Survival to Artificial Gastric Juice of *Lactobacillus delbrueckii* Microencapsulated Using Different Calcium Dosage

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Abstract

Probiotics are important microorganisms that when are administered in adequate amounts (10^6 CFU/mL) provide health benefits to the host. However, the main disadvantage for probiotic bacteria is their low survival rate when exposed to the high acidity levels founded in the stomach. This work focuses on studying the influence of calcium on the microencapsulation of *Lactobacillus delbrueckii* subjected to simulated gastric juices during 120 minutes. The microcapsules were prepared using an internal ionic gelation method. The diameter of the microcapsules, efficiency of microencapsulation and viability of the microencapsulated microorganism were studied as a function of the calcium dosage (10, 30 and 50 mM). The results indicated that the viability was mainly affected by calcium concentration due to high viability values (74%) founded when 30 mM of calcium was employed on the microencapsulation process; however, at 10 and 50 mM of calcium, the viability decreases to 47 and 61% respectively. This behavior could indicate saturation of the active sites by the calcium ion. Conversely, the microcapsules diameter was not affected by the calcium concentration. These results represent an alternative to vehiculate probiotics in food and contribute to possible industrial applications in the development of new alimentary products.

Keywords: low acyl gellan, microcapsules, probiotic
1 Introduction

Over the last decade there has been an increased interest in the role of probiotic bacteria in human health. This has led to industries focusing on incorporating probiotic bacteria in dairy foods and creating new functional food products. Health benefits perceived by consumers due to probiotic intake included therapeutic effects such as alleviating symptoms of serum, reducing the level of cholesterol, irritable bowel syndromes and colon cancer [14, 21]. However, the viability of probiotics often decreases sharply during gastric transit due to the strong acidic conditions [10].

An alternative way to protect probiotic bacteria from deleterious conditions encountered throughout the human gastrointestinal tract is the microencapsulation employing polysaccharides as wall material. Alginates and gellan gum are the main polysaccharides used for the microencapsulation process. Alginates have been used in the food industry due to gelling and non-toxic properties, and are produced by a group of brown algae (Laminaria spp). Alginates are copolymers linear anionic composed by β-D-mannuronic acid (M) and α-l-guluronic acid (G) linked by bonds β-1-4 and structured in homopolymeric (M or G) or heteropolymeric blocks (MG) [16]. Likewise, the gellan gum is an anionic linear heteropolysaccharide produced by the bacterium Sphingomonas paucimobilis and consists of repeating units of 1,3-β-D-glucose; 1,4-β-D-glucuronic acid; 1,4 β-D-glucose; and 1,4-α-L-rhamnose [7].

Microencapsulation is a process by which certain bioactive substances are retained inside a wall system in order to protect them. There are many methods for probiotic microencapsulation including extrusion, spray-drying, complex coacervation and ionic gelation, [15, 17, 19]. Among these methods the ionic gelation (IIG) has been used mainly for probiotics microencapsulation due to its low cost, high viability and cellular retention [3]. IIG is a physicochemical method, which allows the formation of spherical structured systems with controlled size due to the interactions of cations and biopolymers with negative charge [20]. Moreover, IIG facilitates the gradual cell release in the target place [4, 13].

Little research has been carried out focused on evaluating the influence of calcium on the microencapsulation process. Though microcapsules have been found to provide significant microbial protection against simulated gastric juice and bile tolerance [5], there are no systematic reports on survival of Lactobacillus delbrueckii subjected at simulated gastric juices. This paper reports the influence of calcium on the microencapsulation of Lactobacillus delbrueckii subjected to simulated gastric juices during 120 minutes.

2 Materials and methods

Microencapsulation

Microcapsules were obtained using an IIG technique, which is based on the formation of a water–oil emulsion. The dispersion (aqueous phase) was prepared
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with a mixture of LAG/SA at 0.5% w/v. Subsequently, calcium was added at different amounts (10, 30 and 50 mM), then dispersions were shaken by constant stirring at 90°C during 10 min. Afterwards, dispersions were cooled to 30 °C and 1 mL of the cell suspension (*L. delbrueckii*) (8.14 log UFC/mL) was incorporated. Finally, the dispersion was added into the oil phase (sunflower oil containing 0.1% v/v of surfactant) under constant agitation followed by the incorporation of 1 mL of δ-gluconolactone up to pH 4 in order to start the internal ionic gelation process. The microcapsules were harvested by centrifugation at 5000 rpm for 5 min, and the oil residues were removed by adsorption. The microcapsules contained in the aqueous phase were washed three times with saline solution.

**Efficiency of microencapsulation**

The microcapsules were centrifuged in order to separate the free cells from the microencapsulated cells, then the bacterial concentration in the supernatant was determined and the encapsulation efficiency (EM) was calculated as well:

\[
(\%)EM = \frac{[A-B]}{A} \times 100
\]

Where A is the total bacteria concentration in the suspension, and B is the concentration of unencapsulated bacteria found in the supernatant [2, 8]. Determination of free and microencapsulated *L. delbrueckii* was conducted according to plating method.

**Physical examination of the microcapsules**

Thirty micro liter of the microcapsules were employed to determine their diameter using a Leica DM500 microscope coupled to a digital camera. The images were analyzed through the software Image Pro-Plus v 5.1. The average size of microcapsules was established by measuring 100 microcapsules.

**Determination of viability of free and microencapsulated *Lactobacillus delbrueckii* subjected to simulated gastric juices**

Similar concentrations of free and microencapsulated *L. delbrueckii* were subjected to simulated gastric juice (SGJ) during 120 minutes. SGJ is prepared by adjusting the pH of 0.2% (w/v) NaCl solution to 3 through the addition of 1.0 M HCl solution in order to mimic the stomach condition [6]. Next, pepsin was added into the solution until reaching a concentration of 3.2 g/L, and then the mixture was filtered through a 0.22 µm membrane for sterilization. The entire study was performed at 37°C in order to simulate the body temperature. It is interesting to mention that SGJ was prepared on the same day of the analysis. *L. delbrueckii* enumeration was carried out by the drop plate method after 48h incubation at 37°C on MRS agar under anaerobic condition. The viable probiotic cells were counted and expressed in log colony forming units per milliliter (log CFU/mL).
Statistical analysis
The mean of three individual determinations was used to calculate cell counts. Differences between mean values of the viability and efficiency of microencapsulation were determined with the Tukey’s test (p<0.05) and a single factor ANOVA was performed using the software SPSS ver. 17.0.

3 Results
All the microcapsules obtained in this study shows a unimodal behavior and spherical structure with regular surfaces. Microcapsules diameter was not affected by calcium addition due to different calcium concentrations (10, 30 and 50 mM) resulting in the same average diameter (34.65 µm). Figure 1 shows the distributions of the microcapsules obtained with the LAG/SA mixture containing calcium at 30 mM, where it is observed that the diameter distribution ranges from 13 to 65μm, which represents the ideal size for food applications. Also, high microencapsulation efficiency was obtained for all microcapsules manufactured with 10, 30 and 50 mM of calcium.

Figure 1. Size distributions of the microcapsules.

Viability of free and microencapsulated Lactobacillus delbrueckii subjected to simulated gastric juice
The enumeration of L. delbrueckii microencapsulated (7.83 log UFC/mL), was carried out before they were submerged in SGJ, the initial viable count was in agreement with the recommended minimum values for the addition to a food probiotic product, as suggested by Aureli [1], who stated that the ingestion of probiotic cells should be around 8 log CFU/g to obtain beneficial effects on the health.

The viability of microencapsulated Lactobacillus delbrueckii was evaluated before and after being subjected to artificial gastric juice in order to discard any
possible damage caused by the microencapsulation process. Also, it is interesting to mention that there was no significant difference ($p < 0.05$) among efficiency and viability values of *L. delbrueckii* microencapsulated before incorporation to SGJ (data not shown).

### Table 1. Viability of *L. delbrueckii* microencapsulated under influence of simulated gastric juice during 120 minutes

<table>
<thead>
<tr>
<th>Calcium concentration (mM)</th>
<th>Viability (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>$47.82 \pm 0.23^{a}$</td>
</tr>
<tr>
<td>30</td>
<td>$74.12 \pm 0.51^{b}$</td>
</tr>
<tr>
<td>50</td>
<td>$61.45 \pm 0.17^{c}$</td>
</tr>
</tbody>
</table>

Rows with no common letter showed statistically significant difference (significance level <0.05)

After the probiotic immersion in SGJ, the difference of the viability of microencapsulated *L. delbrueckii* became highly significant ($p<0.05$) (see table 1) with respect to the calcium concentration (10, 30 and 50 mM).

The number of microencapsulated cells employing 30 mM of calcium that remain viable after being subjected to SGJ was 6.01 log CFU/mL; followed by cells microencapsulated using calcium at 50 mM (4.98 log CFU/mL) and cells microencapsulated at 10 mM of calcium with 3.88 logCFU/mL. This behavior could indicate saturation of the active sites by the calcium ion on SA and LAG helix when 50 mM of calcium was employed. Microorganisms in free status also were submitted to simulated gastric juice losing completely their viability; which means that microcapsules protected *L. delbrueckii* from the acidic condition found in the stomach.

### 4. Discussion

The average diameter reported in the present study (34.65 μm) is higher to those reported by Homayouni [11] who reported microcapsules with a diameter of 17.89 μm increase the survival of *Lactobacillus casei* and *Bifidobacterium lactis* by 30%. Regarding microencapsulation efficiency, the high values (96.3%) can be attributed to the gelling mechanism of LAG and SA due to both polysaccharides requiring calcium ions to form gel [20]. These results are in agreement with those published by Holkem [10] who found efficiency values of 89.71% for Bifidobacterium BB-12 microencapsulated by IIG using alginate as a wall material.

As can be seen in table 1, the best calcium concentration for increasing *L. delbrueckii* viability was 30 mM, this calcium amount stabilize the gellan three-dimensional network by direct cross-linking [12]; which can decrease the shielding effect caused by the electrostatic repulsion between the carboxyl groups
found in the gellan and SA units, which leads to a greater entrapment of bacteria inside the polymer chain that forms the microcapsule yielding a protecting effect induced by microcapsules.

Okuro [18] found that microencapsulation of *L. acidophilus* along with prebiotic (inulin and polydextrose) protected cells from simulated gastric conditions, while free cells are not detected after 210 minutes of exposure. These findings are contrary to those reported by other authors [9] who stated that microencapsulation does not protect microorganism from acidity due to presence of leaks or pores in the microcapsules. However, other studies found better survival rates for microencapsulated *L. acidophilus* compared to free cells when exposed to gastric and intestinal solutions [22].

The enhancement of the *L. delbrueckii* viability against gastric fluids could be due to the reduction of gastric fluid penetration into the microcapsule core, and the negative charges of the carboxylate groups that enhanced the buffer effect against infiltrated acid. Although, high viability also could be caused by SA particles dispersion which reduces the diffusion of oxygen into the microcapsules and thereby protect microorganisms from oxygen exposure [23]. In spite of protective effect provided by microcapsules there was a significant decrease on the *L. delbrueckii* viability as a result of a little penetration of gastric juices into the microcapsules that killing the probiotic bacteria. Thus, it is possible to apply the microcapsules obtained in this work into food systems.

### 5 Conclusion

Microcapsules based on LAG, SA with 30 mM of calcium were effective to improve the survival rate of *L. delbrueckii* under simulated gastric conditions. These results could represent an alternative to vehiculate probiotics in solid or liquid food due to the ideal microcapsules diameter obtained.

### References


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[12] Y. Huang, J. Tang, G. Swanson and B. Rasco, Effect of calcium concentration on textural properties of high and low acyl mixed gellan gels,


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