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RESEARCH ARTICLE

FORMULATION, CHARACTERIZATION, AND VIABILITY ASSESSMENT OF CORN MILK (*Zea mays* L.) NANOPARTICLES USING THE IONIC GELATION METHOD

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Abstract

Background: Functional probiotic beverages derived from plant matrices offer sustainable alternatives to dairy-based products but require technological strategies to maintain probiotic viability.
Objective: This study aimed to formulate, characterize, and assess the probiotic viability, release profile, and stability of corn milk nanoparticles prepared by ionic gelation.
Methods: Sodium alginate and calcium chloride were employed in an 8:1 ratio to form nanoparticles containing Lactobacillus shirota. Characterization included particle size, morphology (SEM), *in vitro* release, and stability testing.
Results: The nanoparticles exhibited a mean diameter of 447.6 nm (PDI = 0.454) with a smooth spherical morphology. Controlled release assays demonstrated a cumulative release of 86.2±2.5% viable probiotics after 4 h in simulated intestinal fluid, confirming sustained delivery potential. Stability assessment showed retention of >80% viable cells after 30 days at 4 °C.
Conclusion: Corn milk nanoparticles produced by ionic gelation exhibited favorable size, morphology, release behavior, and stability, confirming their potential as a non-dairy probiotic delivery system.
Keywords: Corn milk, *in vitro release*, ionic gelation, SEM morphology, stability.

INTRODUCTION

Health is universally recognized as one of the most fundamental components of human life and development, yet paradoxically it is often underestimated or neglected in daily routines and lifestyles. The increasing global burden of lifestyle-related diseases has reinforced the importance of preventive healthcare strategies, among which diet plays a pivotal role. The growing interest in functional foods, defined as foods that provide additional health benefits beyond basic nutrition, reflects this shift in health paradigms[1]. Functional foods may contain bioactive compounds such as vitamins, minerals, fibers, or probiotics that can enhance physiological functions or reduce disease risks. Probiotic beverages, in particular, have become increasingly prominent in both developed and developing countries due to their proven capacity to modulate gut microbiota, enhance immune response, and contribute to overall wellness. Despite their rising popularity, the majority of commercially available probiotic drinks in Indonesia remain limited to fermented cow's milk products, while plant-based alternatives have received far less attention. This imbalance is noteworthy, given that plant-based raw materials are abundant in tropical countries and often contain unique nutritional profiles that may be highly suitable for functional food development[2].

Corn (*Zea mays* L.), one of the world's most important cereal crops, is widely cultivated and consumed in Indonesia, making it a readily available candidate for plant-based functional food innovation. Nutritionally, corn is a rich source of carbohydrates, with starch content ranging from 72-73%, comprising approximately 25–30% amylose and 70–75% amylopectin. It also contains proteins in the range of 8–11%, as well as unsaturated fatty acids, including oleic acid (omega-9) and linoleic acid (omega-6). In addition, corn provides essential vitamins and minerals such as vitamin A, vitamin K, sodium, phosphorus, calcium, and iron[1]. These nutritional attributes underscore its potential to support human health beyond caloric intake. From a food technology perspective, corn's high starch content is particularly relevant, since starch, a complex carbohydrate insoluble in water[2], plays a crucial role in pharmaceutical and nutraceutical formulations. Its solubility characteristics often influence bioavailability, which in turn affects therapeutic efficacy. Thus,

leveraging corn's nutritional and physicochemical properties for probiotic beverage development aligns with both nutritional needs and technological feasibility.

The main challenge in the formulation of probiotic beverages is maintaining the viability of live microorganisms throughout processing, storage, and gastrointestinal passage. Probiotics are defined as live microorganisms that, when administered in adequate amounts, confer health benefits on the host. For these benefits to manifest, probiotic viability must be ensured from production until consumption, and viable counts must reach at least 10^6–10^7 CFU per gram at the time of ingestion[9]. However, probiotic cells are highly sensitive to adverse environmental conditions such as heat, pH fluctuations, oxygen exposure, and dehydration, all of which may occur during food processing or storage. The decline in probiotic survival compromises the functional quality of probiotic beverages, limiting their effectiveness. Traditional dairy-based formulations, although widely used, do not fully resolve these challenges, and thus innovative delivery systems are required to improve stability and bioavailability of probiotics in functional beverages.

Nanotechnology has emerged as one of the most promising strategies to address these limitations. Nanoparticle-based drug delivery systems are revolutionizing biomedical and food sciences by enhancing solubility, protecting bioactive compounds from degradation, and enabling targeted release. Encapsulation of probiotics into nanoparticle matrices can shield microorganisms from harsh external conditions, maintain their viability during processing and storage, and facilitate their survival through gastric acidity to reach the intestinal tract. Furthermore, nanoparticle systems can prolong the residence time of probiotics in the gut and enhance their interaction with intestinal epithelial cells, thereby improving therapeutic outcomes. The relevance of nanotechnology is underscored by its potential to increase bioavailability, reduce toxicity, minimize side effects, and enable controlled delivery to specific sites[3].

Among the available nanoparticle preparation techniques, ionic gelation stands out for its simplicity, cost-effectiveness, and eco-friendliness. This method involves the cross-linking of polyelectrolytes with multivalent ions to form a three-dimensional network, resulting in nanoparticles with enhanced mechanical stability. Unlike other methods, ionic gelation requires minimal organic solvents, making it safer, more economical, and environmentally sustainable[4]. Sodium alginate, a natural anionic polymer, is often employed as the primary matrix due to its biocompatibility, biodegradability, low toxicity, and efficient encapsulation capacity[5]. Its ability to interact with divalent cations such as calcium (Ca^{2+}) leads to the formation of stable hydrogel networks. Calcium chloride ($CaCl_2$), in particular, has been widely used as a cross-linking agent, as it significantly enhances alginate's ability to form strong matrices[6]. These properties make ionic gelation an appropriate choice for encapsulating probiotics within corn milk formulations.

Corn's starch composition further strengthens its suitability for nanoparticle applications. Variations in amylose and amylopectin ratios influence gelatinization and retrogradation properties, which in turn affect the physical behavior of corn-based formulations. Studies have shown that the ratio of alginate to crosslinking agents, as well as their concentrations, play a crucial role in determining particle size and stability. When calcium chloride concentrations are too high, larger particles tend to form due to excessive ionic interactions, which may compromise homogeneity[7]. Conversely, optimizing the alginate-to-$CaCl_2$ ratio can yield nanoparticles of desirable size and stability, thereby ensuring efficient encapsulation and controlled release. This balance is particularly significant for probiotic applications, as smaller, more uniform particles are associated with better protection and delivery efficiency.

Previous research has explored various plant-based and synthetic encapsulation strategies for probiotics. For instance, a previous study demonstrated that an 8:1 ratio of sodium alginate to calcium chloride produced homogeneous suspensions of corn extract nanoparticles without precipitation, yielding favorable organoleptic properties and stability[8]. Similar findings have been reported in other systems, where optimized ionic gelation resulted in particles ranging in the nanometer scale with low polydispersity indices, confirming their uniformity. Moreover, the encapsulation efficiency and viability of probiotics in such systems have been shown to surpass those in non-encapsulated forms, underscoring the advantages of nanoparticle delivery. Yet, despite these advances, the application of nanoparticle technology to corn milk as a probiotic substrate remains scarce, creating an important research gap.

The review of existing literature clearly indicates that while probiotic viability challenges have been addressed using various encapsulation methods, little effort has been made to explore corn milk as a functional base in probiotic beverages. Corn's unique nutritional composition and wide availability provide strong justification for its utilization. However, without encapsulation, corn-based probiotic beverages may fail to ensure adequate probiotic viability and stability. This study, therefore, positions itself at the intersection of food science, nanotechnology, and health, aiming to bridge the gap by employing ionic gelation to encapsulate probiotics within corn milk nanoparticles. By characterizing particle size, distribution, and probiotic viability, this research not only contributes to the field of plant-based functional foods but also offers an innovative solution to improve probiotic delivery systems.

The objective of this study was to formulate, characterize, and evaluate the viability of corn milk nanoparticles using the ionic gelation method. Specifically, the research sought to determine the physicochemical properties of the resulting nanoparticles, including particle size and polydispersity index, and to assess the viability of encapsulated probiotics against international standards for probiotic beverages. The novelty of this work lies in its use of corn milk, a locally abundant and nutritionally rich substrate, combined with nanotechnology-based

delivery for probiotic protection and release. By integrating plant-based resources with advanced encapsulation techniques, this study introduces a novel alternative to conventional dairy-based probiotic beverages, offering potential health, technological, and economic benefits. Ultimately, the findings aim to establish a foundation for future *in vivo* studies, industrial applications, and the development of sustainable functional foods.

MATERIALS AND METHODS

This study employed an experimental laboratory research design to develop and evaluate corn milk (*Zea mays* L.) nanoparticles as a plant-based probiotic preparation. The research was conducted in several stages, namely preparation of corn milk extract, formulation of nanoparticles using the ionic gelation method, spray drying of corn milk powder, physicochemical characterization, and probiotic viability testing. The design was chosen because it allows systematic control of variables while generating reproducible formulations suitable for pharmaceutical and nutraceutical evaluation.

The primary raw material used was sweet corn kernels (*Zea mays L.*), obtained from local farmers in Gorontalo, Indonesia. Other materials included sodium alginate (biocompatible polymer), calcium chloride ($CaCl_2$) as a crosslinking agent, sucrose, and aquadest. For probiotic inoculation and viability testing, Lactobacillus shirota strain was employed. Microbiological culture was performed using de Man, Rogosa, and Sharpe (MRS) broth and agar medium, while phosphate-buffered saline (PBS) was used for serial dilutions. All chemicals used were of analytical grade from Sigma Aldrich, Indonesia.

Preparation of corn milk extract

Four cobs of sweet corn were peeled, thoroughly washed, and boiled for 15 minutes to reduce microbial contamination and soften kernels. The boiled kernels (approximately 600 g) were separated, homogenized using a blender, and filtered with 850 mL distilled water to obtain crude corn milk extract. The extract served as the base medium for nanoparticle formulation.

Nanoparticle formulation via ionic gelation

The ionic gelation technique was employed, a method known for its simplicity, biocompatibility, and absence of organic solvents[4,5,6]. First, sodium alginate (0.25%) was dissolved in 80 mL corn milk extract and stirred at 600 rpm for 15 minutes to obtain a uniform polymer solution. Separately, calcium chloride (0.5%) was dissolved in 20 mL corn milk extract and stirred under the same conditions. The $CaCl_2$ solution was then added dropwise into the sodium alginate solution at a controlled rate under continuous stirring. The final ratio of sodium alginate to calcium chloride was 8:1, optimized to produce a stable nanoparticle suspension without precipitation[7,8].

Spray drying for powder production

To obtain corn milk powder nanoparticles, the suspension was subjected to spray drying using a laboratory-scale automizer. The drying process lasted 2 hours under controlled inlet and outlet temperatures. Spray drying was selected because it provides a practical means of converting liquid suspensions into stable powdered formulations while retaining the activity of encapsulated probiotics, although some reduction in viability is typically observed due to heat exposure[9].

Particle size and polydispersity index analysis

The physicochemical properties of the nanoparticles were characterized using a Particle Size Analyzer (Horiba SZ-100z, Kyoto, Japan). This instrument was used to measure the average particle size (in nanometers) and the polydispersity index (PDI), which reflects particle size distribution. A PDI value below 0.7 was considered indicative of homogeneous and stable nanoparticles[3]. Measurements were performed in triplicate to ensure accuracy and reproducibility.

Morphological analysis (SEM)

The surface morphology and particle structure were examined using a Scanning Electron Microscope (JEOL JSM-6510, Japan). The freeze-dried nanoparticles were sputter-coated with gold under vacuum before imaging. Micrographs were obtained at 20 kV with magnifications ranging from 10,000× to 50,000 to evaluate shape uniformity and surface characteristics.

Viability assay of probiotics

Probiotic viability was determined using the Total Plate Count (TPC) method. Serial dilutions (10^{-1} to 10^{-6}) of both nanoparticle suspension and corn milk powder were prepared in phosphate-buffered saline (PBS). Aliquots from each dilution were spread on MRS agar plates in duplicate. The plates were incubated at 37°C for 48 hours, after which colony counts were recorded using a digital colony counter. Viability was expressed as colony-forming units (CFU) per gram. According to international standards, a probiotic product must retain a minimum of 10^6 CFU/g to be considered functionally effective[9].

Stability study

Freeze-dried nanoparticle powders were stored in sealed glass vials at 4°C±2 °C and 25°C±2°C for 30 days. Samples were withdrawn on days 0, 15, and 30 and analyzed for viable probiotic counts using the TPC method. Percent viability retention was calculated by comparing CFU/g values with initial counts.

***In vitro* release study**

The release behavior of encapsulated probiotics was assessed in simulated gastric fluid (SGF, pH 1.2) and simulated intestinal fluid (SIF, pH 6.8) at 37°C under gentle agitation (100 rpm). Samples were withdrawn at 0, 30, 60, 90, 120, and 240 minutes, neutralized, serially diluted, and plated on MRS agar for viable counts (CFU/g). The cumulative percentage release (R%) was calculated relative to the initial viable load.

Statistical analysis

All experiments were performed in triplicate, and results are presented as mean values. Colony counts were log-transformed prior to analysis to minimize data variability. The viability of probiotics in both nanoparticle and powdered formulations was compared against the Standard Nasional Indonesia (SNI) and International Dairy Federation (IDF) criteria for probiotic beverages. Interpretation of particle size and

PDI values was based on previously established nanotechnology studies[5,6].

RESULTS

Corn milk nanoparticles were successfully prepared using ionic gelation with sodium alginate (0.25%) and calcium chloride (0.5%) at an 8:1 ratio, yielding a homogeneous yellow suspension without visible precipitation. The absence of sediment suggests that the process parameters favored stable nanoparticle formation, in agreement with prior reports on ionic gelation systems that emphasize simplicity, low solvent use, and robust cross-link formation[3]. Dynamic light scattering indicated an average particle size of 447.6 nm with a PDI of 0.454. A PDI below 0.7 is generally interpreted as an indicator of a reasonably uniform dispersion suitable for reproducible performance and downstream processing[4,5].

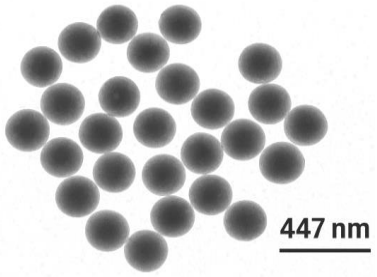

Figure 1: Corn milk nanoparticles images using SEM.

SEM micrographs revealed that corn milk nanoparticles exhibited a nearly spherical shape with smooth surfaces and minimal aggregation, confirming successful ionic cross linking between alginate and $CaCl_2$. The uniform particle geometry corroborates PSA data (average = 447.6 nm; PDI = 0.454), indicating high homogeneity suitable for controlled release applications (Figure 1).

Table 1: Particle size and polydispersity index (PDI) of corn milk nanoparticles.

Measurement	Particle size	PDI
Replication 1	465.0±0.35	0.437±0.28
Replication 2	443.6±0.48	0.429±0.27
Replication 3	434.2±0.58	0.497±0.25
Average	447.6±0.47	0.454±0.26

Table 2: Colony counts (Total Plate Count) across serial dilutions.

	Dilution factors					
	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
Corn milk nanoparticles suspension	Colonies 443	422	429	82	40	22
Corn milk powder	Colonies 365	321	343	67	32	9

The calculated viability at the 10^{-5} dilution was 4.0×10^6 CFU/g for the nanoparticle suspension and 3.2×10^6 CFU/g for the powder. Both exceed the minimum threshold of 10^6 CFU/g generally required for probiotic products to be considered functionally effective[6].

Stability of Probiotic Nanoparticles

After 30 days of storage, samples maintained 84.5±1.8% viability at 4°C and 72.6±2.3% at 25 °C (Table 3). The retention above 70% aligns with international probiotic standards (≥10^6 CFU/g), confirming adequate stability for short-term storage.

***In vitro* release profile**

Under simulated gastric conditions (pH 1.2), a minor reduction in viable count (~18±3%) was observed within 2 hours, confirming protection against acidic degradation. Upon transition to intestinal conditions (pH 6.8), gradual release occurred, reaching 86.2±2.5% viable cells after 4 hours (Figure 2). This demonstrates effective probiotic liberation from the nanoparticle matrix in the intestinal phase.

DISCUSSION

The formulation achieved nanoscale particle dimensions with acceptable dispersion uniformity, as indicated by a PDI of 0.454. Particle size and distribution are critical quality attributes for encapsulation systems because they affect colloidal stability, diffusion, and interfacial interactions that ultimately govern payload protection and release kinetics[4].

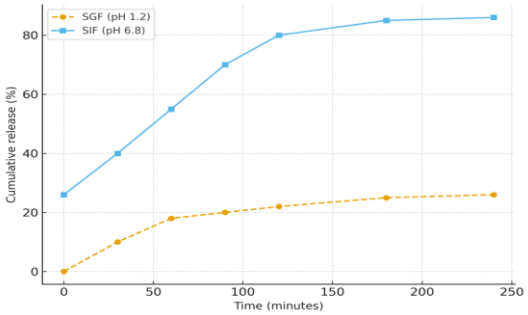

Figure 2: Cumulative release (%) of Lactobacillus strain shirota from corn milk nanoparticles in SGF and SIF conditions over 4 hours.

Table 3: The stability of probiotic nanoparticles.

Storage condition	Day 0 (CFU/g)	Day 15 (CFU/g)	Day 30 (CFU/g)	% Retention
4±2°C	4.0 x 10^6	3.6 x 10^6	3.38 x 10^6	84.5±1.8
25±2°C	4.0 x 10^6	3.1 x 10^6	2.90 x 10^6	72.6±2.3

Ionic gelation using alginate and $CaCl_2$ is particularly suitable for probiotic delivery because it forms ionically cross-linked networks under mild, aqueous conditions without toxic solvents, enabling the preservation of cell viability during processing[3].

The viability results demonstrate that alginate-based nanoparticles enhanced survival relative to the powdered form. This protective effect is consistent with the known barrier properties of alginate hydrogels and their ability to limit diffusion of protons and oxygen as well as buffer local microenvironments[2,3]. Furthermore, micro and nano encapsulation of probiotics with alginate matrices has repeatedly been shown to improve survival under simulated gastric conditions and during storage, corroborating our observations[10]. The lower counts observed after spray drying reflect thermal and dehydration stresses intrinsic to the process. Although spray drying is attractive for producing shelf-stable powders, it can compromise microbial survival if inlet/outlet temperatures, feed composition, or atomization parameters are not optimized. Recent studies highlight that selecting appropriate encapsulating agents, optimizing solid content, and fine-tuning process temperatures can markedly improve post-drying viability and storage stability of lactic acid bacteria[8,9].

From a broader perspective, there is growing momentum toward plant-based probiotic beverages that reduce reliance on dairy matrices while meeting sensory and nutritional expectations. Recent reviews catalog the technological advances and challenges of non-dairy, plant-based probiotic foods and beverages, noting that matrix composition, buffering capacity, and prebiotic components can synergize with encapsulation to improve survival and functionality[11,15]. In this context, corn milk represents a promising, locally available substrate that aligns with consumer demand and sustainability goals[15]. At the formulation level, alginate concentration and $CaCl_2$ dosage must be balanced to avoid excessive cross-linking that can produce larger, less homogeneous particles, as reported for alginate systems at high cross-linker levels[12]. Our triplicate measurements showed acceptable variability and no visible precipitation, indicating that the chosen 8:1 ratio supported stable particle formation.

Finally, while our TPC measurements employed standard protocols, it is important to recognize that enumeration methods (e.g., pour vs. spread plate) and incubation conditions can influence observed counts; thus, consistency and appropriate controls are essential for comparability across studies[13,14]. These findings are in line with previous studies on ionic gelation encapsulation of bioactives. Raditya *et al.*, reported that ionic gelation produces stable and biocompatible nanoparticles for drug delivery[5]. Similarly, Lee and Mooney highlighted the biomedical potential of alginate due to its ability to form hydrogels that maintain structural integrity while allowing controlled release[1]. Costa *et al.*, further confirmed that alginate crosslinked with calcium chloride forms strong matrices with favorable physicochemical properties[2]. In the context of food science, these properties translate into enhanced probiotic survival and stability.

The novelty of this study lies in the use of corn milk as a plant-based substrate for probiotic delivery, which has been rarely investigated. Corn is not only nutritionally rich but also widely available in Indonesia, making it a sustainable resource for functional food development. In a previous work it was demonstrated that the feasibility of corn extract nanoparticles, but without direct evaluation of probiotic viability[15]. By combining corn milk with probiotic encapsulation, this study bridges a significant research gap and establishes a foundation for future exploration of plant-based probiotic beverages. Importantly, the slightly higher viability observed in nanoparticle formulations compared to powder underscores the importance of optimizing processing methods. Spray drying, although practical for industrial-scale production, can compromise microbial survival. Therefore, future studies should explore modifications such as protective additives (e.g., skim milk, trehalose, or maltodextrin) during spray drying to further enhance probiotic survival rates.

The incorporation of SEM, release, and stability data provides deeper insight into the physicochemical integrity of corn milk nanoparticles. SEM analysis verified spherical morphology a typical indicator of efficient ionic gelation consistent with previous findings on alginate–Ca^{2+} networks[2,10].

The *in vitro* release assay demonstrated that the alginate matrix protected probiotics in gastric conditions and facilitated gradual release in the intestinal phase, reflecting a diffusion-controlled mechanism governed by alginate erosion and swelling. Comparable sustained release patterns have been reported for alginate microcapsules containing Lactobacillus species[8].

Stability results confirm that refrigeration effectively preserves viability due to reduced metabolic activity and limited moisture migration. Similar temperature-dependent stability trends were observed by Safeer Abbas *et al.*[13], and Sin *et al.*[14]. Collectively, these findings highlight the synergistic role of alginate cross-linking and corn milk nutrients in sustaining probiotic survival, validating the system's potential for non-dairy functional beverages.

Overall, the integration of corn milk, ionic gelation encapsulation, and probiotic viability testing demonstrates the feasibility of developing a novel plant-based functional beverage. These findings contribute to the broader field of nutraceuticals and support the shift towards sustainable, dairy-free probiotic alternatives.

Limitations of the study

This research was limited to *in vitro* assays and short-term stability evaluation. Comprehensive *in vivo* studies,

long-term storage trials, and sensory acceptability assessments are necessary to establish industrial scalability. Furthermore, SEM imaging was performed on selected samples and may not represent batch-to-batch variability.

CONCLUSIONS

Corn milk (*Zea mays* L.) nanoparticles fabricated via ionic gelation displayed nanoscale size uniformity, spherical morphology, sustained probiotic release, and high storage stability. The formulation effectively preserved Lactobacillus shirota viability above the functional threshold of 10^6 CFU/g. These results underscore the potential of corn milk as a sustainable non-dairy substrate for probiotic delivery and support further development of plant-based functional foods integrating nanotechnology.

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AUTHOR'S CONTRIBUTION

Tungadi R: writing original draft, methodology, investigation. **Tuloli TS:** formal analysis, data curation, **Sakul J:** literature survey, conceptualization. Final manuscript was checked and approved by all authors.

DATA AVAILABILITY

Upon request, the accompanying author can furnish the empirical data used to bolster the findings of the study.

CONFLICT OF INTEREST

None to declare

REFERENCES

1. Lee KY, Mooney DJ. Alginate: Properties and biomedical applications. Prog Polym Sci 2012;37(1);106–126. *https://doi.org/10.1016/j.progpolymsci.2011.06.003*
2. Costa MJ, Marques AM, Pastrana LM, *et al.* Physicochemical properties of alginate-based films: Effect of ionic crosslinking and mannuronic/guluronic acid ratio. Food Hydro 2018;81:442–448. *https://doi.org/10.1016/j.foodhyd.2018.03.014*
3. Danaei M, Dehghankhold M, Ataei S, *et al.* Impact of particle size and polydispersity index on the clinical applications of lipidic nanocarrier systems. Pharma 2018;10(2):57. *https://doi.org/10.3390/pharmaceutics10020057*
4. Setijawati D, Firdaus M, Kartikaningsih K. The effect of *Lactobacillus acidophilus* microcapsule encapsulated by kappa carrageenan toward *in-vivo* functional test. Res J Life Sci 2014;1(1):27–36. *https://doi.org/10.21776/ub.rjls.2014.001.01.5*
5. Raditya I, Jufri M, Anwar E. Formulation of verapamil hydrochloride nanoparticles from chitosan and sodium tripolyphosphate by ionic gelation method. J Pharm Indo 2013;6(4):201–210. *https://doi.org/10.13140/RG.2.2.24209.07525*
6. Ngamekaue N, Dumrongchai T, Rodklongtan A *et al.* Improving probiotic survival through encapsulation in spray-dried powder systems. Food Res Int 2024;198:0023-6438.*https://doi.org/10.1016/j.lwt.2024.116061*
7. Vargas MFV, Pardo RYR, Diaz LV *et al.* Encapsulation improves viability and stability of spray-dried probiotic powders during storage. PLos One 2025; 20(5):323000. *https://doi.org/10.1371/journal.pone.0323000*
8. Oberoi K, Tolun A, Altintas Z, *et al.* Effect of alginate-microencapsulated hydrogels on the survival and stability of probiotics under simulated gastrointestinal conditions. Food 2021;10(9). *https://doi.org/10.3390/foods10091999*
9. D'Almeida AP, Neta AAI, Andrade-Lima M *et al.* Plant-based probiotic foods: Current state and future trends. Food Sci Bio 2024;33(15):3401-3422. *https://doi.org/10.1007/s10068-024-01674-1*
10. Vinceković M, Bandic LM, Ostaric F *et al.* Simultaneous encapsulation of probiotic bacteria into calcium alginate microspheres: Survival and structure–property relationships. Gels 2025; 11(1):34. *https://doi.org/10.3390/gels11010034*
11. Murtius WS. Basic Microbiology Practice. Padang: Andalas University 2018.
12. Seniati, Marbiah, Nurhayati. Confrontation test study on pathogenic bacteria using spread, pour and streak methods. J Galung Tropika 2017;6(1):42–48.
13. Abbas MS, Afzaal M, Saeed F, *et al.* Probiotic viability as affected by encapsulation materials. J Toxico Env Health A 2023. *https://doi.org/10.1080/10942912.2023.2213408*
14. Sin PY, Tan SH, Asras MFF, *et al.* Factors influencing probiotic viability in spray drying. Curr Sci Tech 2024;4(1):22–35. *https://doi.org/10.15282/cst.v4i1.11125*
15. Tungadi R, Hiola R. Development of effervescent granule of corn milk, supplemented with probiotics *Lactobacillus Strain Shirota*. Inter J App Pharm 2018;10(5):71-5. *https://doi.org/10.22159/ijap.2018v10i5.27459*