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Effect of the Encapsulation Process on the Viability of Probiotics in a Simulated Gastrointestinal Tract Model Medium

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Abstract: The primary objective of this study was to investigate the survival rate of three species of encapsulated bacteria (*Propionibacterium acidipropionici*, *Propionibacterium freudenreichii*, and *Propionibacterium thoenii*) in model solutions designed to simulate the acidity levels found within the human gastrointestinal tract. The capsules were prepared by extrusion from gelatin and sodium alginate in a 1:1 ratio on a laboratory encapsulator. The use of gelatin and sodium alginate was found to provide optimum characteristics suitable for encapsulation of live bacteria. Three strains of *Propionibacterium asidirgorionici*, *Propionibacterium freudenreichii*, and *Propionibacterium thoenii* were subjected to in vitro assay to evaluate their probiotic potential. The final cell survival rate of *P. freudenreichii* was 10^5 CFU/mL, indicating that this probiotic is sensitive to acidic medium. The viability of encapsulated probiotics was studied in a model medium simulating the gastrointestinal tract. It was found that alginate–gelatin capsules could provide protection to *P. freudenreichii* from gastric acid and lead to high levels of viable bacteria released in the intestine. Practical application: Encapsulation of *Propionibacterium freudenreichii* into alginate–gelatin capsules produced capsules capable of delivering live probiotic bacteria into the intestine.

Keywords: encapsulation; probiotics; gelatine; alginate; capsule; viability

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1. Introduction

There is now growing scientific evidence that maintaining a healthy gut microflora can provide protection against gastrointestinal disorders, including gastrointestinal infections, inflammatory bowel disease, and even cancer [1,2]. Probiotic bacterial cultures stimulate the growth of beneficial microorganisms, displace potentially harmful bacteria, and strengthen the body's natural defense mechanisms [3–5].

However, despite all the health benefits of probiotics, it has been shown that many probiotic bacteria die in food products after exposure to low pH after fermentation, when interacting with oxygen during the distribution and storage of products in the refrigerator, or when interacting with acid in the human stomach [6,7]. The most promising direction to solve this problem is the use of a bacterial cell immobilization process—encapsulation [8]. Previously, we reported the use of sodium alginate and gelatin capsules for encapsulation. It was found that live bacteria encapsulated in a sodium alginate and gelatin capsule could potentially survive transit through the harsh environment of the stomach and release high levels of live probiotic in the intestine [9,10]. The protective effect of gelatin is found to be due to its ability to fill the porous structure of the sodium alginate capsule [11,12].

To form granules, the cell suspension is mixed with a solution of sodium alginate, and the mixture is dripped into a solution containing a multivalent cation (usually Ca^{2+}

in the form of $CaCl_2$). The drops instantly form gel spheres, capturing the cells in the three-dimensional lattice of ionically cross-linked alginate [13]. Jankowski et al. used a very low concentration of 0.6% for gel formation with 0.3 M $CaCl_2$ [14]. The factors affecting the preparation of granules included alginate (1–2%) and $CaCl_2$ (0.05–1.5 M) concentrations, granule curing time, and cell concentration during probiotic encapsulation. Gels obtained using low calcium ion or alginate that had low mechanical strength were studied [15,16]. $CaCl_2$ 0.5 M and 1–2% alginate gave the highest strength.

Encapsulation using sodium alginate and soy protein isolate as coating materials significantly improved the survival of probiotics under simulated gastrointestinal and thermal conditions. The buffering effect of microbeads prolonged their survival and stability under simulated conditions. The study concluded that encapsulation provides protection during exposure to various hostile conditions [17]. Gelatin was chosen because of its excellent membrane-forming ability, biocompatibility, and nontoxicity [18,19]. The applicability of gelatin as a hydrogel matrix is limited due to its low network hardness. However, its physical properties can be improved by the addition of crosslinking agents. Due to its amphoteric nature, it is also an excellent candidate for interaction with anionic polysaccharides such as alginate, etc. [20,21].

Lactic propionic acid bacteria are involved in the production of various biomolecules and milk fermentation, and are symbiotically found in the gut, benefiting the owner. The propionic acid bacteria are able to produce a wide range of biological compounds that improve human health, such as folate, proline, conjugated linoleic acid, and vitamin B12, and synthesize several different biodefense compounds such as bacteriocins or antifungal compounds [22,23]. These strains produce bifidogenic compounds and show the ability to survive and maintain activity while passing through the digestive tract [24,25].

In this work, in vitro experiments with encapsulated probiotic bacteria were conducted to establish the protective role of capsules for the successful passage of live bacteria through the gastrointestinal tract. Three strains of propionic acid bacteria, *Propionibacterium asidirgorionici*, *Propionibacterium freudenreichii*, and *Propionibacterium thoenii,* were subjected to in vitro analysis to evaluate their probiotic potential.

2. Materials and Methods

2.1. Materials

Sodium alginate, gelatin, and calcium chloride were purchased from KazKhimBaza LLP (Almaty, Kazakhstan). Strains of propionic acid bacteria (*Propionibacterium asidirgorionici*, *Propionibacterium freudenreichii*, and *Propionibacterium thoenii*) were obtained from Barnaul Biofactory LLC (Barnaul, Russia).

2.2. Bacteria Cultivation

For cultivation of propionic acid bacteria, the following medium was used: curd whey 1000 g, magnesium chloride 0.3 g, sodium citric acid tri-substituted 1.0 g, potassium phosphoric acid single-substituted 0.5 g, ascorbic acid 0.1 g, microbiological agar 1.3 g, active acidity (7.0 ± 0.1) pH units.

The propionic acid bacteria cells were grown at 30 ± 1 °C for 24 ± 2 h under periodic culturing conditions with a single neutralization of the culture fluid after 12 h, maintaining the pH at an optimal level saturated with sterile sodium carbon dioxide (Na_2CO_3) solution. After growth, the cells were separated by centrifugation for 10 min (3200 rpm, at 4 °C), the supernatant was collected, and the cell precipitate was washed with buffer solution and redispersed in alginate–gelatin solution to yield approximately 6×10^9 CFU/mL [26].

2.3. Preparation of Encapsulated Probiotics

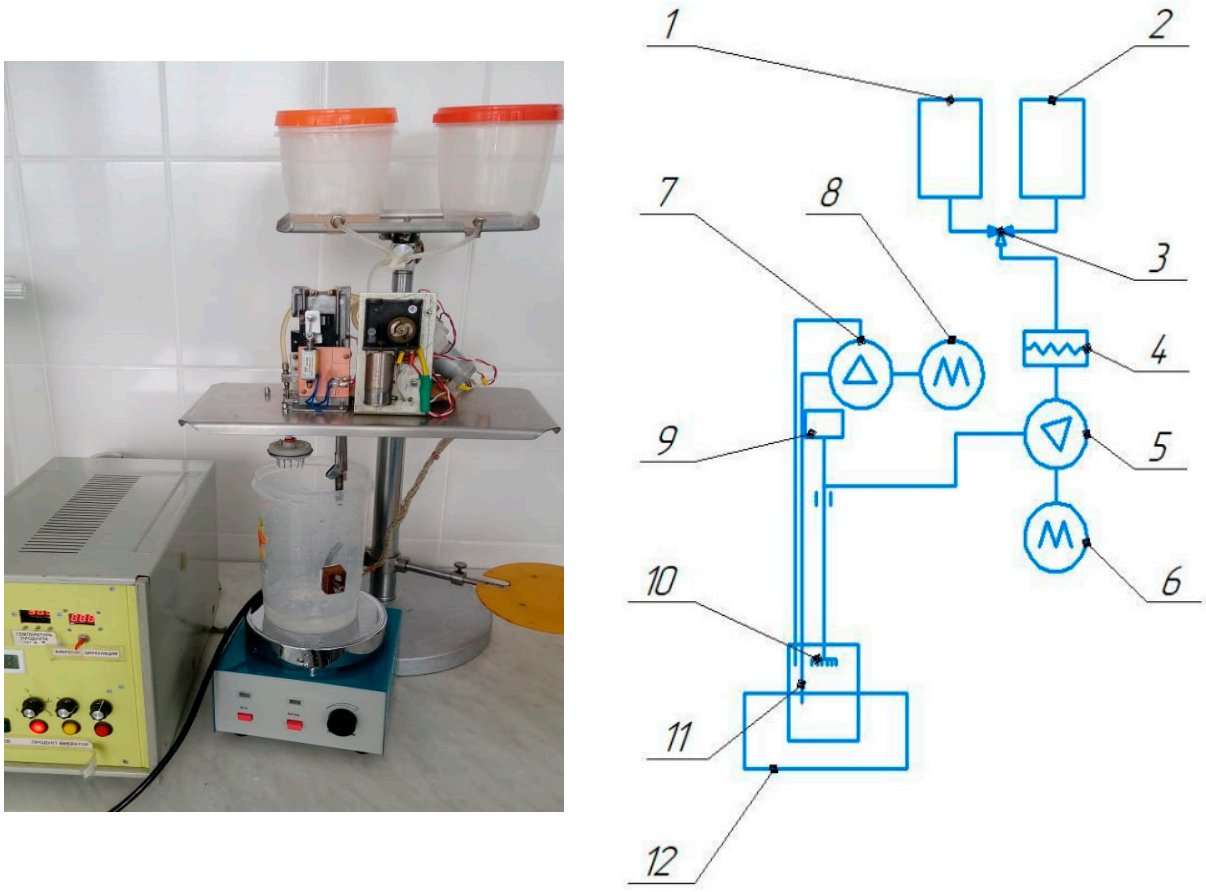
To obtain encapsulated probiotics, a gel-forming mixture of sodium alginate and gelatin was prepared in a 1:1 ratio. The gel-forming mixture was prepared as follows: 88 mL of distilled water (water temperature 60–70 °C) was poured into a 100–150 mL flask and 1 g (1%) of gelatin was added during stirring. The flask with the mixture is put on

an electromagnetic stirrer with heating, then the mixture is heated to 70–80 °C until the complete dissolution of gelatin. After the gelatin is dissolved, 1 g (1%) of sodium alginate is added to the mixture.

The mixture was stirred from time to time until the sodium alginate was completely dissolved. Then the gel-forming mixture is cooled to a temperature of 40 °C. The cell suspension of probiotic in the amount of 10 mL is added to the mixture cooled to 40 °C and stirred for 5 min. The process of formation of encapsulated probiotics is performed by extrusion. The prepared mixture was poured into the working mixture container of the encapsulator and extruded in 100 mL of calcium chloride solution. The capsules were formed immediately after the reaction with calcium chloride solution. After 10 min the capsules were filtered and washed in distilled water.

2.4. Development of Technology for the Production of Encapsulated Probiotics

The capsules were obtained by extrusion on a laboratory encapsulation unit (Figure 1). The machine allows for obtaining capsules with a homogeneous granulometric composition and an elastic gel-like structure. Sodium alginate and gelatin in the ratio of 1:1 were chosen as a gel-forming mixture, calcium chloride was used as a forming liquid.

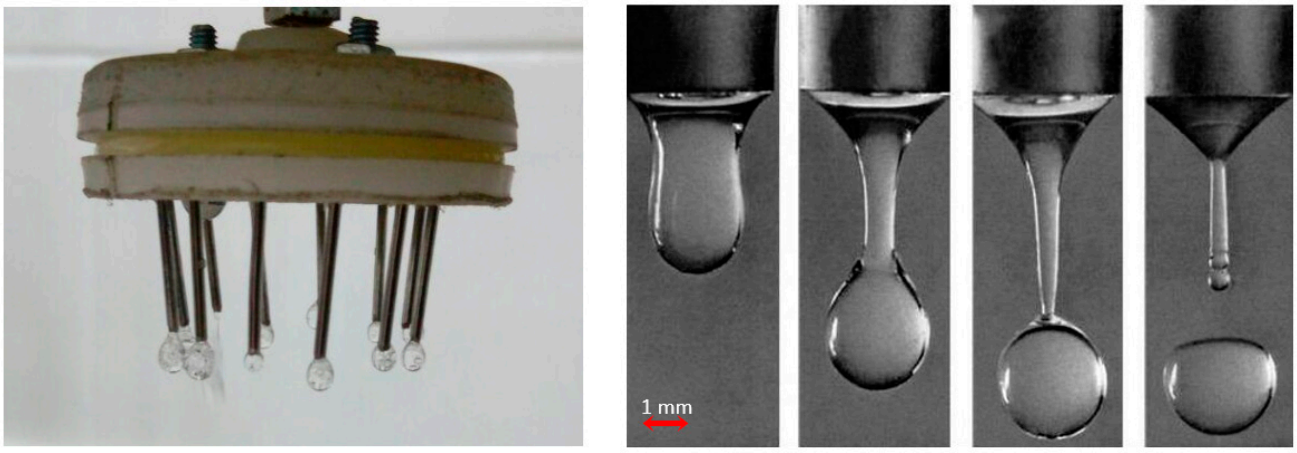


(**a**) (**b**)

Figure 1. General view (**a**) and technological scheme (**b**) of the encapsulator. 1—container for working mixture; 2—container for washing liquid; 3—valve-switch; 4—thermostat; 5—peristaltic pump; 6—peristaltic pump drive motor; 7—circulation pump; 8—circulation pump drive motor; 9—shaker; 10—nozzle with injectors; 11—container for forming solution; 12—container for cooling (ice).

In container 1 for the working mixture, an aqueous solution of gel-forming mixture (a mixture of 1% sodium alginate and 1% gelatin) is poured. The flushing liquid is poured into container 2 for rinsing the system after the work is carried out. Using the valve switch

3, solutions from containers 1 and 2 are fed into the common system. The thermostat 4 is designed to maintain the temperature of the liquid in the system at the proper level. (40 degrees Celsius) [27]. Peristaltic pump 5 supplies liquid to the nozzles with injectors 10, where droplets are formed (Figure 2). Shaker 9 is designed to adjust the droplet size by shaking the droplets off the injectors by shaking the nozzle. Circulation pump 7 is designed to mix the molding liquid, which does not allow drops to stick together when forming capsules. Capsules are formed in the forming liquid, which is calcium chloride, due to the chemical conversion of sodium alginate into sodium alginate chloride when sodium alginate drops react with the forming liquid. To cool the molding liquid, container 11 is placed in ice water (container 12). After the capsules are obtained, the droplets are separated from the forming liquid using a filter screen (not shown in the diagram).



(**a**) (**b**)

Figure 2. Nozzle with injectors (**a**) and droplet-pulling process (**b**).

On the actuator panel, there is a circulating pump, a peristaltic pump with a rotor position sensor of the peristaltic pump, and a vibrator with a system of guides and die mounting. The piping of the circulating pump and a temperature sensor of the cooling solution for encapsulation ($CaCl_2$) are also located on the panel in which they are immersed. The immersion level of the tubes is set in accordance with the conditions of the experiment. The liquid level in the working tank is set by the slope of the overflow pipe located in the side of the working tank. The overflow pipe is designed to maintain the level of liquid in the tank in a predetermined position. When filling the tank with capsules, the level of the liquid will increase, while the excess of it will merge into the container with the cooling solution.

Based on the experimental data obtained, the technology of capsule production was developed. The scheme of the capsule production process is shown in Figure 3. The whole technological process is carried out under aseptic conditions in order to exclude infection of encapsulated probiotics by foreign microflora.

2.5. Determination of Probiotic Cell Resistance to the Conditions of a Model Environment Simulating the Human Gastrointestinal Tract

The probiotics were incubated for 24 h at 37 °C. After cell growth, 1 mL of medium with cells was sampled to determine the initial number of cells by serial dilutions and colony counting. To establish the viability of probiotic cells under acidic conditions, probiotic cells were added to a model medium simulating gastric juice (pH 2.0) and incubated at 37 °C with stirring at 100 rpm. 1 mL of samples were taken after 30 min, 60 min, and 120 min of culturing at pH 2.0. The number of cells of propionic acid bacteria was determined in accordance with regulation GOST 34372-2017 "Bacterial starters for the production of dairy products. General technical conditions" [28].

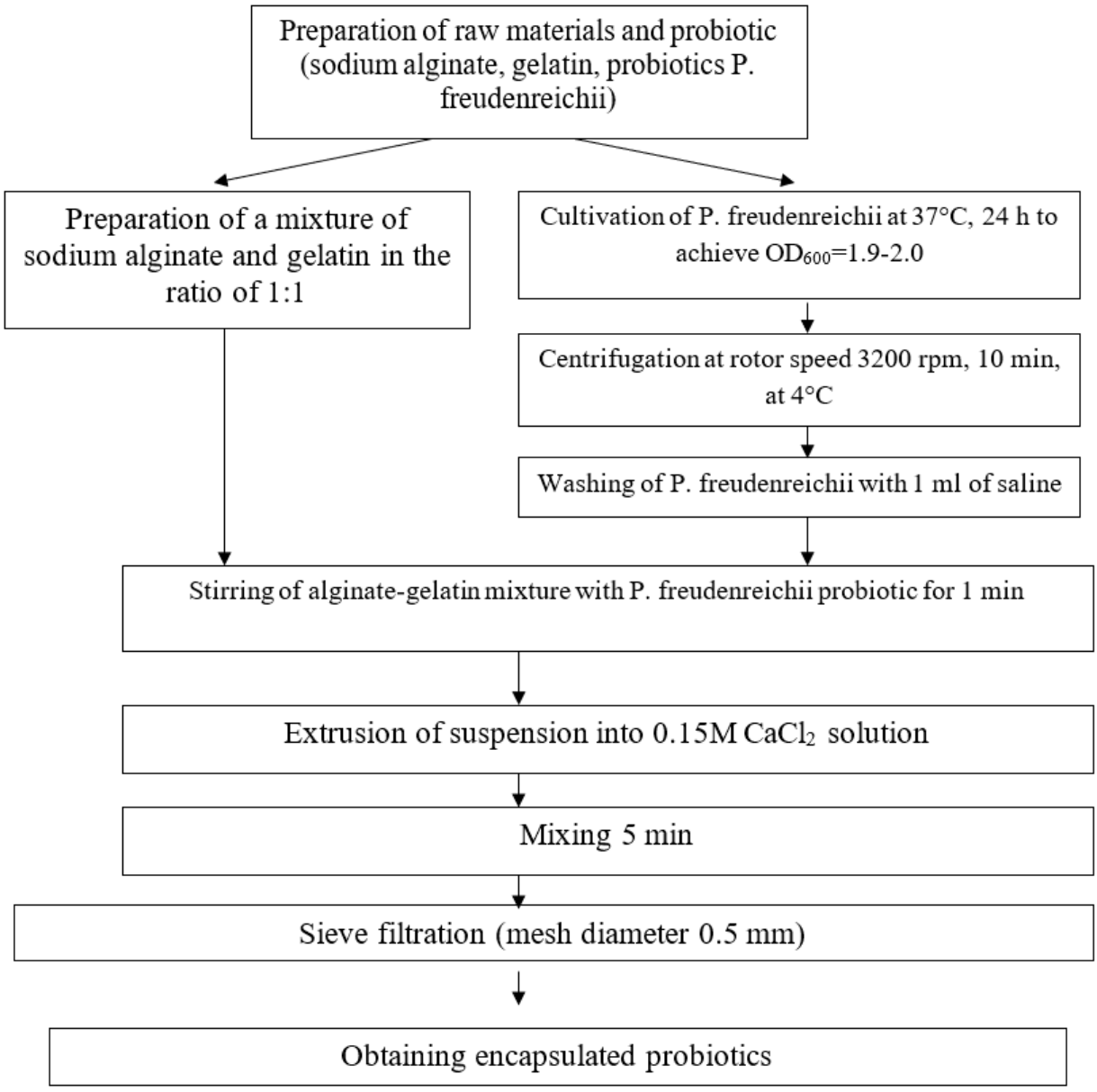


Figure 3. Scheme for the production of encapsulated probiotics.

2.6. Determination of Probiotic Cell Release from Capsules

To study the process of probiotic cell release from alginate–gelatin capsules, we simulated a system as close as possible to the conditions of the gastrointestinal tract. Thus, encapsulated probiotics were first placed in 10 mL of gastric medium (pH 2.0), incubated for 2 h at 37 °C, and stirred at 100 rpm. Then, 1 mL samples were taken after 30 min, 60 min, and 120 min of incubation at pH 2.0. To release the probiotic cells from the polymer, the samples were homogenized using a Stomacher brand homogenizer for 20 min with a stirring speed of 230 rpm. Then, cell counting was performed.

After 120 min of incubation, capsules were transferred from pH 2.0 medium to 100 mL of medium mimicking small intestinal medium (pH 7.2), incubated for 3 h at 37 °C, and stirred at 100 rpm. Then, 1 mL samples were taken after 30 min, 60 min, 120 min, and 180 min of incubation at pH 7.2. Then, half dissolved capsules were subjected to homogenization for 20 min with a turnover rate of 230 rpm. Afterward, cell counting was performed.

2.7. Microscopic Examination

A low-vacuum analytical scanning electron microscope (SEM) "JSM-6390LV" from "JEOL" (Japan), complete with X-ray microanalysis system "INCA ENERGY 250" from "OXFORD INSTRUMENTS" (UK), was used to determine the microstructure of capsules and measure their geometric dimensions.

The sample preparation process for capsules is summarized below:

1. The capsules are placed sequentially in solutions of 30, 50, and 70% isopropyl alcohol. The capsules are soaked for 3 h in each solution and then placed in a vessel with 100% isopropyl alcohol where they are kept for at least 6 h to dehydrate the capsules.
2. The capsules are then placed in solutions of tert-butyl alcohol at 30, 50, and 70% concentration, in the sequence indicated. Then they are placed into 100% tert-butyl alcohol to replace the isopropyl alcohol. Taking into account that 100% tert-butyl alcohol is able to freeze at a temperature of +25 °C, the capsule structure is fixed in a "frozen" form.
3. The "frozen" capsule fixed in tert-butyl alcohol is placed on the freezing table of the MS-2 luge microtome, where the layers of the capsule are cut for access to the internal structure of the capsule.
4. Next, lyophilization is performed. In the lyophilic dryer JFD-320 of "JEOL" company (Japan), under vacuum, there is a transition of tert-butyl alcohol from the frozen state immediately to the vapor state. This makes it possible to remove tert-butyl alcohol from the capsule and keep the capsule structure unchanged.
5. Then, in the vacuum coating equipment JEE-420 of "JEOL" (Japan), the carbon layer is sprayed on the capsule slice's surface, making it possible to obtain more contrasting images.
6. The prepared capsules are fixed on the slide using double-sided carbon tape. Then, the slide is placed in the microscope chamber and the surface of the samples is scanned.

2.8. Determination of Elastic–Plastic Deformation of the Capsule Shells

The elastic–plastic deformation of the capsule shells was determined using an experimental instrument. The instrument will apply force to the capsule shell by pressing under 3–10 g weight mass for 3 min. The capsule shape changes are recorded using an electronic caliper.

$$E = \frac{P \cdot d}{S \cdot f}$$

where P—compression load, N; d—diameter of the capsule, m; S—loading area, m^2; f—difference in capsule size before and after loading, $f = d - h$, m; h—height of the capsule after loading, m.

2.9. Statistics

The results of the measurements were analyzed using Excel 2007 (Microsoft, Washington, DC, USA). The differences between the samples were evaluated using a one-way ANOVA. A p-value < 0.05 was considered statistically significant.

3. Results

3.1. Effect of Incubation Time and pH on the Viability of Probiotics

Three strains of propionic acid bacteria, *Propionibacterium asidirgorionici*, *Propionibacterium freudenreichii*, and *Propionibacterium thoeni,* were subjected to in vitro analysis to evaluate their probiotic potential. Propionibacterium grows within the temperature range of 15–40 °C. The optimum growth temperature for classical propionic acid bacteria is 30 ± 1 °C. The optimal pH value of propionic acid bacteria growth is 6.5–7.0 (maximum—8.0, minimum—4.6) [29,30].

The main factors in the human gastrointestinal tract that affect the survival of probiotics before reaching the gut are the acidity of pH in the stomach and the bile in the

duodenum [31]. To determine the survival of probiotics at low pH values, the in vitro (simulated gastrointestinal tract) system is used. It is known that the acidity of gastric juice in the stomach is normally 1.0 to 2.5 on the hydrogen index (pH) scale when the stomach is empty, and 2.0 to 5.0 when the stomach is full. In addition, the length of time food remains in the stomach varies widely and has been reported to range from 5 min to 2 h [32,33].

At the beginning of the experiment, a gastric juice model medium with pH 2.0 was chosen as a simulated stomach. To establish the viability of probiotic cells under acidic conditions, probiotic cells were added to the model medium simulating gastric juice (pH 2.0) and incubated at 37 °C with stirring at 100 rpm. Then, 1 mL samples were taken after 30 min, 60 min, and 120 min of culturing at pH 2.0.

The ability of propionibacterium strains to tolerate acid is commonly used as one of the criteria for the preselection of probiotic candidates. It was found that propionic acid bacteria strains were able to retain viable cells above 5 lg CFU/mL at pH 3 and 3.5 after 2 h of incubation. From the data provided (Figures 4–6), it can be observed that the survival rate of all three species decreases as the pH level decreases and as the incubation time increases. For example, at pH = 2 and an incubation time of 0 min, the number of viable cells for all three species was 9 lg CFU/mL; however, after 180 min of incubation at pH = 2, the number of viable cells decreased to 4.1 lg CFU/mL for *Propionibacterium acidipropionici*, 4.5 lg CFU/mL for *Propionibacterium freudenreichii*, and 3.5 lg CFU/mL for *Propionibacterium thoenii*.

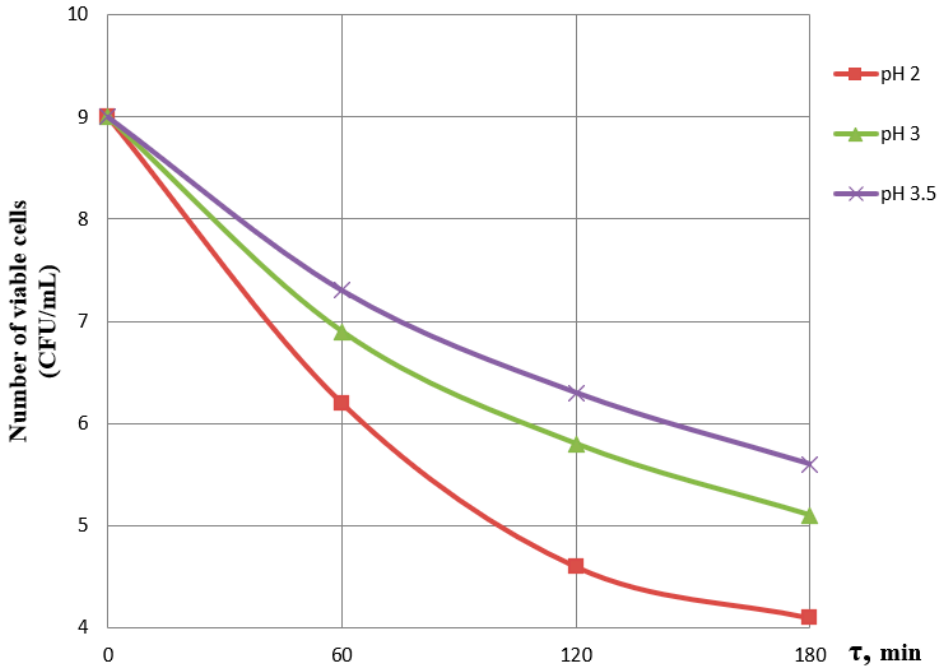


Figure 4. Viability of *Propionibacterium acidipropionici* cells depending on incubation time in simulated stomach medium.

It can also be observed that the survival rate of each species is different at the same pH level and incubation time. For example, at pH = 3 and an incubation time of 60 min, the number of viable cells was 6.9 lg CFU/mL for *Propionibacterium acidipropionici*, 7 lg CFU/mL for *Propionibacterium freudenreichii*, and 6.5 lg CFU/mL for *Propionibacterium thoenii*.

A critical analysis of the data indicates that all three species of bacteria experienced reduced viability when exposed to lower pH levels (pH = 2) and longer incubation times. This observation aligns with the expectation that the acidity of the human gastrointestinal tract, particularly in the stomach, can pose challenges to the survival of bacteria.

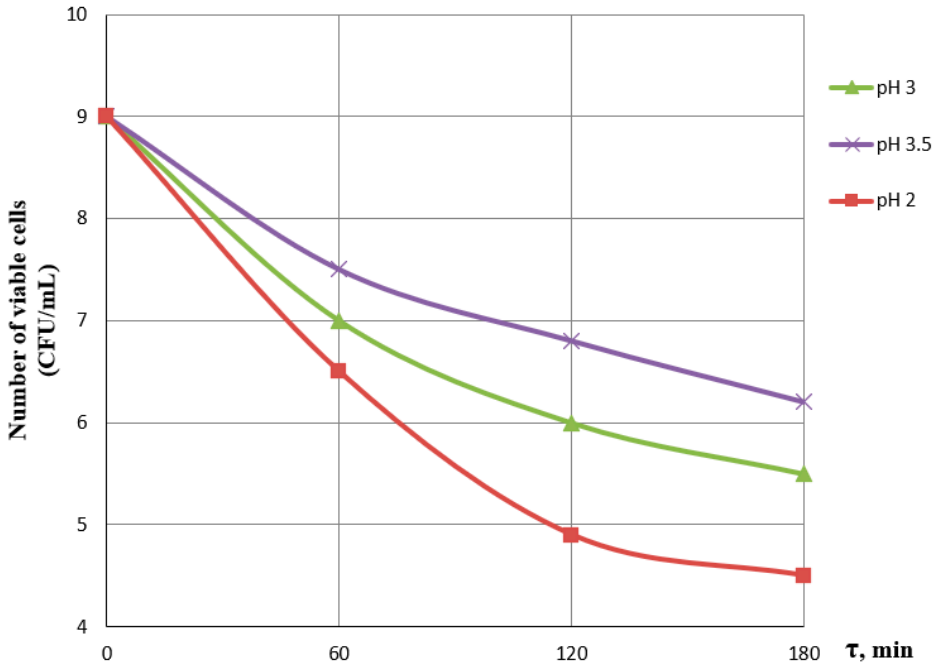


Figure 5. Viability of *Propionibacterium freudenreichii* cells depending on incubation time in simulated stomach medium.

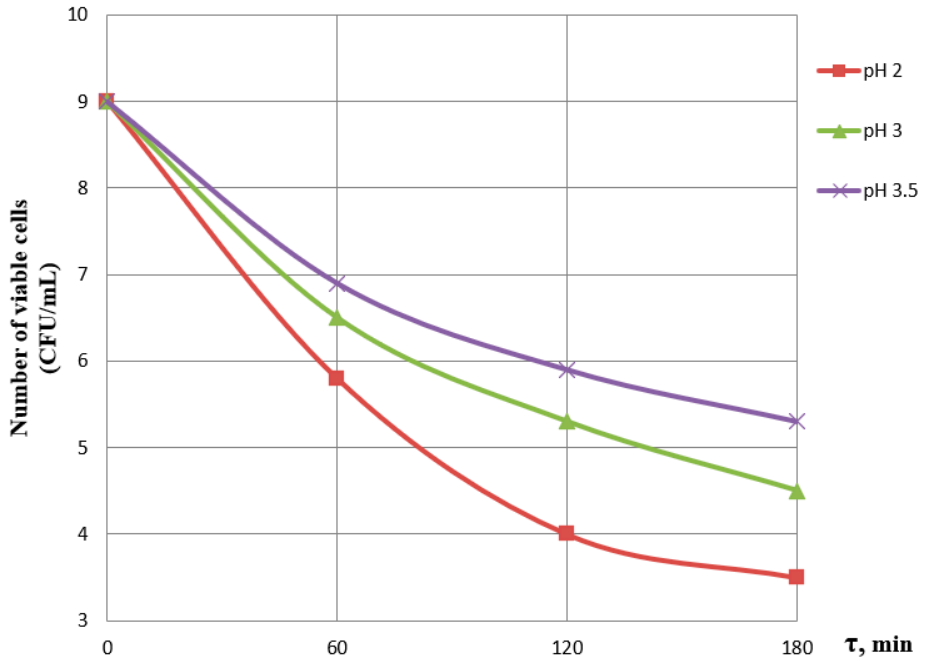


Figure 6. Viability of *Propionibacterium thoenii* cells depending on incubation time in simulated stomach medium.

Moreover, the results highlight species-specific responses to the conditions. *Propionibacterium acidipropionicici* generally exhibited the lowest viability, while *Propionibacterium thoenii* had the highest initial viability and *Propionibacterium freudenreichii* showed intermediate results.

At pH 2.0, all tested strains of propionic acid bacteria showed a progressive decrease in resistance during incubation, which decreased by about 2.5–5 lg units after 2 h. The results of the experimental data to determine the survival rate of propionic acid bacteria

showed that the survival rate of *P. freudenreichii* strain, compared to *P. asidenreichii* and *P. thoenii* strains, is 10^5 CFU/mL. The results are presented in Figure 7.

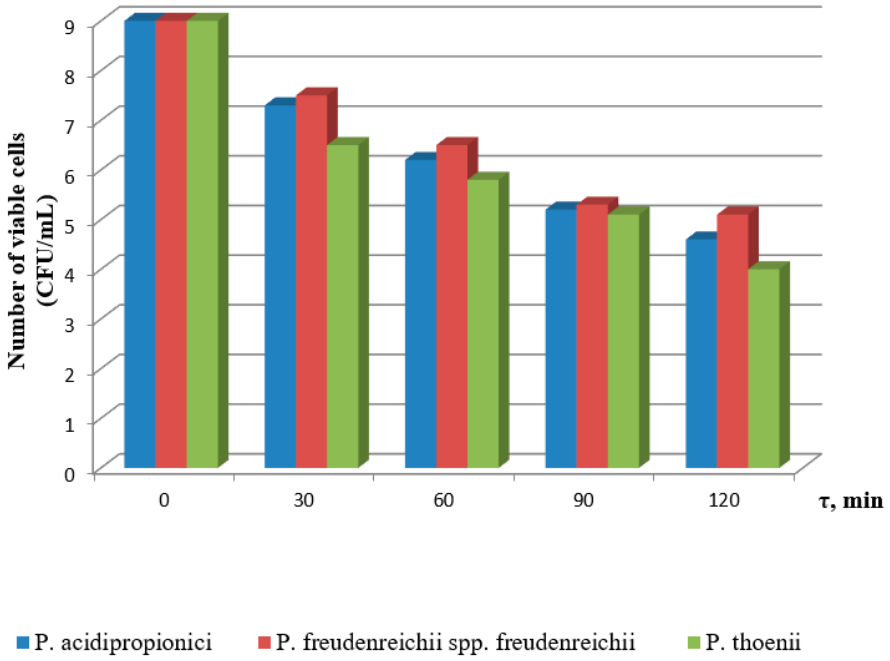


Figure 7. Viability of *P. acidipropionici*, *P. freudenreichii*, and *P. thoenii* cells depending on incubation time in simulated stomach medium (pH 2.0).

These results show that there may be a significant reduction in the viability of propionic acid bacteria at pH 2 after 3 h. Suoumalainen et al. observed that the quantity of propionic acid bacteria was significantly reduced after 3 h at pH 2, but not at pH 3 or 4 [34]. In the same line, Darilmaz and Beyetli found a significant decrease in the number of cells at pH 2 and 3 for *P. freudenreichii* and *P. jensenii* [35]. Zarate et al. noted that *Propionibacterium* strains tolerated well at pH 4, while one strain lost viability at pH 3 [36], while all the strains tested lost viability at pH 2. The difference between the strains tested may be due to the strong strain specificity at acidic pH.

As a result of these studies, it was found that the survival rate of microorganisms—probiotics of the genus Propionibacterium—in acidic conditions varies depending on the species and strain.

The choice of *P. freudenreichii* strain for encapsulation is explained by the fact that, unlike other strains, they are able to produce a number of useful compounds—nutraceuticals—while exhibiting low growth requirements. The final cell survival rate of *P. freudenreichii* was 10^5 CFU/mL, indicating that this probiotic is sensitive to acidic environment.

These studies indicate that the encapsulation process should be used to support the growth of *P. freudenreichii* activity in the gastrointestinal tract.

Encapsulation of *P. freudenreichii* will provide protection from the effects of gastric juice and, thus, allow viable probiotic cells to reach the lower intestine where they can provide a successful therapeutic effect [37].

3.2. Selection of Gel-Forming Mixture for Capsule Production

While alginate is the most suitable material, it has some restrictions. There is a major limitation to the use of alginate: high porosity. Due to the open lattice structure, the pore size distribution is wide, making it difficult to retain cells. Cells can be released into the medium and cause low initial cell loads [38,39]. These defects can be effectively compensated for by mixing with other compounds or coating with other bonds such as gelatin or chitosan.

The pure sodium alginate capsule was found to be porous, as shown in Figure 8. Sodium alginate capsules were obtained and were hard to the touch, with a strong multi-layer shell, and have an irregular shape and heterogeneous structure. This porosity can lead to moisture and oxygen permeation, potentially reducing the shelf life and stability of the encapsulated probiotics. Therefore, gelatin was added to the sodium alginate to fill the porous structure, as shown in Figure 9.

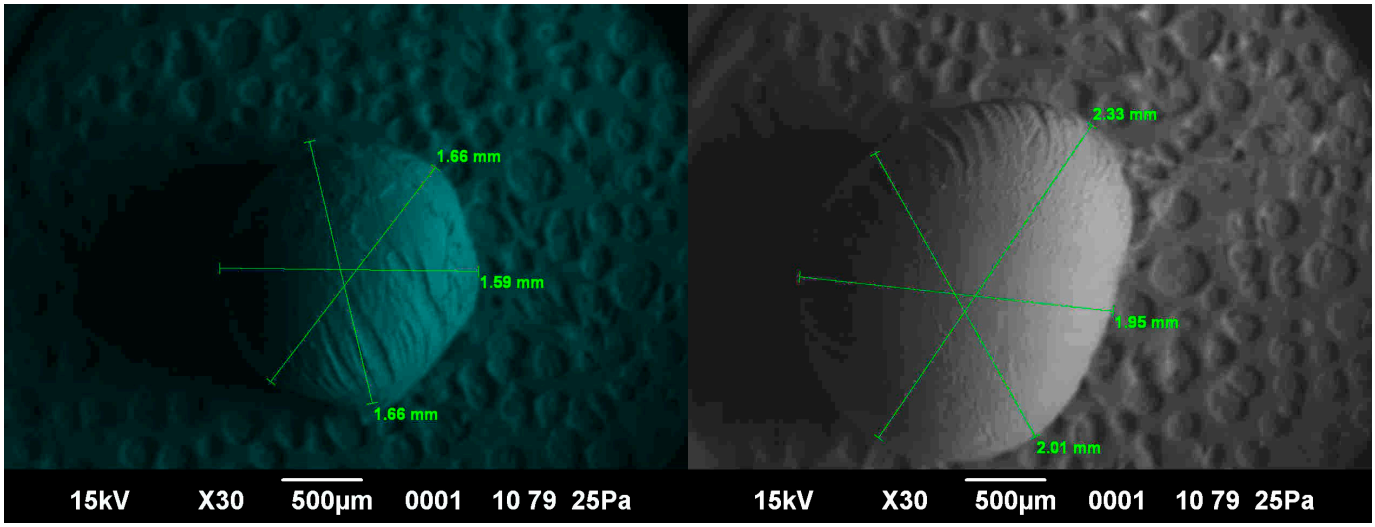


Figure 8. Image of a capsule obtained from sodium alginate without the addition of gelatin.

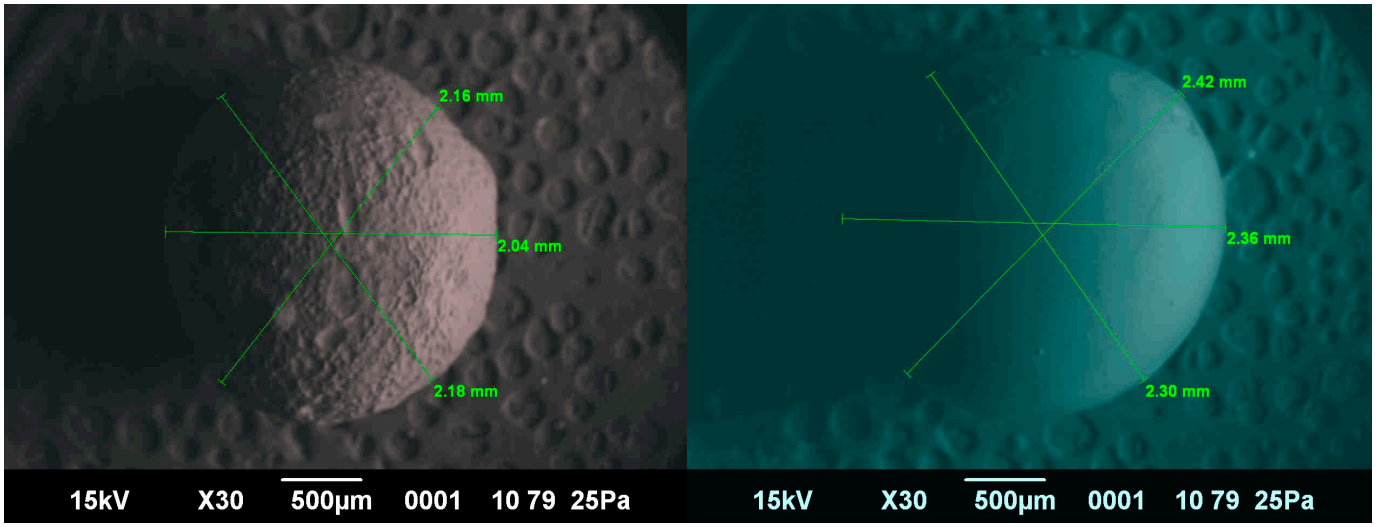


Figure 9. Image of a capsule obtained from sodium alginate with gelatin addition.

When encapsulated with aqueous gelatin solution and aqueous sodium alginate solution in the ratio of 1:1, capsules are obtained as homogeneous in structure, spherical in shape, and with an average size of 2.7 mm, with a homogeneous surface and high elasticity.

At encapsulation in the ratios of 0.5:1, 2:1, 3:1, and 4:1 aqueous solution of gelatin and aqueous solution of sodium alginate, the capsules are formed soft in consistency and inhomogeneous in structure, with a rough surface, not spherical in shape. Figure 10 shows capsules obtained from different ratios of gelatin and sodium alginate.

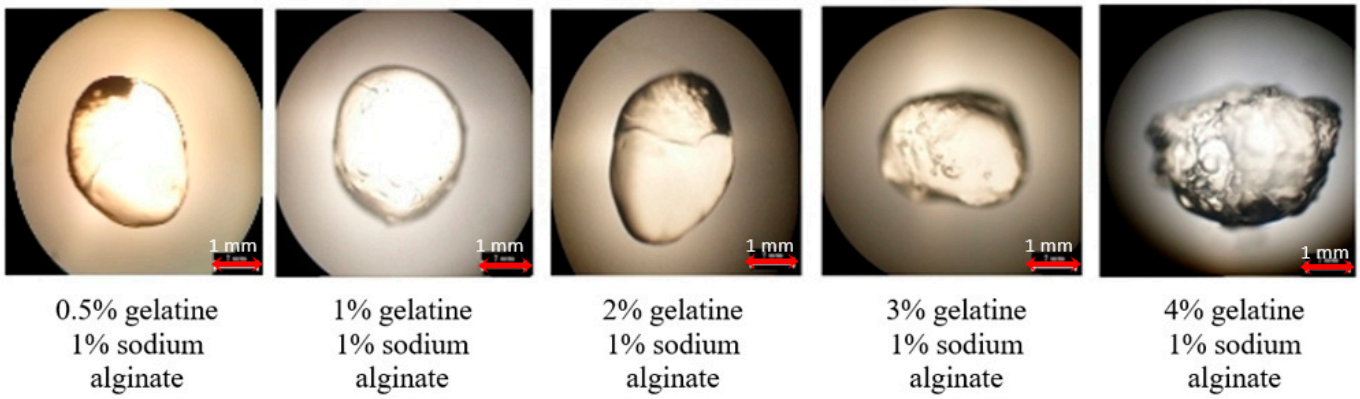


Figure 10. Results of microscopy of capsules made of sodium alginate and gelatin in the ratios of 0.5:1, 1:1, 1:1, 2:1, 3:1, and 4:1.

As can be seen from Figure 10, with increasing concentration of gelatin (4% gelatin and 1% alginate), capsules are formed inhomogeneous in structure, with a rough surface, not spherical in shape. Based on the experimental findings, the optimal composition for the capsules consists of 1% gelatin and 1% sodium alginate. Capsules made of this composition have a rounded spherical shape, are the same size, and are resistant to physical impact [40].

Next, the elasticity of capsules is studied. Elasticity is one of the factors that contribute to the overall quality and performance of encapsulated probiotics [41,42]. The graph shows that the elasticity of capsules obtained at temperatures 20 °C and 30 °C is higher than at temperatures of 40 °C and 50 °C. That is, if we talk about the organoleptic analysis, the capsules obtained at temperatures of 20 °C and 30 °C when added to sour milk products will be felt as unpleasant solid particles, and capsules obtained at temperatures of 40 °C and 50 °C will be felt as soft, elastic balls and, accordingly, have more pleasant organoleptic sensations (Figure 11).

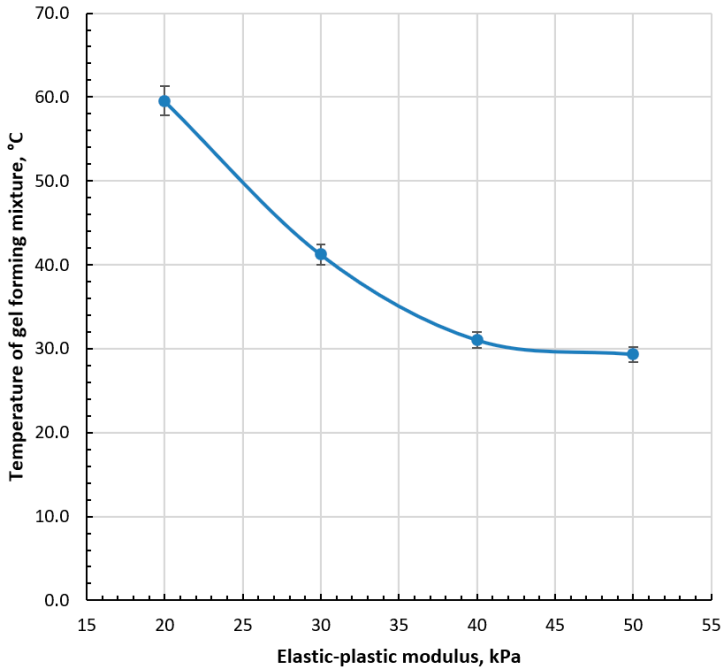


Figure 11. Effect of gelling temperature on the elastic modulus of alginate–gelatin capsules.

3.3. Investigation of the Viability of Encapsulated Probiotics in a Model Medium Simulating the Gastrointestinal Tract

In order to have beneficial health effects, the viability of probiotic bacteria is an important factor in their effectiveness, as they must survive food processing and storage, and pass through the stomach with increased acidity, as well as enzymes and bile salts in the small intestine [43]. After passing through the stomach and upper intestinal tract, *P. freudenreichii* should preferentially adhere to the epithelium of the intestinal tract and grow. As a guideline, the International Dairy Federation has recommended that the bacteria should be active and contain at least 10^7 CFU/mL [44]. Unfortunately, most probiotics, including *P. freudenreichii*, are unable to survive in a high proportion of the harsh conditions of acidity and bile concentration commonly found in the human gastrointestinal tract. Therefore, encapsulation of bacterial cells in alginate–gelatin gels is currently attracting attention to increase the viability of probiotic bacteria in acidic products such as yogurt, and it is a widely used method because this method is very mild and performed at room temperature in aqueous medium by using physiologically acceptable chemicals.

To obtain data on the behavior of the capsules during gastrointestinal simulation, the stability in simulated gastric juice (SGJ) medium (pH 2) and simulated small intestinal (SIJ) medium (pH 7.2) was investigated. In SGJ, the capsules showed swelling without any sign of disintegration within 2 h. The results show that the capsules swelled rapidly during the first 30 min; after 1 h, the swelling of microcapsules reached equilibrium without any erosion.

Encapsulated probiotics were placed in simulated gastric juice medium (SGJ) pH 2.0 and incubated for 2 h; then, the capsules were transferred to simulated small intestine medium (SIJ) of pH 7.2 and also incubated for 3 h. This graph shows the model medium simulating the gastrointestinal tract. The results are shown in Figure 12.

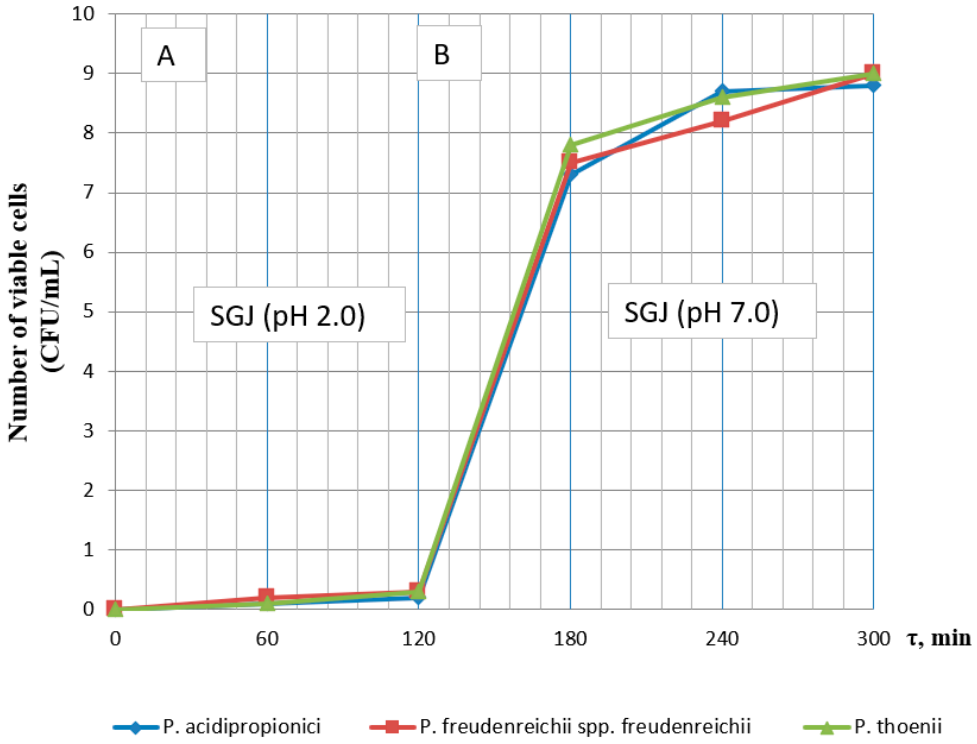


Figure 12. Viability of *P. acidipropionici*, *P. freudenreichii*, and *P. thoenii* cells depending on the time of cell release from capsules in a simulated gastrointestinal tract model medium. (**A**) 0–120 min: time of keeping capsules in solution at pH 2.0; (**B**) 120–300 min: time of keeping capsules in solution at pH 7.0.

While all three probiotic strains demonstrated resistance to the acidic stomach environment (pH 2.0), *P. freudenreichii* and *P. thoenii* exhibited higher cell release rates than *P. acidipropionici*. *P. freudenreichii*, in particular, displayed the highest release, making it a potentially more effective probiotic for delivery to the intestinal tract.

All three probiotic strains exhibited the ability to survive the simulated gastrointestinal conditions. This suggests that encapsulation in gelatin–alginate capsules provide a protective barrier, enabling probiotic delivery to the intestines. The data also indicate a sustained release pattern, with continued cell release beyond the initial exposure to acidic conditions. This extended release may contribute to prolonged probiotic activity in the intestines, which is desirable for probiotic supplements.

When the capsules were placed in SGJ, the alginate component underwent acid-catalyzed hydrolysis as well as the conversion of –COO– to –COOH– groups; the electrostatic attraction between Ca^{2+} and –COO– groups in the egg–box junction almost disappeared and, hence, the beads started to disintegrate [45,46]. This result showed that probiotic cells could be continuously released from microcapsules in SIJ, and the amount and release rate of probiotic cells in SIJ was much higher and faster.

The capsules obtained by extrusion technique were uniformly distributed without signs of collapsed spheres, with a size of 2.5 ± 3.0 mm. The cells of *P. freudenreichii* could be continuously released from the capsules, and the amount and release rate in SIJ (pH 7.2) was much higher and faster than in SGJ (pH 2).

4. Conclusions

Thus, the encapsulation method was found to be very effective in increasing the viability of probiotic bacteria compared to unencapsulated free cells. Alginate–gelatin capsules can potentially be used as a safe and protective delivery agent for viable probiotic bacteria. The data from the viability study showed that the alginate–gelatin capsules provided high probiotic protection, as the number of live microorganisms was at a high level after 3 h of probiotic capsule exposure in a simulated small intestinal environment. This characterizes that the capsules were able to deliver the live microorganisms through low-pH gastric juice to the small intestine where they will exert their functional properties on the human body. The viability data also indicated that the alginate–gelatin capsules provided better protection, suggesting that acid penetration was reduced, possibly due to stronger interactions between the two polymers. Further research is needed to explore the practical applications and health benefits of this delivery method.

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