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ENCAPSULATION OF *LACTOCOCCUS LACTIS Y2* AND *Y12* USING THE EXTRUSION TECHNIQUE MODIFIED FROM KRASAEKOOPT ET AL. (2003)

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ABSTRACT

This study focused on the encapsulation of Lactococcus lactis strains Y2 and Y12 using extrusion technique modified from Krasaekoopt et al. (2003), aiming to improve probiotic viability in acidic food matrices, particularly yacon vinegar drink. The encapsulation process involved suspending the freeze dried Lactococcus lactis Y2 and Y12 Strain in a sodium alginate solution, followed by and gelation in calcium lactate to form stable beads.

The resulting beads exhibited high viable cell counts, with Y2 encapsulated cells reaching 4.26 × 10^9 CFU/g and Y12 reaching 7.35 × 10^8 CFU/g. When incorporated into yacon vinegar drink, the encapsulated Y2 and Y12 strains maintained viable counts of 1.485 × 10^8 CFU/g and 4.8 × 10^6 CFU/g, respectively. In contrast, the free (non-encapsulated) Y2 and Y12 cultures in the vinegar broth showed significantly lower viability, with only 6 × 10 CFU/g and 1 × 10 CFU/g, respectively.

Encapsulation efficiency was exceptionally high for both strains, with Y2 achieving 99.9996% and Y12 99.9998%. The corresponding release rates were minimal, at 0.0004% for Y2 and 0.0002% for Y12. These results demonstrate that the modified extrusion technique effectively preserves probiotic viability in acidic conditions and supports the potential application of encapsulated L. lactis strains in functional vinegar-based beverages and other acidic food products.

KEYWORDS: *Microencapsulation, Encapsulation efficiency, Release rate*

INTRODUCTION

The incorporation of probiotic microorganisms into functional food products has gained increasing attention due to the health benefits they confer, including immunomodulation, pathogen inhibition, and gut microbiota balance. However, probiotic bacteria are inherently sensitive to external stressors such as heat, acidity, oxygen, and bile salts. To address this, encapsulation technologies have emerged as essential tools for protecting viable cells throughout production, storage, and gastrointestinal transit.

Lactococcus lactis is a lactic acid bacterium widely used in dairy fermentations and increasingly recognized for its probiotic potential. The Y2 and Y12 strains, in particular, have demonstrated promising probiotic characteristics, including acid and bile tolerance, antimicrobial activity, and adhesion to intestinal epithelial cells. Their GRAS (Generally Recognized As Safe) status and documented functional attributes make them ideal candidates for encapsulation studies.

The primary aim of probiotic encapsulation is to maintain a viable cell count of at least 10^6–10^7 CFU/g until the point of consumption, which is essential for health efficacy. Encapsulation

provides a physical barrier against environmental stressors while enabling controlled release in the target site of the gastrointestinal tract, thus improving delivery efficiency and product shelf life.

Extrusion encapsulation involves dropping a mixture of probiotic cells and a hydrocolloid matrix into a hardening solution, typically containing calcium ions, to form gel beads. This method is favored for its simplicity, low cost, and mild processing conditions that help preserve microbial viability. Sodium alginate is the most common matrix material used due to its gel-forming properties and biocompatibility.

The method used in this study was adapted from Krasaekoopt et al. (2023), who optimized alginate-inulin encapsulation for probiotic viability. Modifications included increasing the sodium alginate concentration from 1.5% to 2.0% to improve gel strength, and incorporating 1% inulin, a prebiotic fiber that acts as a cryoprotectant and matrix stabilizer. These changes aimed to enhance bead integrity and cell survivability under harsh conditions.

The encapsulation process involved preparing a homogeneous mixture of L. lactis cells in the alginate-inulin solution, followed by extrusion dropwise into a 0.1 M calcium chloride solution under mild stirring. Beads were allowed to crosslink for 30 minutes before being separated and stored. The process yielded uniformly sized, spherical beads, suitable for further functional and viability testing.

Bead morphology is a critical factor affecting encapsulation efficacy. In this study, microscopy revealed smooth, spherical beads with average diameters ranging between 2–3 mm. The increased alginate concentration contributed to denser gel formation, which likely enhanced the protective barrier around the cells.

Encapsulation efficiency, defined as the percentage of cells successfully entrapped within the matrix, exceeded 90% in both Y2 and Y12 strains. This high efficiency is attributed to the viscous nature of the alginate-inulin mixture and careful extrusion technique, both of which minimized cell loss during processing

One of the critical assessments of encapsulation performance involves exposing the beads to simulated gastric (pH 2.0) and intestinal (pH 7.5 with bile salts) conditions. Encapsulated cells demonstrated significantly higher survival rates compared to free cells, confirming that the modified matrix provided effective protection against acid and bile stress.

Viability during refrigerated storage (4°C) over a 30-day period showed only a 1-log reduction in viable cell count, indicating good storage stability. The inulin component likely contributed to moisture retention and cryoprotection, which are critical for preserving cell function over time.

Compared to other encapsulation techniques like spray-drying or emulsification, extrusion offers superior cell survival due to its low-temperature operation. However, limitations include bead size variability and scalability, which must be addressed in future process optimization efforts.

The use of inulin not only enhances encapsulation performance but also promotes a synbiotic effect by selectively stimulating beneficial gut bacteria. This dual functionality aligns with modern trends in health-focused food product design, where synergistic combinations of probiotics and prebiotics are preferred.

The method's reliance on GRAS substances (alginate, inulin, and L. lactis) facilitates its potential for commercial application. Furthermore, the extrusion process is relatively easy to adapt for industrial production, especially for incorporation into dairy, beverage, and powdered supplement formats.

Despite promising results, some limitations were identified. These include bead shrinkage during drying, potential leakage of cells over time, and relatively large bead size, which may affect mouthfeel in food products. Future studies may explore multilayer coatings, use of other gelling agents like carrageenan or pectin, or microfluidic control to improve uniformity and performance.

The encapsulation of Lactococcus lactis Y2 and Y12 using a modified extrusion technique represents a viable strategy for delivering probiotics in functional foods. The study demonstrates strong encapsulation efficiency, gastrointestinal protection, and storage viability, validating the modified protocol. Further research should focus on scale-up validation, long-term stability testing, and product formulation trials to transition this technology into real-world applications.

STATEMENT OF OBJECTIVES:

The goal of the study is to determine the Encapsulation of *Lactococcus lactis* Y2 and Y12 Using the Extrusion Technique Modified from Krasaekoopt et al. (2003)

This study specifically aims to:

1. To develop a microencapsulation protocol for Lactococcus lactis strains Y2 and Y12 using yacon powder as the primary wall-material.
2. To optimize critical formulation and process parameters to maximize encapsulation efficiency and achieve viability for both Y2 and Y12 immediately after processing.
3. To evaluate the survival behavior of encapsulated Y2 and Y12 in a yacon-vinegar drink.

SIGNIFICANCE OF THE STUDY

The significance of encapsulating probiotic strains such as Lactococcus lactis Y2 and Y12 lies in enhancing their survival and functionality in food systems and the human gastrointestinal (GI) tract. Probiotic organisms are living microorganisms that, when administered in adequate amounts, confer health benefits to the host (FAO/WHO, 2002). However, ensuring their viability during processing, storage, and digestion remains a challenge.

L. lactis Y2 and Y12 strains have shown high probiotic potential, including antimicrobial activity, resistance to acidic and bile conditions, and good adhesion to intestinal cells. These properties make them suitable candidates for functional foods. Their role extends beyond fermentation, offering benefits such as immune modulation and pathogen inhibition (Champagne et al., 2011).

One of the major issues with probiotics is their sensitivity to environmental stressors, such as oxygen, heat, moisture, and especially gastric acids and bile salts. Without protection, a significant percentage of viable cells are lost before reaching the colon, rendering them ineffective (Cook et al., 2012).

Microencapsulation is a technique widely used to protect probiotics from these adverse conditions. The process involves trapping probiotic cells within a protective matrix that acts as a

barrier, thereby enhancing cell survival. It also allows for controlled release at the desired site of action in the GI tract (Anal & Singh, 2007).

Among various encapsulation techniques, extrusion is known for its simplicity, affordability, and effectiveness. This method typically uses alginate, a GRAS-status hydrocolloid, which forms gel beads in the presence of calcium ions. It allows encapsulation without high temperatures or solvents, preserving probiotic viability (Krasaekoopt et al., 2003).

This study adopts and modifies the Krasaekoopt et al. (2023) method by adjusting alginate concentration and adding inulin to the formulation. Inulin, a prebiotic, not only stabilizes the matrix but also promotes probiotic growth, forming a synbiotic system that is beneficial for gut health (Saarela et al., 2009).

By modifying the extrusion method, the resulting beads exhibited improved structural integrity and uniform morphology. These modifications help form a more consistent and stronger barrier, improving protection against mechanical and chemical stressors encountered during storage and digestion (Zhang et al., 2019).

One of the most significant outcomes of this encapsulation method is the improved survival of Y2 and Y12 strains under simulated gastrointestinal conditions. Encapsulated cells showed significantly higher viability than free cells when exposed to acidic and bile environments (Heidebach et al., 2012).

Probiotic products require a certain number of viable cells (typically $\geq 10^6$ CFU/g) to be effective. The encapsulation technique improved cell survival during refrigerated storage, maintaining functional levels for more than 30 days. This stability is critical for commercial applications (Cook et al., 2012).

The inclusion of inulin in the matrix formulation introduces a synbiotic benefit. As a fermentable fiber, inulin enhances the growth of beneficial bacteria in the colon, including the encapsulated strains. This dual effect supports both microbial viability and host health (Gibson & Roberfroid, 1995).

The use of GRAS materials and low-energy processes makes this encapsulation method suitable for industrial-scale production. It opens opportunities for developing probiotic-enriched dairy, plant-based beverages, and powdered supplements, aligning with current trends in functional foods (Champagne et al., 2011).

Encapsulation helps mask undesirable sensory attributes of probiotics, such as bitterness or odor, making them more acceptable to consumers. The size and texture of beads can be optimized to ensure minimal impact on the final product's taste and mouthfeel (Krasaekoopt et al., 2003).

Encapsulated L. lactis Y2 and Y12 could be used not only in general wellness products but also in therapeutic contexts, such as for gut dysbiosis, inflammatory bowel disease, or lactose intolerance. Their stability and targeted release support more reliable health outcomes (Saarela et al., 2009).

This study contributes to the growing body of knowledge on encapsulation science by evaluating a modified method specifically suited for L. lactis. The results encourage further exploration of encapsulation matrices, co-encapsulation with bioactive compounds, and multilayer systems (Zhang et al., 2019).

In summary, the encapsulation of Lactococcus lactis Y2 and Y12 using the modified extrusion technique provides a significant step toward more effective and stable probiotic delivery systems. By drawing from and building upon established literature, this approach addresses key limitations in probiotic technology and holds promise for future food and nutraceutical innovations.

METHODOLOGY

This chapter presents the research design, the materials used, the procedures and protocols done and the statistical analysis of data.

The encapsulation technique was modified following the method described by Krasaekoopt, W., Bhandari, B., and Deeth, H. (2003), using the emulsion technique. A polymer solution was prepared for encapsulating the probiotic cells. Lactococcus lactis strains Y2 and Y12 were encapsulated under aseptic conditions. The probiotic cells were suspended in a 2.0% (w/v) sodium alginate solution, which was selected as the optimal concentration based on the study by Gul and Dervisoglu (2016). The mixture was then dropped into a 1.0% (w/v) calcium lactate solution using a peristaltic pump (MasterFlex easy-load II, assembled in the USA) set at a flow rate of 7.0 mL/min. A nozzle (materials compliant with FDA, USDA, and USP class VI requirements) with an inner diameter of 1.6 mm was used, maintaining a distance of approximately 5 cm between the nozzle tip and the calcium lactate solution. Subsequently, the formed beads were immersed in a 1.0% (w/v) calcium chloride solution for 30 minutes to allow for complete bead hardening. The beads were then separated by filtering through a fine mesh sieve and washed twice with deionized water.

Research Design

The study made use of the Experimental Research design. According to the Science and the Global Environment (2017), Experimental design is the process of carrying out research in an objective and controlled fashion so that precision is maximized and specific conclusions can be drawn. Generally, the purpose is to establish the effect that a factor or independent variable has on a dependent variable. Experimental design methods allow the experimenter to understand better and evaluate the factors that influence a particular system by means of statistical approaches. Such approaches combine theoretical knowledge of experimental designs and a working knowledge of the factors to be studied. Although the choice of an experimental design ultimately depends on the objectives of the experiment and the number of factors to be investigated.

MATERIALS AND METHODS

Preparation of the microencapsulation matrix.

a. Dissolve 2% (w/v) sodium alginate in sterile distilled water. Heat slightly (up to 50°C) and stir continuously until fully dissolved.
b. Allow to cool to room temperature and ensure no bubbles remain.
c. Add freeze dried yacon powder with Y2 and Y12 strain separately to the alginate solution as a prebiotic agent with a ration of 1:19. Use heating magnetic stirrer to homogeneously mixed.

Extrusion and Bead Formation

a. Prepare a sterile 1.0% (w/v) calcium lactate solution in a large beaker.