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Research

Microencapsulation of Probiotics for Targeted Oral Delivery: Advances, Applications, and Therapeutic Potential

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Abstract

Microencapsulation of probiotics has revolutionized the landscape of functional foods and therapeutic delivery systems, particularly for gastrointestinal and systemic health. Probiotics are beneficial microorganisms that, when administered in adequate amounts, confer health benefits to the host. However, their survival is compromised by the harsh gastrointestinal (GI) environment, which includes gastric acid, bile salts, and digestive enzymes. Microencapsulation provides a protective matrix that enhances probiotic viability, stability, and site-specific delivery within the GI tract. Encapsulation materials such as alginate, chitosan, and synthetic polymers are used to create microcapsules via methods like spray drying, extrusion, and emulsification. These systems allow controlled and sustained release, which is vital for managing chronic disorders like IBS and IBD. Furthermore, synbiotic formulations combining probiotics and prebiotics enhance colonization and efficacy. This thesis explores the materials, methods, and mechanisms used in probiotic microencapsulation and evaluates their functional applications, especially in targeted therapy and precision nutrition. It also discusses emerging trends in nanotechnology and smart delivery systems tailored to physiological triggers such as pH or enzymes. Finally, it highlights industrial and clinical implications, emphasizing the need for scalable and regulatory-compliant solutions for commercial deployment. The findings underscore microencapsulation's role as a transformative tool for probiotic delivery and gut microbiota modulation.

Keywords: Microencapsulation, Probiotics, Oral delivery, Gut microbiota, Targeted release

INTRODUCTIONS

Probiotics, defined as live microorganisms that confer health benefits when administered in adequate amounts, have garnered significant attention for their role in gut health, immune modulation, and systemic disease management. However, their therapeutic potential is often compromised due to their reduced survival when subjected to the harsh conditions of the gastrointestinal (GI) tract. These conditions include acidic pH in the stomach, bile salts in the intestine, digestive enzymes, and oxygen exposure. To counter these challenges, microencapsulation has emerged as a cutting-edge technique to enhance the viability, stability, and site-specific delivery of probiotics.

Microencapsulation is a process wherein probiotic cells are enclosed within a protective polymeric matrix, such as alginate, chitosan, gelatin, or synthetic polymers like polyvinyl alcohol. This encapsulation matrix serves as a physical barrier against external stressors, helping to deliver a higher number of viable probiotics to the targeted regions in the gut. Moreover, encapsulation allows for controlled and sustained release of probiotics, which is critical for maintaining adequate microbial colonization and therapeutic action.

The application of microencapsulation in oral delivery has been explored through several methods, including extrusion, spray drying, freeze drying, and emulsification. Each method varies in encapsulation efficiency, particle size, and its impact on probiotic viability. Notably, synbiotic formulations those combining probiotics with prebiotic scan be developed using microencapsulation to enhance the synergistic effect of these functional ingredients.

This thesis examines the encapsulation of probiotics using calcium alginate and chitosan, focusing on enhancing survival under simulated GI conditions and refrigerated storage. The use of yogurt as a delivery vehicle is evaluated, along with the protective efficacy of the encapsulating materials. Ultimately, this work aims to validate microencapsulation as a viable and scalable strategy for improving oral probiotic therapy.

MATERIALS AND METHODS

1. Preparation of Buffer Solutions

To maintain physiological conditions, phosphate-buffered saline (PBS) was prepared by dissolving 8 g sodium chloride, 0.2 g potassium chloride, 1.44 g sodium hydroxide, and 0.24 g potassium hydroxide in 800 mL distilled water. The pH was adjusted to 7.0 with hydrochloric acid and made up to 1 L using distilled water. The buffer was sterilized by autoclaving at 121°C for 15 minutes before use.

2. Encapsulation of Probiotics

Microencapsulation was performed using the Inotech Encapsulator IE-50 R with a 300 µm nozzle. A suspension of bacterial cells (~10^{10} CFU/mL) was mixed with 1.8% sodium alginate and 1% Hi-maize starch. Capsules were formed by extrusion into 0.1 M calcium chloride for 30 minutes, followed by a 20-minute immersion in 0.4% chitosan solution to enhance capsule strength.

3. Preparation of Yogurt for Delivery

Three types of yogurts were formulated: (1) control without probiotics, (2) yogurt with free probiotics, and (3) yogurt with encapsulated probiotics. Whole milk (4% fat) with 9% SNF was standardized to 18% total solids and pasteurized at 85°C for 20 minutes. After cooling to 45°C, starter cultures and probiotics were added and fermented to pH 4.6 before refrigeration.

RESULTS

Viability Under Refrigerated Storage

The study demonstrated that CCAS encapsulated probiotics retained significantly higher viability compared to free cells in both stirred and set yoghurts during a 6-week refrigerated storage. Non-encapsulated CSCC 1912 was found to be metabolically active during storage, whereas encapsulated strains remained metabolically dormant, indicating enhanced preservation and longer shelf-life (Adhikari et al., 2003; Kailasapathy, 2002).

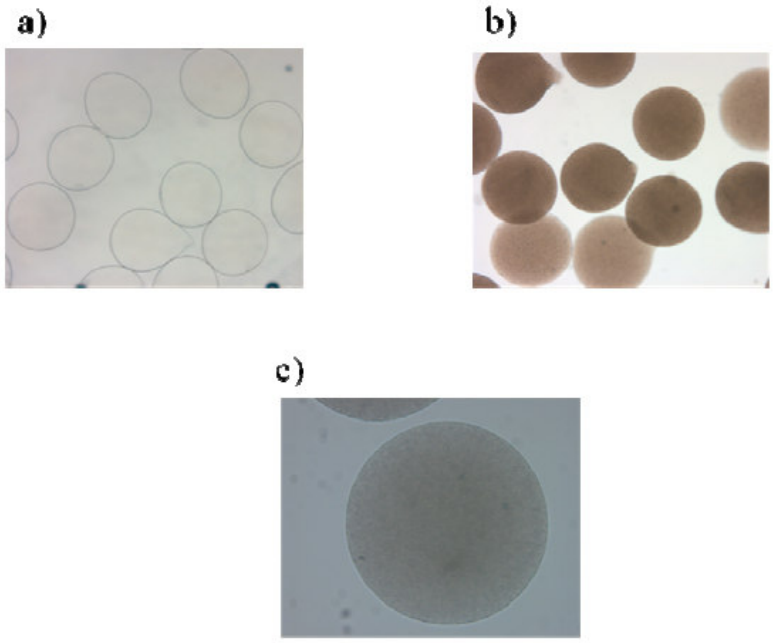

Table 1: Changes in lactic acid and acetic acid levels of stirred yoghurt containing free (non-encapsulated) and CCAS encapsulated probiotics over a 6-week refrigerated storage conditions

Treatments	Lactic acid (mg/100g)				Acetic acid (mg/100g)			
Weeks	**0**	**2**	**4**	**6**	**0**	**2**	**4**	**6**
Control	$^{2}574^{d}$	620^{e}	635^{f}	640^{2fg}	5.8^{e}	10.9^{f}	13.1^{g}	14.0^{g}
Free a*	587^{d}	628^{ef}	640^{fg}	648^{g}	6.2^{e}	11.7^{f}	13.5^{g}	13.9^{g}
Free b*	585^{d}	625^{e}	638^{f}	648^{g}	5.9^{e}	10.6^{f}	12.8^{g}	13.0^{g}
Free c*	580^{d}	620^{e}	632^{ef}	644^{g}	6.6^{e}	12.2^{fg}	13.9^{g}	14.1^{g}
Enc a*	576^{d}	619^{e}	636^{f}	647^{g}	6.0^{e}	11.3^{f}	13.7^{g}	13.5^{g}
Enc b*	570^{d}	622^{e}	629^{f}	642^{fg}	6.0^{e}	10.1^{f}	12.5^{fg}	13.2^{g}
Enc c*	578^{d}	617^{e}	630^{ef}	640^{fg}	6.5^{e}	11.6^{f}	13.0^{g}	13.7^{g}

Protection Against Gastrointestinal Conditions

Encapsulated bacteria using alginate and chitosan showed better resistance to acidic and bile conditions. Under simulated conditions, free probiotics lost viability by 6.36 log CFU/mL, while alginate-, xanthan-, and carrageenan-encapsulated strains showed only a 3.63–4.12 log CFU/mL reduction, indicating effective GI protection (Anal & Singh, 2007; Gombots & Wee, 1998). Further data from this study reveal that microencapsulation using chitosan-coated alginate-starch (CCAS) significantly improved bacterial survival under pH 2.0 and 2% bile salt conditions. For instance, CSCC strains encapsulated in CCAS showed viability losses of only 0.2–0.5 logs, compared to 1.0–1.5 logs in free cells over six hours of bile exposure. This protective capability is attributed to the mechanical stability and reduced porosity of the CCAS matrix, which shields cells from harsh GI fluids and enzymatic degradation. Additionally, SEM and optical microscopy confirmed that encapsulated cells retained their structural integrity post-exposure, reinforcing the viability data. These findings collectively validate that multi-layer encapsulation using biopolymeric coatings such as alginate, xanthan gum, carrageenan, and chitosan offers a robust strategy for maintaining probiotic efficacy through gastrointestinal transit.

Capsule Stability and Dye Retention

Capsules loaded with a fluorescent dye (6-carboxyfluorescein) showed higher retention in alginate and xanthan coatings (22.1% and 18.6% respectively). This suggests reduced permeability and greater barrier integrity, supporting long-term viability under storage and GI stress (Koo et al., 2001; Smidsrod & Skjak-Braek, 1990). Capsule stability is critical for ensuring the protection and controlled release of probiotics in the gastrointestinal

tract. In this study, microcapsules were subjected to simulated gastric and bile conditions to evaluate their mechanical robustness and permeability. A dye permeability assay using 6-carboxyfluorescein (6-CF), a water-soluble fluorescent dye, was employed to assess capsule integrity during storage. Capsules made of alginate, xanthan gum, and carrageenan exhibited significantly lower dye release compared to those formed with guar gum, indicating superior membrane integrity and lower porosity.

Over a two-week period, alginate and xanthan gum-based capsules retained 22.1% and 18.6% more 6-CF, respectively, than guar gum-based capsules. The encapsulated probiotics also showed improved survivability under acidic conditions (pH 2.0) and bile salts. Free cells exhibited a 6.36 log CFU/mL reduction in viability under taurocholic acid exposure, while encapsulated cells showed significantly lower reductions only 3.27 to 4.12 log CFU/mL depending on the gum type

DISCUSSIONS

This study illustrates the promising role of microencapsulation in enhancing the stability and oral delivery efficiency of probiotics. The protective matrix of calcium alginate, reinforced with chitosan, served to effectively shield bacterial cells from gastrointestinal stressors such as low pH and bile salts, which are typically responsible for reduced probiotic viability. The controlled preparation parameters such as alginate concentration, bacterial load, and cross-linking conditions proved vital in achieving optimal encapsulation efficiency and structural integrity. In the context of functional dairy products, encapsulated probiotics demonstrated improved viability over six weeks of refrigerated storage, outperforming free-cell probiotics in both set and stirred yogurt formats.

Moreover, dye diffusion assays and acid/bile challenge tests validated the enhanced resistance provided by encapsulated formulations, thus confirming the superiority of the encapsulation matrix. Notably, the dormant metabolic state of encapsulated cells during storage was associated with extended shelf life and reduced acid production, preserving product quality. These findings reinforce the commercial potential of CCAS microcapsules in dairy and pharmaceutical applications, where product stability and probiotic efficacy are paramount.

Collectively, the use of microencapsulation allows for targeted release and ensures viable colonization in the intestines, paving the way for broader applications including synbiotic therapies and precision nutrition. Future innovations involving smart polymer materials and co-encapsulation strategies may further enhance the therapeutic scope and consumer acceptability of probiotic products. This research thus represents a significant advancement in probiotic delivery science.

CONCLUSION

Microencapsulation presents a transformative strategy for improving the survival, stability, and targeted delivery of probiotics in oral formulations. In this study, the use of calcium alginate and chitosan as encapsulation agents enabled the production of stable microcapsules that successfully maintained probiotic viability through simulated gastrointestinal conditions and prolonged refrigerated storage. The encapsulated probiotics demonstrated a notable increase in survival rates and maintained their structural integrity under acidic and bile conditions, which would otherwise be detrimental to free probiotic cells.

Importantly, the ability of microencapsulated probiotics to remain metabolically inactive during storage without loss of viability ensured the preservation of yogurt quality and extended shelf life. This feature is particularly critical for commercial dairy products and supplements that require consistent efficacy over time. Additionally, encapsulated probiotics showed controlled release properties, making them suitable for site-specific delivery in the intestine, thereby enhancing their interaction with the host's microbiota.

The findings confirm that encapsulation not only addresses common issues of probiotic degradation but also enables product innovation by incorporating bioactives and prebiotics into single delivery systems. Synbiotic formulations, personalized probiotic therapies, and pH-sensitive smart capsules represent future directions that could revolutionize gut health management.

Overall, the research underscores the importance of optimizing encapsulation parameters and choosing appropriate materials to achieve successful oral delivery of probiotics. Microencapsulation stands as a robust, scalable, and consumer-friendly approach that aligns well with the increasing demand for functional foods and nutraceuticals aimed at promoting health and wellness.

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