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Trends in the microencapsulation of probiotics for application in dairy products

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ABSTRACT: Microencapsulation of probiotics has been evolving with reference to encapsulant polymers, encapsulation technologies, release mechanisms, targeted and controlled release within the gastrointestinal tract. An overview of the above is presented with a future prospect of nano encapsulation of physiologically bioactive cellular components of probiotic bacteria which may add synergistic effects in addition to live microencapsulated cells in significantly boosting therapeutic effects.

INTRODUCTION

Microencapsulation (ME) has been utilised as a delivery tool for probiotic microorganism in dairy food matrices to sites of interest within the gastrointestinal tract. The main concerns surrounding probiotic incorporation into food encompasses the inability of the probiotic strains to survive processing of food and during storage. Most food products containing probiotics are reported (1-3) to contain less than the recommended amount of viable probiotic cells at the time of consumption.
Dairy products have a place in delivering probiotic bacteria to the human gut, as these products provide probiotic bacteria with a suitable environment in which their viability is promoted (4). However, probiotics may also show low viability in yoghurt and other fermented milk products due to the acidic environment (5). In the past probiotic microorganisms were selected based on their ability to maintain viability during food processing operations, storage and transport through the gastrointestinal tract as well as providing beneficial health effects. However, more recently, functional efficacy of the probiotic bacterial strain has assumed greater importance, especially clinical evidence for approval from regulatory authorities such as the European Food Safety Authority (EFSA). The efficacy of probiotic strain maybe reduced during food processing, storage and during gastrointestinal transit. Microencapsulation provides the opportunity to protect probiotic cells. New technologies pertaining to the methodology and materials used for microencapsulation have allowed for even the most fastidious microorganisms such as *Bifidobacterium* to survive, for instance, those with a low oxygen tolerance.

EVOLUTION OF MICROENCAPSULATION

Encapsulation enables the separation of a core material such as probiotic bacterial cells from an external environment by a shell-like barrier and subsequently facilitates controlled release of the cells (6), thus encapsulation protects the cells from adverse external environmental conditions and improves their stability.
The Microencapsulation concept was initially derived from simple immobilisation technology where core materials were entrapped using polymers such as alginate. Immobilisation technology was utilised for instance, in continuous dairy fermentation processes and in the treatment of dairy effluents (7). Since this simple technology was initiated, a number of sophisticated techniques with advanced polymer materials for microencapsulation have been evolving as succinct fundamental research areas along with applications in the food and dairy industries (8). There are great limits to polymers available for microencapsulation because they must be of "food grade", and technologies must not be based on use of solvents which can remain even in trace amounts. Nevertheless, the substrate for incorporation of microencapsulated cells in food product matrix has undergone major developments over many years. The interaction of microencapsulated probiotic cells with the highly advanced formulated dairy product matrices and their release into areas of the gastrointestinal tract are not well researched. The stringent food regulations evolving in many parts of the world have called for clinically based evidence for approving a health claim of a probiotic strain for use in dairy products. It may not be possible to transfer health benefits imparted by a specified probiotic bacterial strain in a particular dairy food to another food product, as the bacterial-food interactions may be different in different food matrices. Microencapsulation may be used as a tool to avoid any disadvantageous food product matrix interactions with the probiotic cells. To this date, no research has been published on the interactions of microencapsulated versus free probiotic-food product matrix interactions with reference to viability, release and functional efficacy of probiotic bacterial strains in providing specific health benefits.
Microencapsulation of probiotic bacterial cells began for incorporation into fresh/soft textured short shelf life dairy food matrices with high water activities such as milk, yoghurts etc. However, recent trends indicate a significant expansion of probiotic dairy products in the world market and many new dairy probiotic foods are being developed and marketed. These foods have low water activities ie dry foods with longer shelf lives, for instance, non-fat dry milks and infant milk formulas. In addition, many probiotic foods are being marketed with dairy ingredients such as snack foods, biscuits, cereal bars, chocolates, sports bars etc. which have substantially low water activities, dry or intermediate moisture food matrices and longer shelf lives. Hence the microencapsulation procedures and encapsulated materials also are evolving to accommodate specific requirements of these new versions of probiotic dairy based foods.
The encapsulant materials used also have been evolving from simple polymers such as alginates using cation cross linkingcalcium ions for forming simple beads incorporating bacterial cells. Hydrocolloids such as alginates, carrageenan, pectins, xanthan gum, starch, gelatine, chitosan and carboxymethyl cellulose are food grade stabilisers and are found to be good gelling agents for use in microencapsulation (9). In hydro-gelling (or bead technology), polymers are used in the presence of divalent cations to microentrap cells in beads. The technology has evolved over many years using

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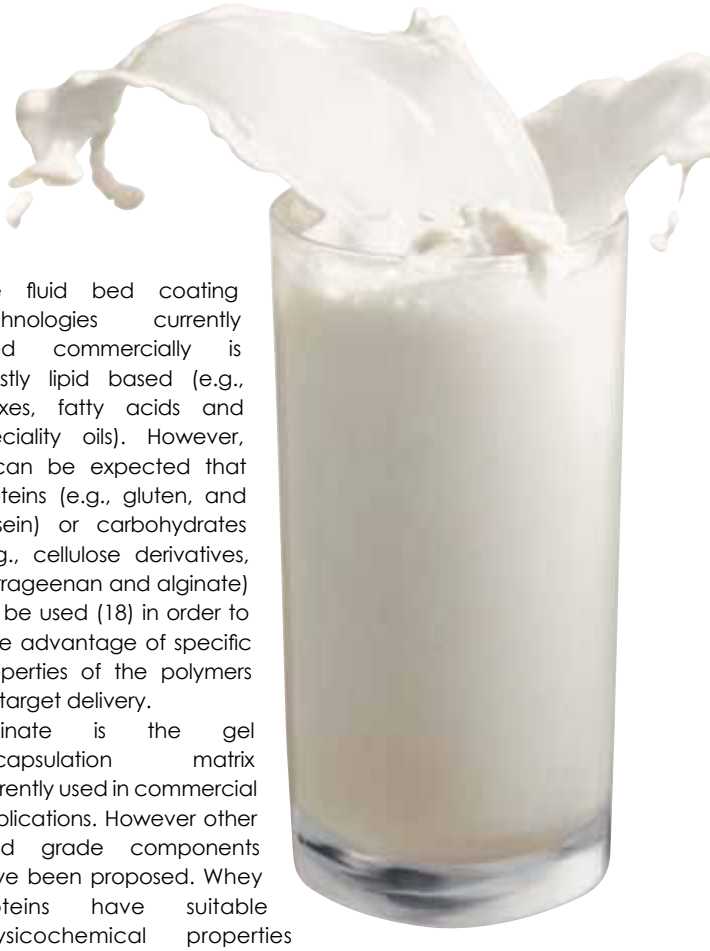
new polymers and encapsulation technologies suitable for spray drying, spray cooling and compression to form free flowing microencapsulated powders that have lower water activities with longer shelf lives. Simple alginate beads have larger pores that may allow more interactions with food components. Hence, coating of beads with polymers evolved, for instance, coating of alginate beads with chitosan (10). Such product is already in the market (Micropharma) where poly-L-lysine is used in the same fashion. Multiple coating of microbeads was developed to strengthen capsules, improve texture and reduce porosity, to make capsules free flowing as a powder in case of spray/freeze dried products and also for targeted delivery within the gastrointestinal tract. More recently, with the evolution of synbiotic technologies for the development of synbiotic dairy foods incorporating probiotics and prebiotics, prebiotic substances are being tested for use as encapsulant materials and targeted delivery in the gastrointestinal tract.

A number of possible delivery mechanisms exist for controlled and targeted release of encapsulated probiotic cells. However, these are not well reported in the literature. A very simple release mechanism is change of pH as in alginate beads, where gelling occurs when calcium ions and alginate polymers associate together for gelling and in alkaline conditions, with the dissolution of the gel and the separation of calcium ions, the alginate polymers destabilise hence allowing release of the entrapped cells (11, 12). This has been reported to be beneficial for delivery to human gastrointestinal tract, as the alginate-calcium beads do not dissolve in the stomach acid and transits to the small intestine where the alkaline pH destabilises the alginate-calcium beads and start releasing the encapsulated cells which may be completed beginning with small intestine and into the colon of the gastrointestinal tract (11, 12). More recently, a number of devices such as drinking straws and bottle caps with microencapsulated probiotic cells have been developed for instant delivery at the time of consumption.

The two technologies used commercially are gel particles and spray-coating. However, many other microencapsulation technologies have been developed on a laboratory scale. They include spray-drying, extrusion and emulsions (8, 9). Spray-drying is a well established technique suitable for large-scale, industrial applications, however, spray-drying as an encapsulated technique for probiotics in food use has not developed, because of low survival rate during drying of the bacteria and low stability upon storage. Freeze-drying is one of the least harmful drying methods for probiotics but is expensive compared to spray-drying (13). Some years ago, a new starch-based technology was developed by VTT Biotechnology, using freeze-drying (14) and spray-drying (15). In this technology large potato starch granules (50-100 micrometers), enzymatically treated to obtain a porous structure, were used as a carrier of the bacterial cells. Crittenden et al. (16) spray-dried probiotics in the presence of an O/W emulsion, but combined this with Maillard reaction products between protein and carbohydrates to improve film-forming and oxygen scavenging properties of the shell. It was reported that the encapsulated probiotics by this method were more stable upon storage at 25C and 50 percent relative humidity than non-encapsulated (free cells) ones. The encapsulated probiotics were also more stable in "in vitro' gastrointestinal conditions. More recently, a new process known as 'spray freeze drying (SFD) has been reported to produce microcapsules of *Lactobacillus paracasei* with high viability (> 60 percent) (17). The spraying stage did not affect viability of the bacteria. In the freezing stage, high osmotic pressures originated by elevated trehalose concentrations helped preserving viability.



The fluid bed coating technologies currently used commercially is mostly lipid based (e.g., waxes, fatty acids and speciality oils). However, it can be expected that proteins (e.g., gluten, and casein) or carbohydrates (e.g., cellulose derivatives, carrageenan and alginate) will be used (18) in order to take advantage of specific properties of the polymers for target delivery.

Alginate is the gel encapsulation matrix currently used in commercial applications. However other food grade components have been proposed. Whey proteins have suitable physicochemical properties and have the ability to form gels, which can be generated by heating the protein solution with subsequent cooling and acidification. Recently, Doherty et al. (19) reported that hydrolysed or denatured whey protein isolate were suitable for cell immobilisation of *L. rhamnosus* GG with reference to enhancing survival during storage at 37°C for 14 days as well as providing thermal protection at 57°C. More recently, a new encapsulation method to yield milk-protein based, water-insoluble microcapsules was developed by means of an enzyme-induced gelation mechanism. Transglutaminase (TGase) is a transferase that forms both inter-and intra- molecular isopeptide bonds in and between many proteins by cross linking of the amino acid residues of protein bound glutaminase and lysine (20). Heidebach et al. (21) reported a novel process of microencapsulation based on transglutaminase-catalysed gelation of casein suspensions containing *L. paracasei* ssp. *paracasei* F19 and *B. lactis* Bb12. Analysis of living cell numbers after incubation of free and encapsulated probiotics in simulated gastric juice without pepsin at pH 3.5 and pH 3.6 (37°C, 90 min) demonstrated a protective effect due to microencapsulation. This study showed that entrapment of probiotic cells in a dense casein matrix can protect the live cells from damage at pH levels similar to those in the human stomach. Other technologies for microencapsulation of probiotic bacteria include submerged co-extrusion, twin screw extrusion and compression coating (22).

Application of microencapsulation of probiotic bacteria in dairy foods includes yoghurt (23, 24), cheese (25, 26), frozen dairy desserts (27, 28) and powdered formulations (29, 30).

FUTURE PERSPECTIVES

Microencapsulation technology will continue to evolve with sophisticated encapsulants and technologies as the food-pharmaceutical interphase narrows and new breed of probiotic therapeutic agents evolve. The assessment of industrial feasibility of microencapsulation technology

for providing cost-effective large scale quantities of microencapsulated probiotic products for specific clinical and or commercial use will assume more importance. More research is needed on the interaction of microencapsulated materials with different food matrices, smart polymers for coating the microcapsules for targeting areas in the gastrointestinal tract and sustained delivery of viable probiotic cells. The probiotic paradox is that both live and dead cells in probiotic products may generate beneficial biological responses (31). This concept is currently speculative as it lacks confirmation using human clinical trials. However the effect of probiotics could be a dual one where live probiotic cells might well influence the gastrointestinal microflora and have an immunomodulatory effect, whereas the cell components of dead cells could exert an anti-inflammatory response (31). In this respect nano encapsulation that could encapsulate bioactive cellular components of probiotic bacteria will assume importance in the near future.

REFERENCES AND NOTES

1. H. Iwana, H. Masuda et al., *Bifidobacteria Microflora*, **12**, pp. 39-45 (1993).
2. S. Rybka, G.H. Fleet, *Food Australia*, **49**, pp. 471-475 (1997).
3. N.P. Shah, *J. Dairy Sci.*, **83**, pp. 894-907 (2000).
4. R.P. Ross, G. Fitzgerald et al., *Aust. J. Dairy Technol.*, **57**, pp. 71-80 (2002).
5. K. Kailasapathy, *Therapeutic Microbiology: probiotics and related strategies*, pp. 97-118 (2008).
6. K. Kailasapathy, *CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources*, **4**, pp. 1-19 (2009).
7. C.P. Champagne, C. Lacroix et al., *Crit. Rev. Biotechnol.*, **14**, pp. 109-134 (1994).
8. A.K. Anal, H. Singh, *Trends in Food Science & Technology*, **18**, pp. 240-251 (2007).
9. K. Kailasapathy, *Current Issues in Intestinal Microbiology*, **3**, pp. 39-48 (2002).
10. W. Krasaekoopt, B. Bhandari et al., *Int. Dairy J.*, **14**, pp. 737-743 (2004).
11. C. Iyer, K. Kailasapathy et al., *Lebens Wissens Technol.*, **37**, pp. 639-642 (2004).
12. C. Iyer, M. Phillips et al., *Letters in App. Microbiol.*, **41**, pp. 493-497 (2005).
13. B.E. Chavez, A.M. Ledeboer, *Drying Technology*, **25**, pp. 1193-1201 (2007).
14. P. Myllarinen et al., Patent. F1104405 (W 9952511 A1) (2000).
15. K. O'Riordan, D. Andrews et al., *J. App. Microbiol.*, **91**, pp. 1059-1066 (2001).
16. R. Crittenden, R. Weerakkody et al., *App. Environ, Microbiol.*, **72**, pp. 2280-2282 (2006).
17. D. Semyonov, O'Ramon et al., *Food Res. Intl.*, **43**, pp. 193-202 (2010).
18. C.P. Champagne, D. Fustier, *Current opinion in Biotechnol.*, **18**, pp. 184-190 (2007).
19. S.B. Doherty, V.L. Gee et al., *J. Microbiol. Methods*, **80**, pp. 231-241 (2010).
20. G.A.H. De Jong, S.J. Koppelman, *J. Food Sci.*, **67**, pp. 2798-2806 (2002).
21. T. Heidebach, P. Forst et al., *Int. Dairy J.*, **19**, pp. 77-84 (2009).
22. E.S. Chan, Z. Zhang, *Food Bioprod. Process.*, **80**, pp. 78-82 (2002).
23. K. Sultana, G. Godward et al., *Int. J Food Microbiol.*, **62**, pp. 47-55 (2000).
24. K. Kailasapathy, *Lebensmittelwissenschaft und Technologie*, **39**, pp. 1221-1227 (2006).
25. K. Kailasapathy, L. Masondole, *Aust. J. Dairy Technol.*, **60**, pp. 252-258 (2005).
26. J. Darukaradhya, Masters Hons. Thesis. University of Western Sydney, NSW, Australia (2005).
27. N.P. Shah, R.R. Ravula, *Aust. J. Dairy Technol.*, **55**, pp. 139-144 (2000).
28. G. Godward, K. Kailasapathy, *Milchwissenschaft*, **58**, pp. 161-164 (2003).
29. P. Siuta-Cruce, J. Goulet, *Food Technology*, **55**, pp. 36-42 (2001).
30. P. Capela, T.K.C. Hay et al., *Food Research Intl.*, **39**, pp. 203-211 (2006).
31. C.A. Adams, *Nutrition Research Reviews*, **23**, pp. 37-46 (2010).