

## CHARACTERIZATION AND ANALYSIS OF MICROENCAPSULATED PROBIOTICS USING MILK BASED EXTRUSION METHOD

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**Abstract**—Probiotics, an essential part of human life, still remains insufficient in foods we consume. They are active live microorganisms that enrich the gut microbiota. They are of various types and *Lactobacillus* are one among them. Probiotics have the potential to activate the immune system by enriching the gut by interacting with stomach epithelial cells. Goat milk provides a medium of abundant *Lactobacillus* species as they are rich in medium fatty acid chains and linoleic acid. Microencapsulation is a process of immobilization. Among other methods, sodium alginate-calcium chloride bead formation is preferable. Sodium alginate is the pre-eminent in this process as it is nontoxic and biocompatible. Vitamin B12 is incorporated into the *Lactobacillus* beads as they are essential macromolecules. Vitamin B12 is a major compound required for DNA synthesis and survival of *Lactobacillus*. They act as cofactor for enzymes involved in metabolic pathway. Incorporating vitamin B12 into microspheres prevent them from decaying and enhance survivability. Characterization tests assess the properties of microspheres and determine the level of consumption. These tests examine the capacity of the beads in target delivery of probiotics to gut. Stability tests are performed for assessing the release profile of microspheres in releasing the probiotics to target. Stability tests are performed in phosphate buffer and skimmed milk under the observation for a period of time. Survivability tests are performed in stimulated intestinal juice to determine the survivability of beads in extreme conditions in stomach. Stimulated intestinal juice contain HCL, bile salt and pancreatin that mimic the intestinal juice. Other tests are also performed such as FTIR, SEM, ZETA potential for further morphological studies and their enlarged examination. These tests and assessments are required for the probiotic consumption. The results of stability and survivability can be obtained in CFU by MRS agar plating. The results are compared between microencapsulated cells and free cells.

### INTRODUCTION

Encapsulation is an efficient process for trapping bioactive compounds. In this world of consumption of food of different varieties, lack of nutrition and beneficial compounds is an emerging problem. Among them probiotics is one of the substances that is lagging. To overcome this problem probiotics can be isolated and microencapsulated to ensure availability for people. Probiotics is available from a wide range of sources but the challenge is making them sustainable throughout the ingestion and digestion process. Most of the probiotics show difficulty in survivability among the extreme conditions. To ensure its damage by external environment, encapsulation method is useful.

Microencapsulation by sodium alginate is most preferable as it is biocompatible and nontoxic. It also eases the formation of hydrogels with calcium chloride. Also, the physical properties of sodium alginate can be tailored by adjusting their concentration. Calcium chloride serves as a crosslinker. This crosslinking leads to hydrogel matrix formation and exhibit controlled release profile (Gange *et al.*, 2024).

Probiotics are microorganisms that enrich gut microbiota and interact with immune systems. They reduce oxidative stress. *Lactobacillus* is known to possess probiotic activity. They can adhere to intestinal epithelial cells to activate the immune system. They release antimicrobial compounds that can fight against pathogen (Nyugen *et al.*, 2023).

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Goat milk is known as non-bovine milk that has low Ph which creates environment for the growth of *Lactobacillus*. Goat milk also has a medium fatty acid chain that promotes *Lactobacillus* growth (Rasika, *et al.*, 2020).

These resulting microspheres are free of any nutrients. Vitamin B12, also known as cyanocobalamin, acts as an important substrate for many pathways in the metabolism of *Lactobacillus*. Incorporation of vitamin B12 in the encapsulated microspheres help enrich the species in the microspheres, thus extending the shelf life of the *Lactobacillus* and maintaining a healthy environment.

Characterization of the microspheres is important to investigate the compounds present in the sample. Characterization can be utilized to examine and ensure the available compounds in the sample and for further proceeding with the project. Characterization including FTIR, SEM, ZETA, Brine shrimp toxicity test, Survivability in intestinal juice and Stability tests are performed. FTIR is used to differentiate the compounds present that can be utilized for incorporating into the food. SEM imaging can be used for morphological study of the structure of capsules. It can be used for structural analysis of microspheres. ZETA potential test is used for analyzing the surface charge of the microspheres. The result in a positive value can assure a stable microsphere at different conditions. Brine shrimp toxicity test is performed for the toxicity analysis of the sample and its level for consumption. Survivability test in intestinal juice can ensure stability in harsh in intestinal acidic condition and their prevention from decay and damage. Stimulated intestinal juice is prepared for performing survivability test. Also, stability test in milk is performed for a period for analyzing its target release profile. Stability test is also performed in phosphate buffer comparing with the free cells and microencapsulated cells for comparison purposes (Li *et al.*, 2022).

## METHODOLOGY

### Materials

- Goat milk
- MRS broth
- Vitamin B12
- Vitamin C
- Sodium alginate
- Calcium chloride
- *Lactobacillus* species
- MRS agar
- Phosphate buffer
- Stimulated intestinal juice.

## METHODS

### Sample Collection

The fresh goat milk is collected from a nearby farm at Sulur. It is stored in the refrigerator at 4 °C to prevent spoilage and maintain fresh. This milk is used for *Lactobacillus* isolation and microsphere formation.

### Serial Dilution

From the collected milk, 1 ml of goat milk samples is taken in the test tube for performing serial dilution. Serial dilution is done in 10 test tubes for dilution. The test tube of dilution  $10^{-3}, 10^{-4}$  is taken for inoculation in agar plates and incubated for 24hr at 34 °C.

### Inoculation

Inoculation is performed in MRS broth. The broth is prepared by adding 10g of MRS agar to 150 ml of distilled water. The inoculated broth is used for mass production of *Lactobacillus* under incubated condition in shaker at 34 °C (Wang *et al.*, 2017).

### Pellet formation of *Lactobacillus*

After 48 hr, dense growth is obtained and it is centrifuged at 10,000rpm for 5 mins. The pellet is washed in saline water and repeated the process multiple times (Taye *et al.*, 2021).

### Incorporation of Vitamin B12

Vitamin B12 powder of 0.5mg is incorporated in this procedure for the enrichment of *Lactobacillus* in the microspheres to be prepared (Paulo *et al.*, 2017).

### Beads formation of *Lactobacillus*

Sodium alginate of 1.76g is taken and dissolved in 50ml of water. *Lactobacillus* pellet is added to the sodium alginate solution along with the Vitamin B12. Calcium chloride of 0.10g is added to 20 ml of water. Sodium alginate solution is taken in syringe for extruding the solution in the calcium chloride solution which forms the beads like structure as a result of microencapsulation. They are kept undisturbed for 30 mins and then isolated (Cui *et al.*, 2023).

### Stability Test

The microspheres are suspended in phosphate buffer of pH 8 for 10 mins. The suspended beads are plated in MRS agar to examine the stability of the microspheres. The stability of *Lactobacillus* beads is kept under observation in skimmed milk at 4 °C for four weeks. Viable *Lactobacillus* counts were determined at intervals weekly by dissolving beads and plating in MRS agar. The percentage of viable cells were calculated to the initial count. This test is attempted to evaluate the ability of beads to secure probiotics during storage (Ahmadi, *et al.*, 2025)

$$\text{Encapsulated efficiency} = \frac{\text{Number of colonies from the beads}}{\text{Number of colonies from cell suspension}}$$

### Survival Test in Stimulated Intestinal Juice

Stimulated intestinal juice was prepared by dissolving 0.8 g bile salts, 0.01 g pancreatin and 0.08 g sodium chloride in 10ml phosphate buffer. The microspheres of quantity 2 g were incubated in sterile stimulated intestinal juice at 37 °C under gentle shaking in shaker for 1hr. The released viable *Lactobacillus* was enumerated by plate counting method on MRS agar. Survivability was expressed as the percentage of viable cells remaining after incubation in stimulated intestinal juice compared to the initial count. By this test, assessment of protective effect of microspheres of *Lactobacillus* probiotic on survival in the intestinal harsh environment (Anitha *et al.*, 2022).

### Survival Test of Probiotics based on Storage

The microspheres were stored in sterile skimmed milk at 4p C. Viable probiotic counts were determined after 20 days by dissolving the beads and plating on MRS agar. The log CFU/g and percentage of viable cells were calculated over storage period to assess the protective effect of microencapsulated probiotic (Da Silva, *et al.*, 2021)

## RESULTS AND DISCUSSION

### Incorporation of Vitamin B12 and Vitamin C

The spherical alginate beads successfully incorporated in lactobacillus along with Vitamin B12 (75% encapsulation efficiency) and Vitamin C (82%). The encapsulated *Lactobacillus* demonstrated good viability (>85% survival) after encapsulation. In simulated Intestinal fluid, a gradual release of both vitamins was observed over 6 hours, suggesting a controlled delivery. Storage studies at

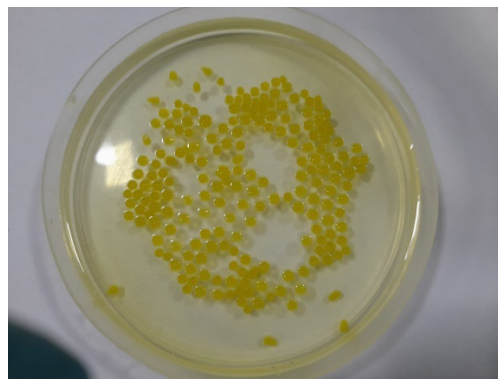


Fig. 1. *Lactobacillus* beads after incorporation of Vit B12

4 °C showed minimal loss of vitamin content and maintained probiotic viability for 2 weeks. This co-encapsulation strategy shows promise for delivering both probiotics and essential vitamins.

### Stability Test

Stability test has been performed in phosphate buffer and the beads showed survival after 10 mins with viable count.

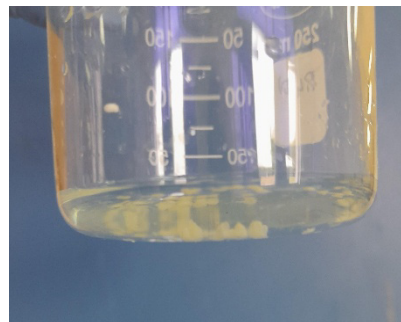


Fig. 2. Beads in phosphate buffer after 10 mins

### Survival test in Simulated Intestinal juice

*Lactobacillus* loaded beads exhibited significantly survivability in simulated intestinal juice (SIJ). After 4 hours of incubation in SIJ (pH 6.8 with 0.3% bile salts), the viable *Lactobacillus* count remained high, with a survival rate of  $75 \pm 5\%$ . The encapsulation matrix provided substantial protection against the bile salts and alkaline pH of the intestinal environment. This indicates the potential of the beads to effectively deliver visible probiotics to the lower gastrointestinal tract. The protective effect of the matrix was evident compared to non-encapsulated cells, which showed significantly lower survival rates under the same conditions.

### Survival tests of probiotics based on storage

*Lactobacillus*-loaded beads demonstrated excellent

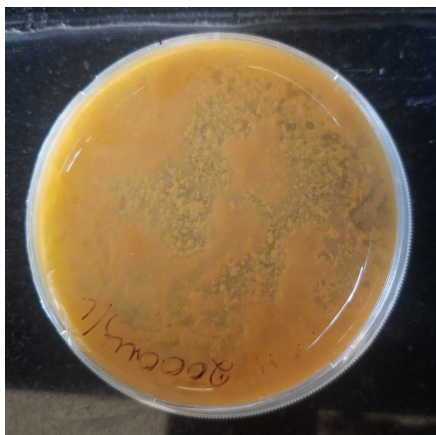
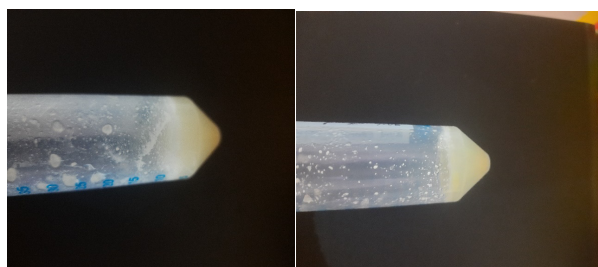


Fig. 3. Survivability of *Lactobacillus* beads in Intestinal juice



a) Survivability of free *Lactobacillus* cells in milk storage      a) Survivability of Microencapsulated cells in milk storage

survivability when stored in skimmed milk at 4 °C. Over a period of four weeks, the viable probiotic count within the beads showed a minimal reduction of less than 0.5 log CFU/g. The skimmed milk provided a protective and nutrient-rich environment that supported the long-term viability of the encapsulated bacteria. This suggests that skimmed milk is a suitable medium for storing and delivering these probiotic-loaded beads, maintaining their therapeutic potential over an extended period.

### CONCLUSION

The *Lactobacillus* vitamin  $\beta$ 12 incorporated beads showed better survival result than normal *Lactobacillus* intake in stimulated intestinal juice which determines the efficiency in target delivery of the probiotics. Also the stability tests shows the stability of bead and its capacity to thrive harsh situations.

**Conflicts of Interest:** The authors declare that there is no conflict of interest.

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