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Effects of Sucrose, Skim Milk and Yeast Powder on Survival of *Lactobacillus rhamnosus* GG Encapsulated with Alginate during One-week Storage at room Conditions

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Abstract

Background and Objective: During the manufacture and storage of probiotic products, there are always possibilities that probiotics be exposed to harmful stresses such as high temperature, low pH, various osmotic pressures and high oxygen levels, which can decrease the number of living cells. Encapsulation is one of the methods used to protect living cells such as probiotic bacteria from environmental challenges. Up-to-date, various compounds with abilities to protect bacteria during freeze-drying and to increase viability during storage have been identified. In this study, alginate with calcium chloride was used to encapsulate probiotic bacteria using extrusion method. Then, effects of sucrose and skim milk as cryoprotectants and yeast powder containing beta-glucan as prebiotic on the survival of these bacteria were investigated.

Material and Methods: The Homogeneous solution of bacteria with alginate and cry protectants were manually extruded into a calcium chloride solution. After 30 min of agitation, shaped beads were separated. For the comparison, bacterial population were enumerated in the primary culture, after encapsulation, freeze-drying and 1 week of storage at room conditions (N0, N, NF and $N1_w$, respectively). To assess colony-forming unit per ml of the samples, first 1 g of fresh beads was dissolved in 9 ml of trisodium citrate and then serial dilution and pour plate techniques were carried out. Plates were incubated for 24-48 h and colonies were counted.

Results and Conclusion: Results showed that encapsulation of bacteria with alginate and calcium chloride alone was 51% effective while use of yeast powder and sucrose with alginate increased the encapsulation efficiency to 97 and 99%, respectively. Furthermore, use of skim milk with alginate and sucrose resulted in the highest survival rate after 1 week of storage at room conditions. Therefore, encapsulation of probiotic bacteria with alginate layer and yeast powder containing prebiotics, sucrose and skim milk can be effective in survival of these bacteria.

Conflict of interest: The authors declare no conflict of interest.

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1. Introduction

Probiotics are susceptible to a variety of environmental factors, including water availability, redox potential, temperature, humidity and acidity [1]. One of the easiest and the most successful ways to protect probiotic bacteria from environmental stresses such as high temperature, low pH, variable osmotic pressures and high oxygen levels that usually occur during the manufacture and storage of probiotic products is use of physical coatings for the living cells [2]. This technique, known as microcoating, has recently been interested and is addressed as one of the most effective methods. Microcoating is defined as the preservation of solids, liquids or gases in a small space with

a semi-permeable wall that can release its contents under certain conditions. The resulting capsule is usually from a few micrometeres to a few millimeters in diameter and is made up of liquid precursors that solidify during the drying process [3-5]. These capsules are usually spherical in shape. Various types of encapsulating materials have been used to trap probiotic bacteria, including biopolymers, fats and milk proteins. However, alginate is the most popular encapsulating material due to its low toxicity and immunogenicity, biocompatibility and relatively low costs [6]. Extrusion is one of the common and cost-effective methods for the preparation of alginate hydrogels and microcoatings [7,8]. Numerous reports are available on the encapsulation of probiotic bacteria, including *Bifidobacterium animalis* [9], *Lactobacillus* (*L.*) *acidophilus* [10], *L. casei* [11], *L. paracasei* [12], *L. bulgaricus* [13] and *L. plantarum* [14], to increase and preserve their viabilities using extrusion methods. However, freeze-drying is commonly used to achieve dry powders, which is based on water sublimation under freezing conditions. Cells are frozen at -196 °C during the freezing process and the ice crystals are then sublimated under vacuum. During the freezing stage of this process, cells may be damaged. Formation of ice outside the cells induces increased osmotic pressure, which can causes further water to exit the cells and ultimately dries them out [15]. Speed of the freezing process plays a critical role in this technique and viability of the cells largely depends on the speed. Even though rapid freezing may damage the cells, cells are mostly damaged during slow freezing due to the formation of large crystals. Furthermore, the larger the capsules, the greater the damages caused by the ice crystal formation. Therefore, cell size includes significant effects on cell viability during freeze-drying. For example, cocci bacteria include higher chances of surviving low tempratures than that the bacilli bacteria do due to their smaller spherical shapes [16]. Novelty of this study included use of yeast powder containing beta-glucan and its effects on the bacterial survival after 1 w of storage at room conditions. The aim of this study was to investigate effects of alginate alone and in combination with yeast powder, sucrose and skim milk on the viability of encapsulated *L. rhamnosus* GG under freeze-drying and storage conditions.

2. Materials and Methods

2.1. Materials

Sodium alginate LB (low viscosity) provided by Duchefa Biochemie (Haarlem, the Netherlands), MRS broth, sodium chloride (NaCl), calcium chloride dihydrate ($CaCl_2.2H_2O$), agar-agar for microbiology and tri-sodium citrate dihydrate ($C_6H_8Na_3O_7.2H_2O$) were purchased from Merck, Germany.

2.2. Growth curve

To generate the growth curve using optical density (OD), bacteria were first inoculated into MRS broth from the initial stock for 16 h at 37 °C. When OD of the growth solution reached 1, 1.5% v v^{-1} of the first culture were inoculated into fresh MRS broth and incubated at 37 °C for 8 h. Then, ODs of the latter culture were recorded hourly. All ODs were measured at a 600 nm, using Bel Spectrophotometer V-M5 (Italy). Every hour, number of the living bacteria was counted using serial dilution and pour plate methods. Plates were incubated at 37 °C for 48 h.

2.3. Bacterial encapsulation

In this study, extrusion method was used to encapsulate *L. rhamnosus* GG. To prepare an appropriate microbial suspension for coating, a single colony from a solid bacterial culture was inoculated into 40 ml of MRS broth using smear loop and then incubated at 37 °C for 8 h. When OD reached 1 at 600 nm, number of the living bacteria was 10^{10} CFU ml^{-1}. Culture media were centrifuged at 3000 rpm for 10 min at 4 °C before washing twice with physiological saline. To find the optimal concentrations of alginate and calcium chloride for encapsulation and their effects on the formation and structure of beads, various concentrations of these chemicals were investigated. Alginate solutions were added to the bacterial precipitates at concentrations of 1-3%. Then, homogeneous solution was extruded manually into calcium chloride solution with concentrations of 0.05-0.5 M using syringe. After 30 min of aggitation, shaped beads were separated and divided into four parts (each 1 g). Each part was used to 1) assessment of the best formulation, 2) calculation of the encapsulation proportion, 3) investigation of the survival proportion after freeze-drying, and 4) investigation of the survival proportion after storage at room temprature. It has been reported that the weight of wet beads after drying decreases by 99% and each 1 g of the wet beads contains approximately 60 beads. To maintain the conditions throughout the experiments, formed beads were divided into groups of 60 (equivalent to 1 g of fresh beads) and assessed separately. To assess the bacterial population, serial dilutions with physiological serum were carried out after adding ۹ ml of tri-sodium citrate solution and sufficient aggitating for the beads to fully dissolve. Then, pour plate method was used to inoculate specific dilutions using MRS agar. Prepared plates were incubated at 37 °C for 2 d [17].

2.4. Assessing characteristics of the beads

Based on the protocol in Section 2.3, samples were prepared with various proportions of alginate and calcium chloride (Table 1). The best proportion of alginate and molarity of calcium chloride were assessed based on morphology and dissolution time of the beads in tri-sodium citrate solution.

Morphological characteristics*:* Formed beads were mostly spherical and a special plate was used to measure their sizes. Plates included a checkered piece of paper with an exact size attached to its bottom.

Table 1. Characteristics of the samples with various proportions of alginate and calcium chloride

Sample number	Alginate (%)	$CaCl_2$ (M)	Dissolution time in trisodium citrate solution (min)
1	1%	0.05	10
2	2%	0.05	18
3	3%	0.05	25
4	1%	0.1	12
5	2%	0.1	20
6	3%	0.1	48
7	1%	0.5	24
8	2%	0.5	55
9	3%	0.5	180

Then, five wet beads were randomly chosen and transferred onto the screen and their sizes were checked.

Solubility characteristics: Briefly, 1 g of each sample was weighed and dissolved in 9 ml of 0.1 M tri-sodium citrate solution. Then, condition of the beads was checked every 5 min [17].

2.5. Effects of various encapsulation formulations on the bacterial viabiliy

Based on the results in Section 3.2, 2% concentration of alginate and 0.1 M calcium chloride were used for monolayer encapsulation. Effects of monolayer formulations such as alginate, alginate-yeast, alginate-yeast-sucrose, alginate-sucrose-skim milk and alginate-sucrose on encapsulation efficiency and bacterial viability were investigated. Moreover, a group of bacteria were encapsulated in two layers. Briefly, 0.1% alginate and 0.1 M calcium chloride were used in the second layer. After freeze-drying, CFU counting from the beads at various stages was carried out and the bacterial viability was assessed [18].

2.6. Investigation of the bacterial encapsulation efficiency

The CFU counts were reported 18 h after the initial culture from 1 g of wet beads. To assess encapsulation efficeancy of the bacteria (EE%), a colony of the bacteria was inoculated into 40 ml of liquid culture media using smear loop and then incubated at 37 °C for 24 h. Bacterial populations were shown as N0 and N at 18 h after initial culture and after encapsulation, respectively. Then, proportion of the encapsulation was calculated using Eq. 1 [17].

$$EE\% = \frac{N}{N0} \times 100 \quad \text{Eq.1}$$

2.7. Investigation of bacterial viability after freeze-drying

To assess viability of the bacteria after freeze-drying (F%) using Eq. 2, N and NF were considered as the bacterial population in 60 beads before and after freeze-drying, repectively.

$$F\% = \frac{NF}{N} \times 100 \quad \text{Eq.2}$$

2.8. Investigation of the bacterial survival at room temperature

Briefly, 60 dried beads (1 g of wet beads) was stored at room conditions. After 1 w, beads were dissolved in 9 ml of tri-sodium citrate solution and CFU counting was carried out. Assessing bacterial populations after freeze-drying and after 1 w of storage at room conditions as NF and N1W, respectively, survival proportions (1 W%) were reported using Eq. 3.

$$1\,W\% = \frac{N1W}{NF} \times 100 \quad \text{Eq.3}$$

3. Results and Discussion

3.1. Growth curve

To assess the harvest time and relative assays, growth curve of the bacteria was first investigated. Figure 1 shows a growth curve with various growth phases for *L. rhamnosus* GG for OD and CFU ml^{-1}. Bacteria included a 4-h lag time before reaching their maximum growth rate. They reached the stationary phase after 12 h. Since the stationary-phase cells were the best choice for the freeze-drying process [19], cells were harvested for encapsulation after 16-18 h.

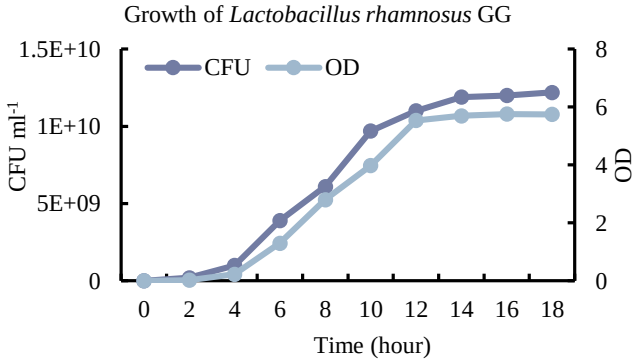

Figure 1. *Lactobacillus rhamnosus* GG growth chart for OD and CFU ml^{-1}

3.2. Effects of alginate and calcium-chloride concentrations on bacterial encapsulation

Several studies have shown positive effects of alginate on encapsulation of probiotic bacteria and their survival. For example, encapsulation of *L. casei* with quince seed gum-alginate preserved *cell* viability of the recommended level of 6-7 log CFU g^{-1} during the storage of 60 d [20]. Similarly, Xu et al. reported that encapsulation of *L. casei* with a pea

protein isolate-alginate hydrogel matrix resulted in 59.9% survival rate during the storage at -15 °C [21]. However, it seems necessary to use appropriate proportions of alginate and calcium chloride to prepare beads with appropriate sizes and physical characteristics. Alginate concentration affects swelling behavior and structure of the beads by affecting hydrogen bonds and thus mediates the release of probiotics [22]. Therefore, the best proportion of alginate and molarity of calcium chloride were assessed based on morphology and dissolution time of the beads in tri-sodium citrate solution. Results of the experiments are summarized in Table 1. Wet beads were usually spherical in shape with an average diameter of 3 mm. Furthermore, the average diameter of dry beads was 1 mm. Therefore, it was detected that size of the beads decreased by 66% after drying. Table 1 shows dissolution time of the samples in tri-sodium citrate. With increasing concentrations of the alginate and calcium chloride, prolonged dissolution times were observed. However, other factors such as viscosity and hardness of the beads should be addressed. Figure 2 demonstrates beads prepared with various quantities of alginate and calcium chloride (Fig. 2a-i). Results showed that 2% alginate and 0.1 M calcium chloride were the best samples of beads. In a study by Cordoba et al. to assess the appropriate concentration of alginate for the preparation of capsules using extrusion method, it was revealed that concentrations of more or less than 2% alginate included negative effects on the characteristics of calcium-alginate hydrogels [23]. The current results showed that the use of alginate with a concentration of greater than 2% was viscous and cannot be extruded (Fig. 2a, c, g) and alginate less than 2% formed weak beads that could not well protect the bacteria (Fig. 2d, f, h, i).

Moreover, high proportions of alginate and molarities of calcium chloride led to the formation of hard beads that could not dissolve in tri-sodium citrate solution and hence were not useful. Similarly, Li et al. reported that increasing concentration of calcium ions in calcium-alginate gelatin capsules decreased the average diameter, prolonged dissolution time, decreased permeability and swelling and increased cross-sectional area and contraction of the capsules [24]. Yuan et al. reported that increases in calcium ion concentration was positively correlated with loss of bacterial survival and concentrations greater than 1.5 M significantly decreased cell survival after freeze drying [25].

3.3. Effects of various formulation in bacterial encapsulation efficiency

After assessing the best proportion of alginate and concentration of calcium chloride (2% and 0.1 M, respectively), protective effects of other materials such as sucrose, yeast powder containing beta-glucan (yeast beta-glucan) and skim milk were studied to increase the encapsulation efficiency and bacterial survival. As seen in Fig. 3, various formulations included various proportions of bacterial encapsulation. Formulations containing sucrose and yeast included the highest encapsulation quality (Fig. 3) as A and Y, and A, S and Y formulations included 97 and 99% encapsulation efficiencies, respectively. These results showed that addition of other substances (especially polysaccharides) to the encapsulation formulation caused further cohesion and the formation of stronger matrices, which could entrap further bacteria inside [26]. Jouki et al. reported that 95.2% of *L. casei* encapsulated in quince seed gum-alginate (QSG-A) formulation were entrapped [20].

Comparing monolayer samples to bilayer samples in Fig. 3, it can be concluded that monolayer samples included higher proportions of encapsulation than those the double-layer samples did. While bilayer samples included less pores than those the monolayer samples did, preparation time for the beads in monolayer method was shorter and the beads were less submerged in various solutions, resulting in less bacterial leakages to the outside and greater populations of live bacteria. Similarly, Mirmazloum et al. reported that the encapsulation proportion of *L. acidophilus* in calcium alginate beads in monolayer formulations was more than that in bilayer formulations (83 and 74%, respectively) [27]. These results showed that by addition of other substances especially polysaccharides to the encapsulation formulation caused further cohesion and formation of stronger matrices, which could entrap further bacteria inside. At the same time, addition of further ingredients such as powdered milk decreased the encapsulation proportion by maintaining structure of the capsules and closing pores of the alginate gels.

3.4. Effects of various formulations on the bacterial survival after freeze-drying and storage

In this study, use of preservatives with alginate included positive effects on encapsulation performance (Section 3.3). Thus, various formulations of alginate with protective materilas were assessed to investigate which formulation could achieve the highest proportion of bacterial survival after freeze-drying and 1 w of storage at room conditions (Table 2). The monolayer (A, alginate; A.S, alginate-sucrose; A.Y, alginate-yeast; A.Y.S, alginate-yeast-sucrose; and A.S.SM, alginate-sucrose-skim milk) and bilayer (monolayers with a second layer of alginate) formulations were investigated in these experiments (Fig. 4).

These findings suggest that coating cannot protect the bacteria from freeze-drying and that protective substances such as sucrose or skim milk should be used instead. A minimum bacterial decrease of 2 log CFU ml^{-1} and maximally 3 log CFU ml^{-1} was reported primarily after freeze-drying (Fig. 4a,b).

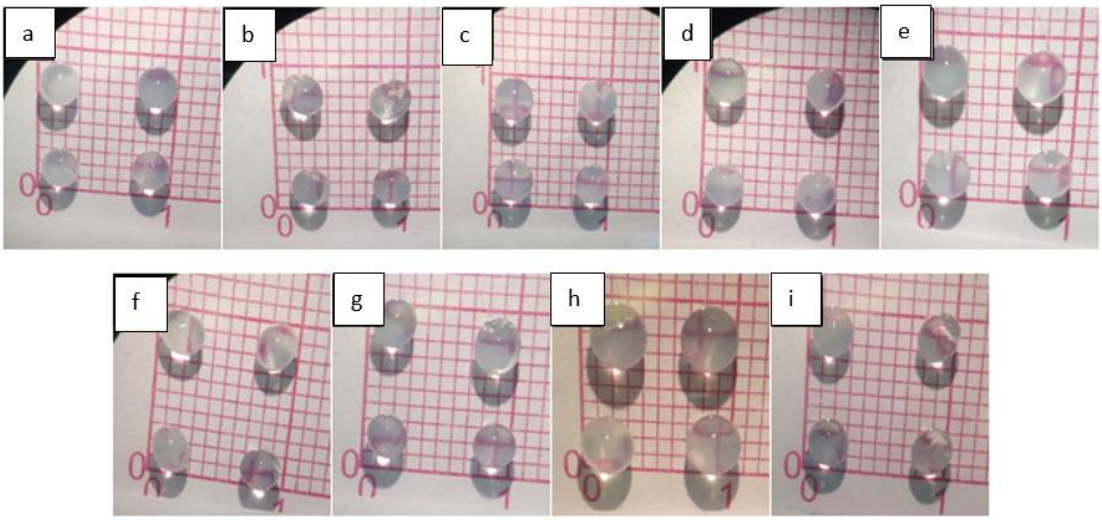

Figure 2. Effects of various concentrations of alginate and calcium chloride on the characteristics of beads. a) Alginate 3% and $CaCl_2$ 0.05M, b) alginate 2% and $CaCl_2$ 0.5M, c) alginate 3% and $CaCl_2$ 0.5M, d) alginate 1% and $CaCl_2$ 0.1M, e) alginate 2% and $CaCl_2$ 0.1M, f) alginate 1% and $CaCl_2$ 0.05M, g) alginate 3% and $CaCl_2$ 0.1M, h) alginate 2% and $CaCl_2$ 0.05M, and i) alginate 1% and $CaCl_2$ 0.5M

Table 2. Compositions of various samples and their experimental results

Number	Formulation		N ($\times10^9$)	NF ($\times10^7$)	N1w ($\times10^7$)	Viability Proportion After freeze drying	Survival Proportion After 1 week Storage
1	Monolayer	A	5.2	0	0	0%	0%
2		A+Y[2]	5.4	0	0	0%	0%
3		A. S	5.1	9.1	0.18	1.8%	2%
4		A+S[4]+Y	5.2	6.76	0	1.3%	0%
5		A+S+SM[5]	5.1	0.5	0.5	0.1%	100%
6		A. S+SM	5.1	5.1	5.1	1%	100%
7	Double layers	A. S+A*	5.1	0.22	0.004	0.04%	1.9%
8		A. S+Y+A*	5.3	0.9	0.017	0.17%	1.3%
9		A. S+SM.Y+A*	5.2	6.76	0.025	1.3%	0.38%
10		A+Y+A*	5.1	3.57	0.035	0.7%	1%

A: Alginate, Y: Yeast powder, S: Sucrose, A.S: Combination of alginate and sucrose, SM: Skim Milk, SM.Y: Combination of Skim Milk and Yeast powder, A*: The second layer of alginate.

It is well-known that carbohydrates can protect bacteria against harmful effects of dehydration during drying processes [28]. Studies have compared effects of carbohydrates as cryoprotectants [29,30]. In the current study, sucrose was used as protectant to encapsulate bacteria in addition to alginate. Based on the findings, presence of sucrose in encapsulation formulations increased bacterial viability up to 1.8% in monolayer beads after freeze-drying with a shelf-life of 100% (Table 2, No. 3). It was explained that sucrose could be a good protector for the bacteria in freeze-drying conditions, preserving bacterial survival after freeze drying and storage. Similarly, Liu et al. reported that use of 48.5 $g.l^{-1}$ sucrose, 30 $g.l^{-1}$ gelatin and 28.4 $g.l^{-1}$ glycerol as protectants resulted in greater than 90% cell survival rates and preserved activities of *Enterobacter* spp. even after 18 m of storage [31]. Jouki et al. reported protective effects of sorbitol on alginate-skim milk and glycerol beads as protectant for *L. plantarum* in freeze-dried yogurt powders and showed that sorbitol-enriched microcapsules included longer shelf-lives, compared to other formulations. They reported that the survival rate of free bacteria was 67.1% while that of bacteria encapsulated with cryoprotectants was 89.4% and the survival rate of bacteria encapsulated with cryoprotectants enriched with sorbitol was 91.2% [32]. In another study by Shu et al., it was detected that cryoprotectants containing 13% of trehalose, 7.5% of lactose and 21% of skim milk increased the survival rate of freeze-dried *L. acidophilus* to 93.9%, compared with 36.6% in the control sample [33]. In other formulations, yeast powder and skim milk were added to sucrose-alginate separately and in combinations to assess improvements of bacterial viability. Results showed that in formulation of sucrose and yeast powder containing beta glucan in monolayer encapsulation, viability rates after freeze drying and 1 w of storage at room conditions significantly decreased (Table 2, No. 4). By adding protectants such as skim milk or yeast powder, the osmotic pressure balance around the bacteria might be disturbed, which led to cell dehydration and bacterial death [34].

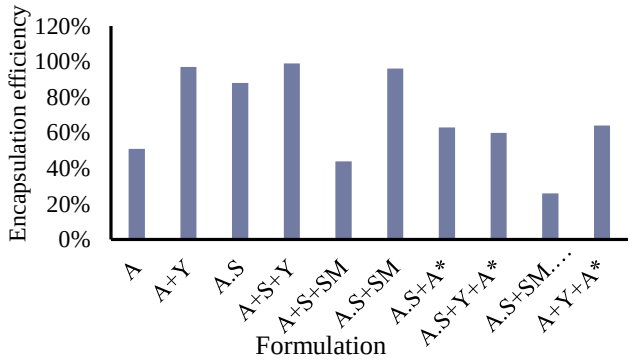

Figure 3. Comparison of encapsulation efficiencies in monolayer and bilayer beads (A, alginate; Y, yeast extrat; S, sucrose; A.S, combination of alginate and sucrose; SM, skim milk; and SM.Y, combination of skim milk and yeast extract). Formulations containing two A represent a bilayer coating with alginate (A*, the second layer of alginate).

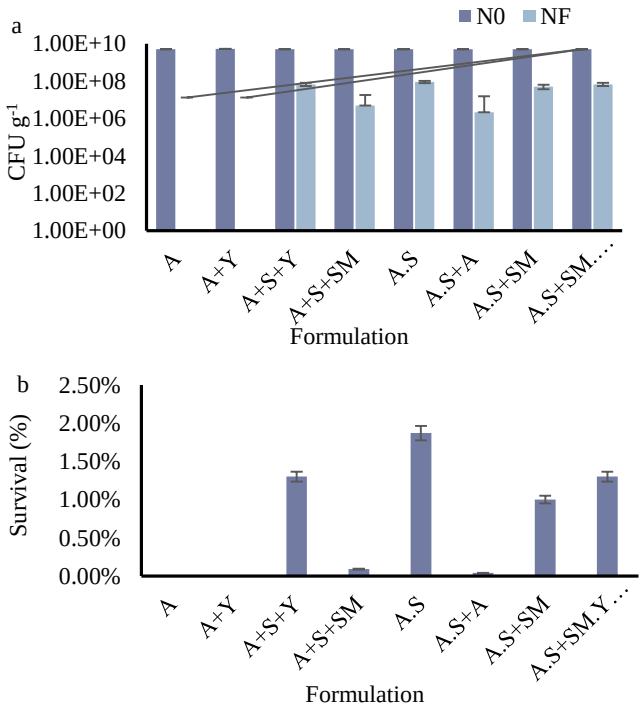

Figure 4. Effects of various formulations in bacterial viability after freeze drying. a) Number of live bacteria and b) proportion of bacterial viability

Shao et al. reported that yeast extracts included negative effects on cold tolerance of *L. delbrueckii* and decreased their viability during lyophilization [35]. Furthermore, it was demonstrated that combination of skim milk with sucrose in monolayer encapsulation decreased the cell viability after freeze drying. However, use of skim milk preserved 100% survival of the remaining cells during 1 w of storage at room conditions (Table 2, No. 5). In other words, skim milk did not improve the cell viability rate after freeze drying but included positive effects on preserving the cell survival after 1 w of storage. Zarate et al. reported that lyophilization of *L. paracasei*, *L. salivarius* and *L. acidophilus* with lactose or skim milk significantly decreased their viabilities [36].

As shown in Table 2, use of skim milk with sucrose in monolayer or bilayer encapsulation preserved 100% cell survival after 1 w of storage at room conditions, indicating positive effects of skim milk on cell survival during storage. Yue et al. reported use of 10% (w v^{-1}) skim milk and 10% (w v^{-1}) sucrose as cryoprotectives to increase the survival of freeze-dried *L. salivarius* [37]. In another formulation, a combination of sucrose, β-glucan of yeast powder and skim milk were used for two-layer encapsulation. Results revealed that this formulation (compared to alginate-sucrose formulation) did not include positive effects on improvement and preserve of cell viability after freeze drying and storage (Table 2, No. 9). In general, it was detected that the viability proportion was lower in two-layer encapsulation than single-layer one in all samples after freeze drying (Fig. 5a, b).

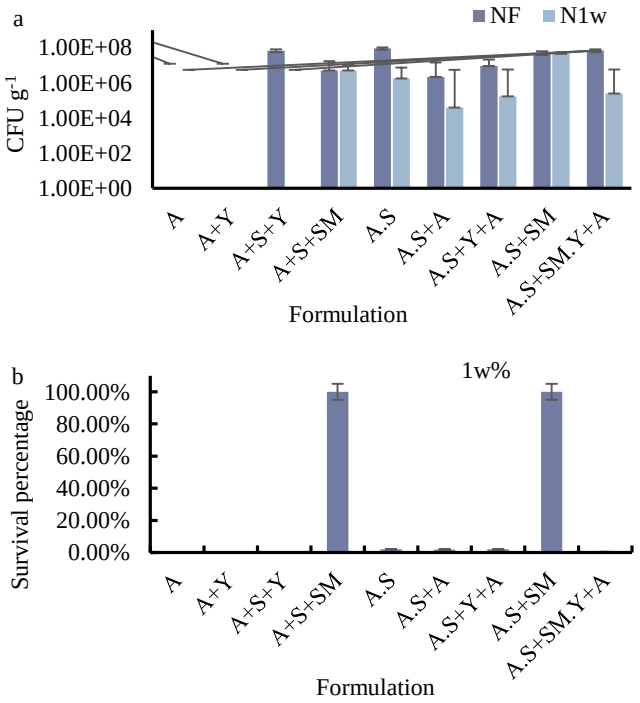

Figure 5. Effects of various formulations in bacterial survival in monolayer and bilayers samples after 1 w of storage at room conditions. a) Number of surviving bacteria and b) proportion of surviving bacteria

It can be concluded that increases in processing time and transferring beads in further aqueous solutions in bilayer encapsulation method resulted in beads to absorb further water, causing rupture of the cell membrane during freeze drying and significantly decreasing bacterial viability [38]. However, results showed that the survival rate was higher in bilayer encapsulated samples than monolayer ones after 1 w of storage at room conditions. In fact, presence of the second layer of alginate could prevent leakage of the nutrients to the outside, prolonging survival of the cells that survived after freeze drying [39].

4. Conclusion

Recently, needs to store pharmaceutical products and mostly probiotic food products at room conditions have increased, urging needs of further studies in this field. In the present study, various formulations were investigated for the *L. rhamnosus* encapsulation and the best formulation based on encapsulation proportion included alginate, sucrose and yeast powder for monolayer beads. The present results showed that sucrose increased survival of bacteria after freeze drying. By the addition of yeast powder containing β-glucan, survival rate decreased, compared to sucrose. Moreover, alginate, sucrose and skim milk included the highest survival rate after 1 week of storage at room conditions. However, further studies are needed to ensure cell longer survival rates at room conditions.

5. Acknowledgements

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6. Conflict of Interest

The authors gratefully acknowledge the National Institute of Genetic Engineering and Biotechnology (NIGEB) for providing laboratory facilities.

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اثرات ساکارز، شیر بدون چربی و پودر مخمر بر زنده‌مانی *لاکتوباسیلوس رامنوسوس GG* ریزپوشانی شده با آلژینات در مدت نگهداری یک هفته‌ای در شرایط اتاق

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چکیده

سابقه و هدف: در هنگام تولید و نگهداری فراورده‌های زیست‌یار[۱]، همیشه این احتمال وجود دارد که زیست‌یارها در معرض تنش‌های مضر گوناگون مانند دمای بالا، pH پایین، فشارهای اسمزی گوناگون و سطوح بالای اکسیژن قرار گیرند، که می‌تواند تعداد سلول‌های زنده را کاهش دهد. ریزپوشانی[۲] یکی از روش‌هایی است که برای محافظت سلول های زنده مانند باکتری‌های زیست‌یار در برابر چالش‌های زیست‌محیطی مورد استفاده قرار می‌گیرد. تا به امروز، ترکیبات گوناگون با توانایی محافظت از باکتری‌ها در هنگام خشک کردن انجمادی و افزایش زنده‌مانی در هنگام ذخیره‌سازی شناسایی شده‌اند. در این مطالعه، از آلژینات به‌همراه کلرید کلسیم برای ریزپوشانی باکتری‌های زیست‌یار به‌روش اکستروژن استفاده شد. سپس، اثرات ساکارز و شیر بدون چربی به‌عنوان محافظ برودتی و پودر مخمر حاوی بتا-گلوکان به عنوان کمک زیست‌یار[۳] بر زنده‌مانی این باکتری‌ها بررسی شد.

مواد و روش ها: محلول همگن باکتری با آلژینات و محافظ برودتی به‌صورت دستی در محلول کلسیم کلرید اکسترود شد. پس از ۳۰ دقیقه هم زدن، دانه‌های تشکیل شده جدا شدند. برای مقایسه، جمعیت باکتری در کشت اولیه، پس از ریزپوشانی، خشک‌کردن انجمادی و یک هفته نگهداری در شرایط اتاق (به ترتیب، N0، N، NF، $N1_w$) شمارش شد. برای شمارش تعداد باکتری از روش تهیه CFU استفاده شد، به این ترتیب که ۱ گرم از دانه‌های تازه تشکیل شده در ۹ میلی‌لیتر تری سدیم سیترات حل و سپس رقت‌سازی سریالی و پورپلیت انجام شد. پلیت‌ها به‌مدت ۲۴ تا ۴۸ ساعت گرمخانه‌گذاری و کلنی‌ها شمارش شدند.

یافته‌ها و نتیجه‌گیری: نتایج نشان داد که ریزپوشانی باکتری‌ها با آلژینات و کلسیم کلرید به‌تنهایی ٪۵۱ مؤثر بود، در حالی که استفاده از پودر مخمر و ساکارز به‌همراه آلژینات راندمان ریزپوشانی را به‌ترتیب به ۹۷ و ۹۹ درصد افزایش داد. علاوه براین، استفاده از شیر بدون چربی با آلژینات و ساکارز منجر به بالاترین میزان زنده‌مانی پس از ۱ هفته نگهداری در شرایط اتاق شد. بنابراین ریزپوشانی باکتری‌های زیست‌یار با پوشش آلژینات و پودر مخمر حاوی کمک زیست‌یارهای ساکارز و شیر بدون چربی می‌تواند در زنده‌مانی این باکتری‌ها موثر باشد.

تعارض منافع: نویسندگان اعلام می‌کنند که هیچ نوع تعارض منافعی مرتبط با انتشار این مقاله ندارند.

[۱] Probiotic products
[۲] Encapsulation
[۳] Prebiotic