



Protecting probiotics by microencapsulation

Probiotics are live bacteria which transit the gastrointestinal tract and in doing so benefit the health of the consumer¹. An approach currently receiving considerable interest is the provision of a physical barrier against adverse environmental conditions for the living probiotic cells.

In the past, microorganisms were immobilised or entrapped in polymer matrices for use in bio-technological applications. The physical retention of the cells in the matrix facilitated the separation of the cells from their metabolites. As the technique of immobilisation or entrapment improved, the immobilised cell technology has evolved into encapsulation of live bacterial cells².

Encapsulation is the process of forming a continuous coating around an inner matrix that is wholly contained as a core of encapsulated material within the bead, whereas immobilisation refers to the trapping of material within or throughout a matrix. A small percentage of immobilised material may be exposed at the surface, while this is not the case for encapsulated material. Encapsulation tends to stabilise cells, potentially enhancing their viability and stability in fermented foods as well as in the gastrointestinal tract.

Why encapsulate probiotic bacteria?

In the recent past, there has been an explosion of probiotic-based health products mostly in the form of fermented dairy products as well as dietary supplements. To exert positive health benefits, these organisms have to reach their site of action in the gastrointestinal tract alive and establish themselves in certain numbers.

As a guide, the International Dairy Federation has recommended 10⁷ CFU/g

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at the point of consumption³. In order to produce therapeutic benefits, the suggested minimum level of probiotic bacteria in a food is 10⁶ viable cells per gram of a product⁴.

Many reports indicate that there is poor survival of probiotic bacteria in health products^{5,6}. Further, the survival of these bacteria in the human gastrointestinal system is not well documented. Viability and metabolic activity of probiotic bacteria in a food product or supplement at the point of sale are important considerations for their efficacy; these bacteria have to survive the duration of the shelf life, as well as transit through high acidic conditions (pH 1-3) of the stomach, enzymes such as lysozymes, bile salts in the small intestine, and toxic metabolites such as phenols produced during the digestion process.

A number of factors have been reported to affect the viability of probiotic cultures in food products and supplements⁷. These include acid, hydrogen peroxide, dissolved oxygen, temperature, concentration of lactic and acetic acids, and buffers such as whey protein concentrates.

Survival of encapsulated probiotic bacteria in dairy foods

The aim of this study was to increase the viability and survival of probiotic bacteria when incorporated into dairy products such as yoghurt, cheese and ice cream. In an earlier study⁸, several probiotic

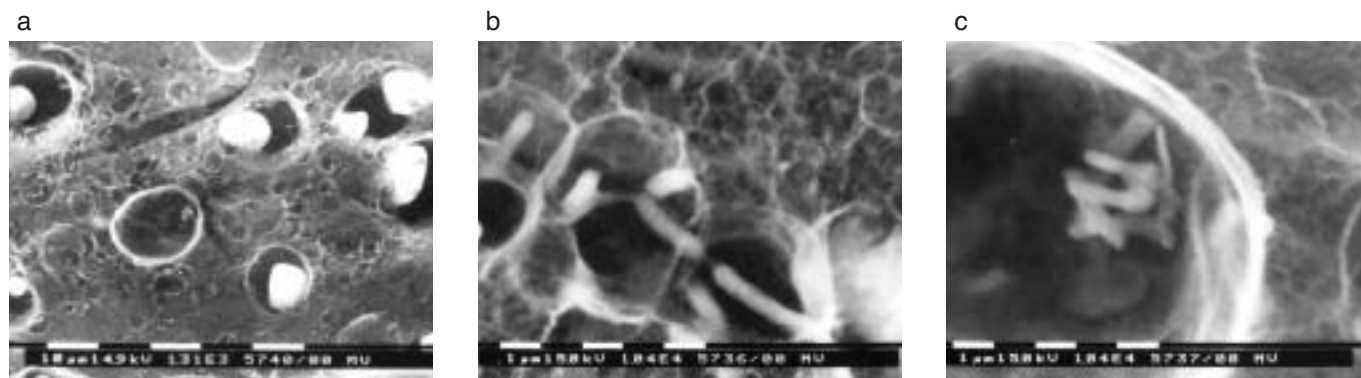
cultures from the CSIRO starter culture collection were screened for tolerance to acid, bile, oxygen, sugar and low temperatures and *Lactobacillus acidophilus* CCCC 2401 and *Bifidobacterium infantis* CCCC 1912 were selected as the tolerant strains. These strains were then given additional protection by encapsulation.

A mixture containing 2% alginate (Germantown, Australia), 2% Hi-Maize™ resistant starch (Starch Australia Ltd) and 0.1% probiotic culture was made in one litre of sterile milli-Q water. The culture mixture was then added to one litre of canola oil containing 0.2% Tween 80 and emulsified. 0.1 M calcium chloride solution was added quickly along the side of the beaker and mixed well, then allowed to separate into oil and water phases over 30 minutes, during which time the alginate-starch-probiotic culture formed as micro beads and settled to the bottom of the calcium chloride layer. The oil layer was drained and the encapsulated beads were collected by centrifugation and allowed to harden in 0.1M calcium chloride solution overnight.

In our study⁷, four batches of set yoghurts were made using yoghurt culture *Streptococcus thermophilus* DD134, and incorporating probiotic bacteria, *L. acidophilus* CCCC 2401 and *B. infantis* CCCC1912 in encapsulated and co-encapsulated states. Survival of probiotic bacteria was monitored over a period of 8 weeks of storage at 5°C.

The encapsulated cells showed some decrease in numbers with *L. acidophilus* CCCC 2401 decreasing by 0.4 log and *B. infantis* CCCC 1912 decreasing by 0.56 log. However, free cell numbers of *L. acidophilus* CCCC 2401 and *B. infantis* CCCC 1912 decreased by 1.44 log and 0.92 log respectively.

Figure 1. Section of alginate microcapsules showing: a). the starch grains in cavities, b). *L. acidophilus* and c). *B. infantis* located in the alginate matrix.



The results therefore showed that encapsulation of the probiotic cells ensured better viability of cells in yoghurt. Co-encapsulation of both *L. acidophilus* CSCC2401 and *B. infantis* CSCC1912 did not increase viability compared to individual encapsulation of these probiotic bacteria. This study showed that encapsulation improves the viability of probiotic bacteria in yoghurt and therefore makes it a better probiotic carrier.

Survival of probiotic bacteria was also monitored in ice cream incorporating probiotic bacterial cultures (*L. acidophilus* CSCC2401 and *B. infantis* CSCC1912) in encapsulated and co-encapsulated states, over a period of 24 weeks of storage at -20°C. The results showed that free cells survived better (approximately one log difference) than freshly encapsulated cells in ice cream⁷. Co-encapsulation of *L. acidophilus* CSCC2401 and *B. infantis* CSCC1912 enhanced survival of both strains as compared with individual encapsulation of the same strains.

This result shows that probiotic bacteria may not survive well in a low pH product such as yoghurt (pH <4.5); however, in a neutral pH product such as ice cream, they could survive well. Also, the high content of fat in ice cream (not <10%), could act as an encapsulant material for the probiotic bacterial cells. Hence, encapsulation may be not essential in the case of ice cream and similar high fat frozen dairy desserts.

Survival of probiotic cultures in either encapsulated or co-encapsulated states (*L. acidophilus* CSCC2401 and *B. infantis* CSCC1912) in Cheddar cheese was monitored over a period of 24 weeks of maturation at 8-10°C. The results showed that after 6 months' maturation, the encapsulated cell counts decreased by approximately 1-2 log cycles compared to free cell counts⁷. *L. acidophilus* strains showed better survival than Bifidobacterium strains during storage of cheese.

This study showed that free cells of probiotic bacteria survived better than encapsulated cells in Cheddar cheese matrix, hence encapsulation does not significantly help to increase the survival of probiotic bacteria during Cheddar cheese maturation and storage.

Conclusion

These encapsulation studies with three major dairy products show that the characteristics of the food matrix should be taken into consideration when deciding on encapsulation of the probiotic bacterial cells. Factors such as pH, texture of the food matrix and composition of the products such as high fat are important considerations in deciding on the encapsulation of probiotic cells to enhance their survival.

Food matrix could also influence the viability and the survival of encapsulated bacteria. For example, a Cheddar cheese matrix is a tightly structured one and it is possible that the physical barrier in this

case may inhibit the release of cell metabolites from the encapsulated bacteria, causing a build up of acids immediately surrounding the bacteria leading to loss of viability. Also, different strains of probiotic bacteria will survive differently in the encapsulated states. The size of the encapsulated beads may influence the sensory acceptance of the encapsulated products by the consumers.

References

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