al.*, 2004) which share affinity for calcium thus destabilizing the alginate gel (13) resulting in release of entrapped cells.

All of the solutions were sterilized in 121 °C for 15 min (Krasaekoopt *et al.*, 2004) then cooled to environment temperature. Before the onset of the coating process, washed activated probiotic cells was mixed with sterile sodium alginate solutions.

Sample Preparation:

Fresh strawberries without any signs of mechanical damage or fungal decay were selected and sanitized by immersion in 10 mgL⁻¹ 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 probiotic contained sodium alginate solution, (b) allowing 1 min for dipping off the residual solution and (c) submerging them for 2 min in the solution of calcium chloride (Olivas *et al.*, 2007). All above steps were performed under perfectly sanitary conditions.

Coated fruits after drying on sterile filter papers, stored in a dry and clean plastic container at the refrigerator (5 °C) for 14 days.

Microbiological Analysis:

L. acidophilus enumeration was conducted on probiotic contained sodium alginate solutions prior to coating and coated strawberries at 0, 3, 7, 10 and 14 days of refrigerated storage period.

5 g of coated fruit was liquefied in 45 mL of sterile sodium citrate solution at pH 6.0. *L. acidophilus* was enumerated on MRS agar at 37 °C under aerobic conditions for 72 h (Krasaekoopt *et al.*, 2004).

Scanning Electron Microscopy:

Thin layers of coated samples (with 2 % and 3 % used sodium alginate solutions) were cut with a sterile blade and dried overnight at room temperature. Then, they were coated with gold in a Jeol JFC-1100 ion sputter for 10 min, to increase electron conductivity, and examined in a Jeol JSM-6300 scanning electron microscope.

Statistical Analysis:

Data were recorded as mean ± S.D of three replicates. 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

Electron microscopy examination (Fig. 1,2) clearly showed the presence of *L. acidophilus* in the immobilizing support of calcium alginate on fruit surface. A marked difference was observed in microstructure of gels with different sodium alginate concentrations which it agreed with this previous finding that concentration of sodium alginate was an important variable for formation of calcium alginate gels (Thu *et al.*, 1996).

The viable counts of *L. acidophilus* ($\log \text{cfu mL}^{-1}$) in initial sodium alginate solutions and in the fruit coatings during 8 days of refrigerated storage are shown in table 1.

The initial cell counts of *L. acidophilus* in 2 and 3 % sodium alginate solutions were around $10 \log \text{cfu mL}^{-1}$. There was no significant ($p < 0.05$) difference between probiotic load of sodium alginate solutions definitely because of the same rate of *L. acidophilus* inoculations.

The viable counts of immobilized *L. acidophilus* were significantly different between two samples with two different applied sodium alginate solutions over the storage time. The initial probiotic load of coated sample with 2 % sodium alginate was about 2.5 log cycle lower than of coated fruit with 3 % sodium alginate. Similar differences between two samples were also observed in the other analytical days. This finding agreed with the observed scanned microstructure of two coating gels showing the entrapped cells in the gel nets. Obtained electron micrographs (1 and 2) showed that the probiotic load of the gel with low porosity (3 % sodium alginate) was high in comparison with that of the gel with much porosity. In this relation other researchers reported that bacteria (1–3 μm size) are well held in the alginate gel matrix (Klenin *et al.*, 1983) and the alginate gel with less porous matrix is more protective to the bacteria (Prakash and Jones, 2005; Thu *et al.*, 1996).

On the other hand, no significant reduction ($p < 0.05$) of immobilized cells were observed in two samples during 8 days of sample storage at 5 °C. It indicated that low temperature of the refrigerator had no adverse affect on the viability of entrapped and immobilized *L. acidophilus* in alginate gel so they remained in high counts at the end of their storage period as the 0 day. It approves this statement that entrapment and immobilization of bacterial cells in the gel beds like the microencapsulation technique seems to be the most promising technology to protect bacterial cells from adverse environment (Kailasapathy, 2002).

Conclusion:

The above results demonstrate that calcium alginate is an effective bed for probiotic entrapment on the surface of fruits like strawberry. Application of high concentrations of sodium alginate results in higher probiotic cell entrapment so the load of probiotic cells rises on the coated fruit. The survival of immobilized probiotic bacteria in the alginate gel on the surface of strawberry remained unchanged over the 8-day storage period at the refrigerator.

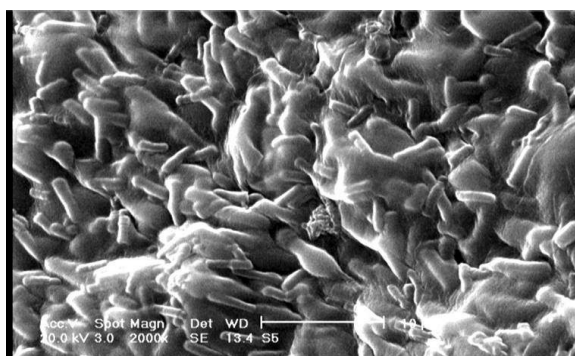


Fig. 1: Electron micrograph at 2000 \times , showing immobilized *L. acidophilus* in the alginate gel using 2 % sodium alginate.

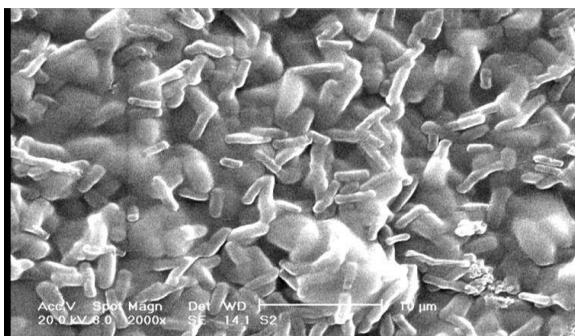


Fig. 2: Electron micrograph at 2000 \times , showing immobilized *L. acidophilus* in the alginate gel using 3 % sodium alginate.

Table 1: viability of *L. acidophilus* in initial sodium alginate solutions and in the fruit coatings during storage at 5 °C for 8 days.

	Sodium alginate (%)	Viable counts of <i>L. acidophilus</i> (log cfu mL ⁻¹)				
		In initial sodium alginate solution	In fruit coating			
			Storage time (day)			
		0	2	4	6	8
2	9.71 ± 0.36 ^a	7.71 ± 0.63 ^a	7.66 ± 0.05 ^a	7.82 ± 0.55 ^a	7.62 ± 0.59 ^a	7.41 ± 0.62 ^a
3	10.09 ± 0.20 ^a	10.36 ± 0.15 ^b	10.31 ± 0.24 ^b	9.89 ± 0.41 ^b	9.73 ± 0.23 ^b	9.66 ± 0.05 ^b

Means in same column with different letters (a-b) differ significantly ($p < 0.05$).

REFERENCES

- Anil Kumar, A. and S. Harjinder, 2007. Recent advances in microencapsulations for industrial applications and targeted delivery. *Trends in Food Science and Technology*, 18: 240-251.
- Chan, E.S. and Z. Zhang, 2005. Bioencapsulation by compression coating of probiotic bacteria for their protection in an acidic medium. *Process Biochemistry*, 40: 3346-3351.
- El Gaouth, A., J. Arul, R. Ponnampalam and M. Boulet, 1991. Chitosan coating effect on storability and quality of fresh strawberries. *Journal of Food Science*, 56(6): 1618-1620.
- Goderska, K., M. Zybals and Z. Czarnecki, 2003. Characterization of microencapsulated *Lactobacillus rhamnosus* LR7 strain. *Polish Journal of Food and Nutrition Science*, 12(53): 21-24.
- Kailasapathy, K., 2002. Microencapsulation of probiotic bacteria: Technology and potential applications. *Current Issues in Intestinal Microbiology*, 3: 39-48.
- Klenin J., J. Stock and K.D. Vorlop, 1983. Pore size and properties of spherical Ca-alginate biocatalysts. *European Journal of Applied Microbiology and Biotechnology*, 18(1): 86-91.
- Krasaekoopt, W., B. Bhandari and H. Deeth, 2003. Evaluation of encapsulation techniques for probiotics for yogurt. *International Dairy Journal*, 13: 3-13.
- 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 Journal*, 14: 737-743.
- Mancini, F. and T.H. Mchugh, 2000. Fruit–alginate interactions in novel restructured products. *Nahrung*, 44(3): 152-157.
- Olivas, G.I., D.D. Mattinson and G.V. Barbosa- C´anovas, 2007. Alginate coatings for preservation of minimally processed ‘Gala’ apples. *Postharvest Biology and Technology*, 45: 89-96.
- Prakash, S. and M.L. Jones, 2005. Artificial cell therapy: New strategies for the therapeutic delivery of live bacteria. *Journal of Biomedical Biotechnology*, 1: 44-56.
- Sime, W.J., 1990. Alginates. In *Food gels*, Ed., P. Harris. Elsevier Applied Science: London, pp: 53-58.
- Smidsrod, O. and G. Skjak-Braek, 1990. Alginate as immobilization matrix for cells. *Trends in Biotechnology*, 8(3): 71-78.
- Thu, B., O. Smidsrod and G. Skjak-Braek, 1996. *Progress in Biotechnology 11, Immobilized Cells: Basics and Applications*, Eds., R.H., Wijffels, R.M., Buitelaar, C., Bucke and J., Tramper, Elsevier Science B.V., pp: 19-31.
- Vennat, B., F. Lardy, A. Arvouet-Grand and A. Pourrat, 1998. Comparative texturometric analysis of hydrogels based on cellulose derivatives, carraghenates and alginates: Evaluation of adhesiveness. *Drug Development and Industrial Pharmacy*, 24: 27-35.