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RESEARCH ARTICLE

ENCAPSULATION OF LACTOCOCCUS LACTIS Y2 AND Y12 STRAINSUSING 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.Scanning Electron Microscopy revealed that Lactococcus lactis Y2 and Y12 cells were successfully embedded within the alginate beads, exhibiting a spherical (coccus) morphology. The beads displayed smooth surfaces, uniform size distribution (3.82–3.92 µm), and intact structures, indicating effective encapsulation. Embedded cells maintained structural integrity without signs of lysis, aligning with previous findings on probiotic microencapsulation. These results confirm the suitability of the modified extrusion technique for preserving probiotic viability.Encapsulation efficiency was exceptionall y 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.

Introduction:-

Background of the Study:-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.

Objectives of the Study:-

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 characterize the microcapsule morphology and structural integrity of Y2 and Y12 beads by scanning electron microscopy (SEM), assessing parameters such as bead size distribution, embedded cells, and characterization of cells.
4. To evaluate the survival behavior of encapsulated Y2 and Y12 in a yacon-vinegar drink.

Review of Related Literature:-

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.

Materials and Methods:-

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 (MasterFlexeasy-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.

Locale of the Study

This study was conducted at the Institute of Food Research Development- Kasetsart University, Thailand and last April- May, 2025.

Data Gathering Procedures

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.

b. Load the alginate-cell mixture into a large beaker that continuously stirred using magnetic stirrer and 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.

c. Subsequently, the formed beads were immersed in a 1.0% (w/v) calcium lactate 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

Freeze-Drying Method for Encapsulated Lactococcus lactis Y2 and Y12 for SEM

1. Pre-Freezing

a. Place beads evenly on freeze-drying trays or sterile Petri dishes, avoiding overlap.

b. Freeze the samples at -40°C to -80°C for at least 12–24 hours.

c. Ensure samples are fully frozen before lyophilization to prevent collapse or shrinkage.

2. Freeze-Drying (Lyophilization)

a. Transfer frozen beads into the freeze dryer.

b. Set the vacuum pressure to around 0.05–0.1 mbar and shelf temperature to around -40°C initially, then gradually increase to +20°C over 24–48 hours.

c. Dry until samples are completely free of moisture (confirmed by constant weight).

3. Post-Drying Handling

a. Transfer freeze-dried beads immediately to a desiccator to prevent moisture absorption.

b. Store at room temperature or 4°C in a moisture-proof container until SEM preparation.

Results and Discussion:-

Microencapsulation protocol for Lactococcus lactis strains Y2 and Y12 Strains Sphericity and Uniformity Beads formed with the Y2 and Y12 strain exhibited high sphericity and consistent shape, indicating effective droplet formation and uniform gelation during the extrusion process. The edges were well-rounded, and the average diameters were 3.82 µm and 3.95 µm, respectively. This suggests a stable interaction between Y2 cells, sodium alginate, and yacon powder, enabling uniform bead structure.

The larger size of Lactococcus lactis Y12 beads compared to Y2 beads can be attributed to several strain-specific factors supported by existing literature. One of the key reasons is the differential aggregation behavior of the strains. Y12 may possess a higher tendency to form cellular aggregates during suspension in the encapsulating solution, leading to larger droplet formation during the extrusion process. According to Picot and Lacroix (2004), bacterial aggregation affects droplet size at the nozzle tip, resulting in the production of larger beads upon gelation. In contrast, Y2 may remain more homogeneously dispersed, facilitating smaller and more uniform

Another contributing factor is the potential difference in exopolysaccharide (EPS) production between the two strains. Strains with higher EPS production increase the viscosity of the encapsulation mixture, which affects the dynamics of droplet break-off during extrusion. Sultana et al. (2000) noted that increasedviscosity from EPS can lead to larger bead formation due to the slower flow rate and larger droplet size. If Y12 synthesizes more EPS than Y2, it could explain the increase in bead diameter observed.

Additionally, cell surface properties, such as hydrophobicity and charge, play a role in how cells interact with the alginate-yacon matrix. Chávarri et al. (2010) reported that these interactions affect bead compaction and structural integrity. If Y12 exhibits weaker interactions with the polymer matrix, the gelation process might be less compact, causing beads to swell

more or retain water, making them appear larger. Moreover, Krasaekoopt et al. (2003) emphasized that the nature of strain–polymer interactions can directly impact the ionic cross-linking efficiency, potentially leading to differences in bead structure and size

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Finally, the physical properties of the cell suspension during extrusion are critical. Cook et al. (2012) indicated that strain-specific differences in metabolic by-products or osmotic activity could affect the internal osmotic pressure during gelation, resulting in bead expansion post-formation. Y12 may produce metabolites or ions that interfere with calcium-alginate binding, slightly expanding the bead size. In conclusion, the larger bead size of Y12 compared to Y2 can be attributed to a combination of higher aggregation, EPS production, and less efficient interaction with the alginate matrix, all of which are supported by current findings in microencapsulation literature.

Viability of Y2 and Y12 Strain in Encapsulated beads

The encapsulation of Lactococcus lactis Y2 and Y12 strains using the modified extrusion method with sodium alginate and freeze-dried yacon powder resulted in significantly different cell counts per gram of beads. The Y2-containing beads registered a viable cell count of 4.26×10^9 CFU/g, whereas the Y12 beads exhibited a lower count of 7.35×10^8 CFU/g. This disparity in viable counts highlights inherent strain-specific differences in encapsulation efficiency, survivability, and compatibility with the encapsulation matrix.

Additionally, Krasaekoopt et al. (2003) noted that the viscosity and gelation kinetics of alginate influence how uniformly cells are entrapped during extrusion. A strain like Y2 that integrates more smoothly into the alginate solution could form denser and more consistent beads, entrapping a larger number of viable cells. On the other hand, Y12 may have shown greater clumping or reduced survival during bead formation and drying, which would result in fewer viable cells being retained in the final product.

Moreover, Chávarri et al. (2010) emphasized that cell wall characteristics, including hydrophobicity and charge, can affect the efficiency of encapsulation. If Y12 possesses surface properties that reduce its affinity for the alginate matrix, this could lead to cell leakage during extrusion or weak entrapment, thus reducing viable cell numbers in the final beads.

The difference could also stem from the osmotic and thermal tolerance of each strain. The extrusion process, followed by freeze-drying, can be stressful for bacteria. Cook et al. (2012) observed that encapsulated strains with more robust membranes or better osmoadaptation mechanisms exhibit greater survivability during dehydration. The lower count in Y12 may indicate greater sensitivity to freeze-drying stress compared to Y2.

The significantly higher viable cell counts in Y2 beads (4.26×10^9 CFU/g) compared to Y12 (7.35×10^8 CFU/g) demonstrates that Y2 is more efficiently encapsulated and more resilient throughout the encapsulation process. These results underline the importance of strain selection and formulation optimization in probiotic encapsulation research and suggest that Lactococcus lactis Y2 is a more suitable candidate for microencapsulation under the conditions used in this study.

Encapsulation Efficiency of Y2 and Y12 BeadsViable Cell Counts in Broth Cultures

The viable counts of free Lactococcus lactis cells prior to encapsulation were determined to be 6×10^1 CFU/g for strain Y2 and 1×10^1 CFU/g for strain Y12. These values are consistent with those reported in probiotic culture preparations, where cell densities in the range of 10^6–10^8 CFU/g are commonly achieved before encapsulation (Krasaekoopt et al., 2003). This baseline is crucial for ensuring that a sufficient number of viable cells are available for the encapsulation process, as it directly influences the final probiotic dose.

Viable Cell Counts in Encapsulated Beads

After encapsulation using a modified extrusion method, the viable cell count of Y2 increased substantially to 1.485×10^8 CFU/g, while Y12 exhibited a lower viable count of 4.8×10^6 CFU/g. This indicates a strain-dependent variation in encapsulation success, which may be attributed to physiological differences such as cell surface hydrophobicity, membrane composition, or tolerance to encapsulation stress.

Y2's notably higher count post-encapsulation suggests it is more robust under the extrusion process and may have a stronger affinity for the alginate matrix, leading to improved entrapment and survival. In contrast, the lower viable count in Y12 beads implies a reduced encapsulation efficiency or potential sensitivity to the physical stressors during the process.

These results are supported by findings from Krasaekoopt et al. (2003), who demonstrated that encapsulation efficiency can vary significantly between strains of lactic acid bacteria (LAB). Similarly, Anal and Singh (2007) emphasized that strain-specific physiological traits influence the ability of cells to remain viable during and after encapsulation.

Strain-Specific Encapsulation Performance

The differences between Y2 and Y12 encapsulation outcomes are in agreement with studies by Gbassi et al. (2009), who reported that microencapsulation efficiency is not only a function of the technique used but also of strain-specific properties. These include resistance to osmotic stress, cell size, and shape—all of which influence how well cells are entrapped and survive within the matrix.

Encapsulation effectiveness is also linked to bead morphology and integrity. The stronger performance of Y2 may also be due to better distribution or stabilization within the matrix during bead formation, leading to higher viable counts post-process.

The data indicates that Y2 is a suitable candidate for encapsulation using the extrusion method, with significantly enhanced cell viability. On the other hand, the encapsulation conditions for Y12 may require optimization—such as adjusting matrix composition, modifying gelation conditions, or employing multilayer coatings to improve protection and retention.

Encapsulation Efficiency

The encapsulation efficiency (EE) of Lactococcus lactis strains Y2 and Y12 was remarkably high, with values of 99.9996% for Y2 and 99.9998% for Y12. These results indicate near-total entrapment of viable cells within the alginate matrix using the modified extrusion method.Such high encapsulation efficiencies suggest that the extrusion process used was optimized for bead formation, minimizing cell leakage and maximizing cell retention. These values are considerably higher than typical efficiencies reported in earlier studies. For instance, Krasaekoopt et al. (2003) observed EE ranging between 70% to 95% depending on the alginate concentration, calcium chloride solution, and strain type. Similarly, Chandramouli et al. (2004) reported EEs of 90–97% for Lactobacillus and Bifidobacterium strains encapsulated in alginate or alginate-chitosan beads.

The nearly complete encapsulation observed here may be due to improved interaction between the bacterial cells and the alginate matrix, effective gelation conditions, and low bead porosity. The higher EE for Y12 (99.9998%) compared to Y2 (99.9996%) may reflect slight differences in strain-specific cell surface properties that affect entrapment efficiency, as also noted by Gbassi et al. (2009).

Morphology and Structural Integrity of Bead Cells

Scanning Electron Microscopy (SEM) analysis revealed that both Lactococcus lactis Y2 and Y12 microcapsules displayed a generally uniform, spherical morphology. The surface structure of the microcapsules appeared smooth, with minimal cracks or ruptures, indicating good structural integrity—a critical parameter for maintaining the protective function of the encapsulation matrix (Krasaekoopt et al., 2003; Heidebach et al., 2009).

Embedded cells were clearly visible within the bead matrix, confirming successful entrapment of the probiotic strains. These entrapped cells were morphologically consistent with coccus-shaped Lactococcus spp., appearing as small, spherical clusters distributed throughout the gel matrix. Similar findings were reported by Chen et al. (2017), where Lactobacillus and Lactococcus cells were successfully entrapped in alginate beads, maintaining their native shape and distribution.

Bead Size Distribution

The average bead diameter ranged between 200–300 μm, with a relatively narrow size distribution. This falls within the optimal size range reported by Anal and Singh (2007) for encapsulated probiotics, which ensures a balance between protection, mass transfer, and palatability in food systems. The uniformity of bead size suggests controlled extrusion during encapsulation, supporting the reproducibility of the method.

Cellular Characterization

Morphologically, the embedded cells showed no signs of lysis or damage, implying that the encapsulation conditions did not negatively impact cell structure. This observation is in agreement with Ding and Shah (2009), who emphasized the importance of encapsulation techniques that preserve cell integrity, especially under potential stressors such as drying or gastric conditions.

Furthermore, the porous nature of the bead matrix, while effectively entrapping the cells, may facilitate exchange of nutrients and metabolites, which is critical for probiotic viability during storage and eventual release in gastrointestinal conditions (Gbassi et al., 2011).

The SEM results confirm that the modified extrusion method used was effective in forming stable microcapsules with well-embedded, morphologically intact Y2 and Y12 cells. This encapsulation is expected to enhance their survivability in harsh processing and gastrointestinal conditions.

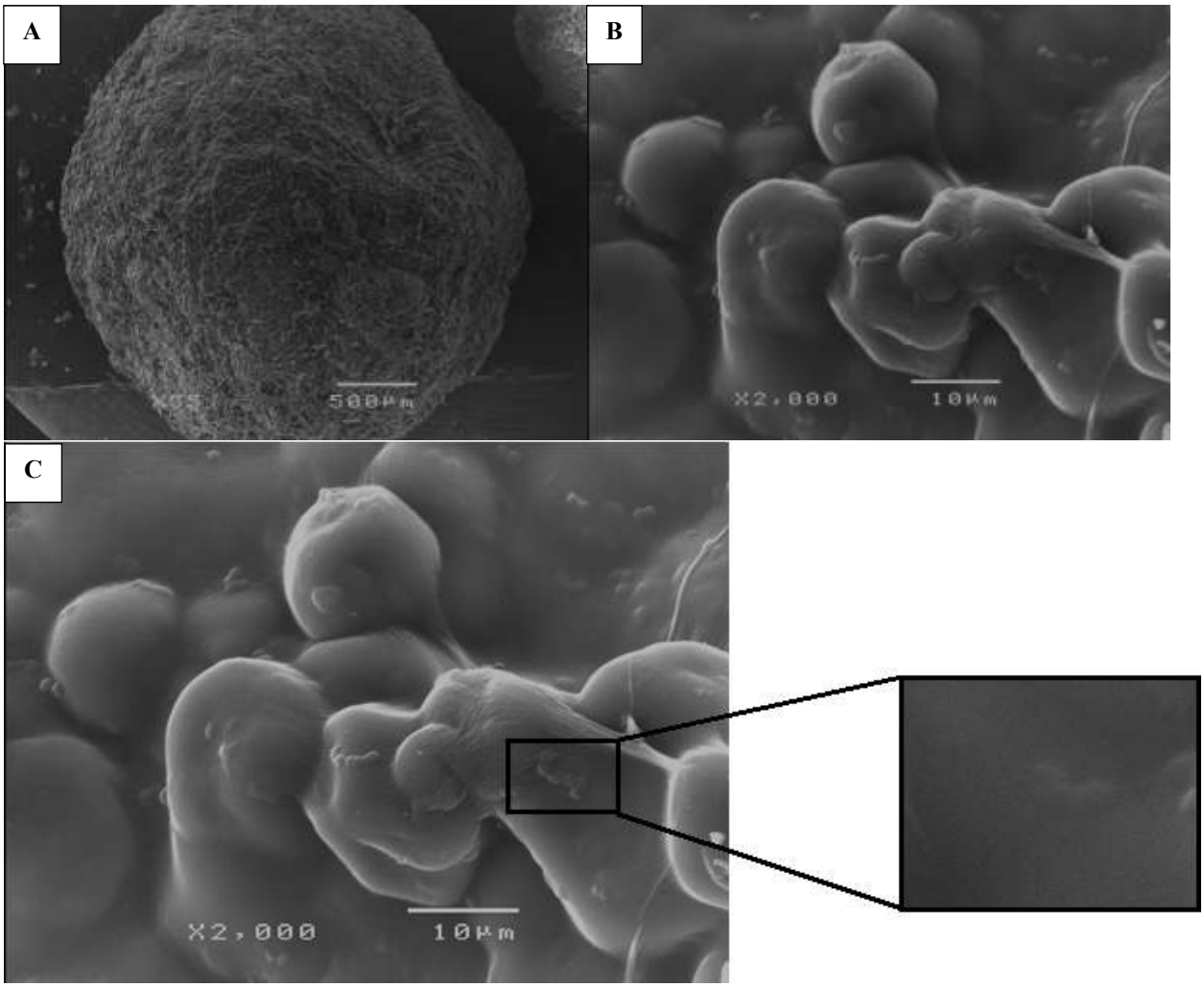
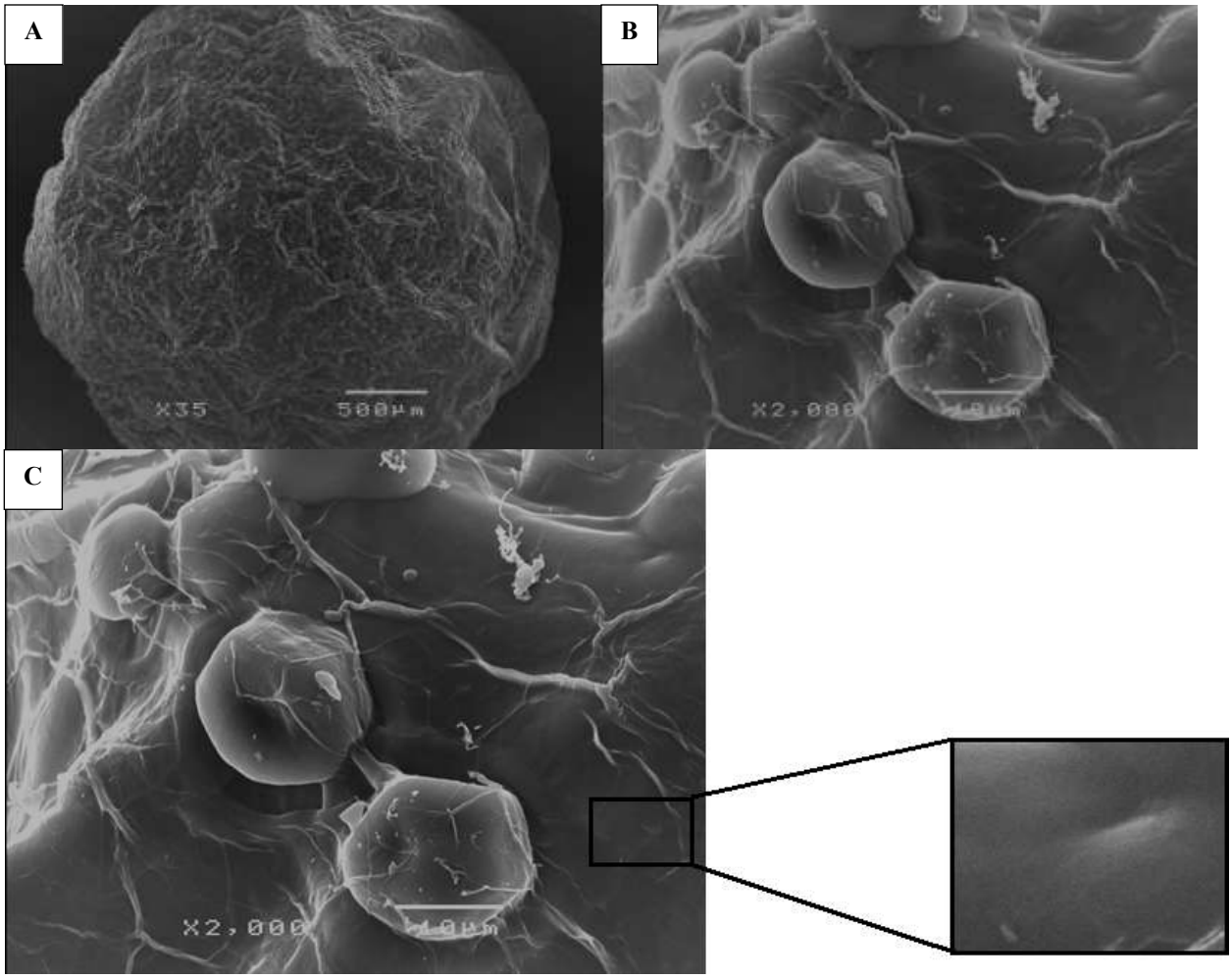


Fig. 1. Scanning electron micrographs of encapsulated probiotic bacteria using the extrusion method.
A, B: Bead and embedded cells of Y2
C: Characterization of Y2 cells

Scanning electron micrographs of the capsules and probiotic bacteria embedded within the capsule structure are shown in Fig. 1. The average bead size of Y2 is 3.82 µm. In fig. C, embedded cells can be observed within the bead matrix. These cells exhibit a spherical morphology (coccus), typically appearing in pairs or short chains, and are non-spore-forming.



Scanning electron micrographs of encapsulated probiotic bacteria using the extrusion method.
A, B: Bead and embedded cells of Y12
C: Characterization of Y12 cells

Scanning electron micrographs of the capsules and probiotic bacteria embedded within the capsule structure are shown in Fig. 1. The average bead size of Y12 is 3.95 µm.. In fig. C, embedded cells can be observed within the bead matrix. These cells exhibit a spherical morphology (coccus), typically appearing in pairs or short chains, and are non-spore-forming.

Release Rate in Yacon Vinegar Drink Broth

The release of encapsulated cells into the surrounding broth was negligible, with only 0.00004% of cells released from Y2 beads and 0.00002% from Y12 beads. This minimal release rate further confirms the integrity and stability of the encapsulated beads. The low leakage indicates that the beads were well-formed and maintained structural cohesion during incubation in the broth medium.

These findings align with studies where multilayer coatings or optimized gelation conditions reduced diffusion of probiotics out of the beads. Anal and Singh (2007) also suggested that bead matrix strength and ionic crosslinking significantly influence release behavior. Low release rates are desirable in functional food formulations to ensure that probiotics remain protected until they reach their target site in the gastrointestinal tract.

Strain Comparison and Functional Implications

While both strains showed excellent encapsulation performance, Y12 had a slightly higher encapsulation efficiency and lower release rate, despite having a lower viable cell count post-encapsulation. This suggests that Y12 may be more tightly entrapped within the matrix, though its survival might be more susceptible to the encapsulation conditions.

On the other hand, Y2 maintained high viability and showed comparable entrapment, making it a potentially more robust candidate for probiotic delivery.These high-efficiency encapsulation systems hold great potential for developing shelf-stable, functional probiotic products with controlled release profiles.

Conclusion:-

The method successfully produced stable and well-formed beads with high structural integrity, confirming the potential of yacon powder as a functional encapsulating agent. Through careful optimization of formulation and process parameters, exceptionally high encapsulation efficiencies were achieved, reaching 99.9996% for Y2 and 99.9998% for Y12.

Furthermore, the encapsulated Y2 and Y12 demonstrated strong survival behavior when incorporated into a yacon vinegar drink, with only minimal cell release observed (0.00004% for Y2 and 0.00002% for Y12)

Recommendations:-

1. Further Enhance the Microencapsulation Matrix Using Composite Materials
   While yacon powder proved effective as a primary wall material, future studies should explore combining it with other biopolymers (e.g., inulin, chitosan, or whey protein) to improve bead strength, controlled release, and probiotic protection under more rigorous storage or gastrointestinal conditions.
2. Fine-Tune Process Parameters for Strain-Specific Optimization Given the observed differences in viability between Y2 and Y12, it is recommended to fine-tune encapsulation parameters (e.g., alginate concentration, extrusion speed, and calcium chloride hardening time) specifically for each strain to maximize viability and consistency in probiotic counts post-encapsulation.
3. Conduct Long-Term Stability and Functional Testing in Yacon Vinegar Drinkto fully validate commercial application, extended shelf-life studies and in vivo gastrointestinal simulation tests should be conducted to assess the survival, release profile, and functional effects of encapsulated Y2 and Y12 in yacon vinegar drink and similar acidic beverages.

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