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Microencapsulation of *Lactobacillus acidophilus* by ammoniacum gum enriched with nanoselenium : investigating the survival rate in simulated gastrointestinal tract and storage period

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ABSTRACT

The increasing demand for hydrocolloids with specific functionality has led researchers to seek new hydrocolloid sources. One of the methods to enhance the viability of probiotics under stressful conditions is microencapsulation, to deliver these bacteria to the body in sufficient numbers and utilize their health-promoting effects. The aim of this study is to investigate the feasibility of *Lactobacillus acidophilus* microencapsulation by ammonium gum (AMG) and its enrichment using nanoselenium, and determine its viability under stressful conditions. AMG was extracted from the mucilage of *Dorema Ammoniacum* plant using a solvent and then precipitated by alcohol. 1%, 5%, and 10% w/v solutions of AMG were prepared, and then nanoselenium particles synthesized at a concentration of 1.5% were added to the bacterial suspension with an average count of 6.85×10^{11} log CFU g^{-1}. The results showed that with an increase in the concentration of gum, the encapsulation efficiency significantly increased from 66% to 81%. The viability rate for 1%, 5%, and 10% concentrations of AMG under refrigeration conditions was 62.43%, 72.37%, and 81.83%, respectively. Under simulated gastric conditions at the pH levels used in this study, the counts of viable cells after 3 h of incubation for 5% and 10% concentrations of AMG remained higher than 7 log CFU g^{-1}. After 6 h of incubation in a 10 g L^{-1} solution, the free cells showed a decrease to 5.93 log CFU g^{-1}, while for microencapsulated cells at concentrations of 1%, 5%, and 10%, it was only 3.93 log CFU g^{-1}, 3.15 log CFU g-1, and 1.9 log CFU g^{-1}, respectively. The results showed that AMG has very good encapsulation properties, which can be used in many food formulations for these purposes.

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1. Introduction

Hydrocolloids refer to a group of polysaccharides and proteins that, by dissolving or dispersing in water, significantly increase viscosity and are widely used in various industries today with functions such as thickening, gel formation, films and emulsions, stabilizing foam and dispersions, preventing the formation of ice and sugar crystals, antimicrobial agents, dietary fiber, as well as encapsulation (controlled release of flavors and bioactive substances) [1].

Ammoniacum mucilage, known as "ushaq," "Vasha," or Persian ammoniacum, is obtained from the stems and leaves of the *Dorema Ammoniacum* plant, belonging to the Apiaceae (*Umbelliferae*) family. This plant is one of the most important medicinal plants growing in dry and semi-dry regions of Central Asia, including Iran, Afghanistan, and Pakistan. In Iran, it is native to Yazd, Kerman, Isfahan, and Semnan. The plant grows up to 2.5 meters tall and exudes milky sap in spring and early summer. The leaves are twisted together, and the hollow, knotty, and blue-lined stems are about 5 centimeters thick. To obtain the mucilage, the plant stems are split, and a mucilaginous substance emerges, which hardens upon exposure to air. The mucilage appears as a bright mass with cracks and shiny yellow lines on its surface [2].

Selenium (Se) is a key element for maintaining the health of mammals, and Se deficiency in the diet is a major global health problem. Se deficiency makes individuals susceptible to several chronic diseases, including diabetes, cardiovascular diseases, and cancer. It also affects the immune and reproductive systems, leading to male infertility and increased risk of miscarriage in females [3].

In order to prevent the reduction of probiotics' viability during processing and passage through the digestive system, some technologies are available. Microencapsulation is a technique that can protect live probiotic cells against undesirable environmental stresses [4]. The choice of materials is an important factor in microencapsulation. This substance must be food grade and be able to trap and protect probiotics. The most common wall materials used for probiotic microencapsulation are carbohydrates such as alginates, carrageenans, and other gums [5]. The aim of this study was to investigate the possibility of microencapsulation of *Lactobacillus acidophilu*s probiotics by AMG, enriching it using nanoselenium, and determining its viability under stressful conditions.

2. Material and methods

2.1. Synthetize of Selenium Nanoparticles

Nanoparticles were synthesized by reducing selenium dioxide (SeO_2) solution using ascorbic acid in the presence of polyvinyl alcohol (PVA) stabilizer. Equal amounts (10 mL) of 0.02 M selenium dioxide, 0.08 M ascorbic acid, and 1.0 M polyvinyl alcohol solutions were prepared. The Se solution was placed on a magnetic stirrer, and the ascorbic acid and polyvinyl alcohol solutions were slowly (drop by drop) added to the Se solution simultaneously. Upon the formation of Se nanoparticles, the solution turned orange-red, indicating the formation of nanoparticles. The resulting red solution was dried by freeze-drying [6].

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2.2. Extraction of Gum from Ammoniacum Plant's Exuded Mucilage

The mucilage prepared from the Ammoniacum plant, which tended to be yellow, was manually cleaned, and then it was ground by a laboratory mill to prepare the powder. After cleaning, the mucilage was mixed under continuous stirring conditions at a ratio of 1 to 10 with previously heated distilled water. The water-mucilage mixture was gently stirred at a temperature of 50 °C for 2 h. The prepared solution was kept for 24 h at a temperature of 4 °C for complete hydration. Then, to remove the extra particles, the solution was centrifuged (20 °C, 30 min, and 300 g) and passed through filter paper. In the next step, 96% ethanol was used in a ratio of 1 to 3 to deposit the extracted gum. The deposited gum was filtered with a linen cloth and then washed twice with 70% ethanol, twice with 96% ethanol, and once with acetone at a temperature of 25 °C for 10 min each time. This washing process was carried out to completely remove potential residues of the skin, improve the color of the produced gum, and purify the minerals. After washing, the obtained gum was dried in an oven (Fan Azma Gostar, Iran) at a temperature of 38 °C. The dried gum was milled and then stored in a dry and cool place for further experiments [2].

2.3. Probiotic bacteria activation

The purchased strain of *Lactobacillus acidophilus* in lyophilized form was opened under a laminar hood and transferred into the MRS broth culture medium. Then, it was placed in an incubator at a temperature of 37 °C for 24 h to grow. To ensure the purity of the bacteria, a microbial suspension solution was prepared, and the bacterial purity was confirmed. The plates were placed in the incubator at a temperature of 37 °C for 24 h for bacterial colonies to grow. All equipment and culture media were sterilized at a temperature of 121 °C using an autoclave for 20 min. The microbial suspension prepared on MRS broth culture medium for use in microencapsulation was centrifuged at a speed of 400 g for 10 min to separate the bacterial cells from the surrounding culture medium solution. Then, the bacterial cells were washed with 0.85% sterile sodium chloride solution [7].

2.4. Preparation of Suspension for Microencapsulation

1, 5 and 10% w/v solutions of AMG were prepared under homogenization conditions at a temperature of 60 °C and autoclaved at 105 °C for 5 min. The reason for choosing a high concentration of gum was the low viscosity of AMG and its higher protective effect. The centrifuged bacterial cells with an average cell count of 6.85×10^{11} log CFU g^{-1} were added to the suspension.

2.5. Microencapsulation process using a spray dryer

At this stage, the prepared suspension was fed into a spray dryer machine (Dorsa tech, Iran) at a pressure of 6-5 bars, an inlet temperature of 100-90 °C, an aspirator of 90%, and a pump suction of 25% (equivalent to a solution suction rate of 6 mL per minute). Initially, distilled water was used to adjust the inlet temperature to the desired value, and then the main suspension solution was injected into the spray dryer machine. The dried powder was collected from the bottom cyclone collector of the machine and weighed. Then, each sample was separately poured into three falcon tubes, covered with

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parafilm, and placed inside aluminum foil. Subsequently, they were stored at a temperature of 4 °C [8].

6.2. Moisture content and water activity

The water activity of the powders was measured using the AquaLab Pawkit device. The moisture content of the powder was measured according to the AOAC standard (2005).

7.2. Encapsulation efficiency

To assess the viability of bacteria during the spray drying process, microbial counting was performed immediately after drying. The encapsulation efficiency was calculated using the following equation:

$$\text{Encapsulation yield (EY)} = \left(\frac{\log N}{\log N_0}\right) \times 100$$

Where, N is the number of live cells in the powder (CFU g^{-1}) and N_0 is the number of live cells in the encapsulating material before drying [9].

8.2. Examination of the viability of encapsulated *L. acidophilus* bacteria during refrigerated storage

The viability of encapsulated probiotic bacteria during one month of refrigerated storage (4 °C) was investigated. 1.0 gr of the bacterial powder obtained was poured into 9.9 mL of physiological saline solution for complete homogenization. Then, for complete release of the encapsulated bacteria, it was placed in an incubator with a speed of 10 g at a temperature of 37 °C for 30 min. After preparing serial dilutions, they were cultured in MRS agar medium using the pour plate method. Then, they were incubated at 37 °C for 72 h. All counts were performed three times, and plates containing 300-30 colonies were counted [10].

9.2. Examination of the viability of *L. acidophilus* bacteria encapsulated under simulated gastric conditions

The simulated gastric solution containing NaCl was prepared by suspending pepsin (3 g L^{-1}) in saline solution (5 g L^{-1} NaCl) and adjusting the pH to 2 or 3 using 5 mol L^{-1} HCl solution. After filtration and sterilization, 1.0 gr of encapsulated *L. acidophilus* samples or 1 mL of free cell suspension separately were added to test tubes containing 9/9 mL of simulated gastric solution and placed in a 37-°C incubator. After 0.5, 1, 2, and 3 h of incubation, the samples were immediately cultured for counting the number of viable cells anaerobically on MRS agar medium [11].

10.2. Evaluation of *L. acidophilus* viability in simulated bile conditions

The resistance of encapsulated *L. acidophilus* in the presence of bile salts is investigated according to the simulated gastric method, with the difference that 9.9 mL of bile salt solution containing 5 or 10 gr per liter of bile salts were used [11].

11.2. Scanning Electron Microscopy

The morphology of probiotic powders was observed under a scanning electron microscope (JSM-6610LV, JEOL Ltd. Japan).

11.3. Statistical Analysis

ANOVA method was used for data analysis. If the F-values in ANOVA were significant ($P<0.05$), the Duncan method was used to compare the means. All tests were performed in three repetitions.

3. Results and discussion

3.1. Particle Size and Dispersion of Nanoselenium

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To confirm that the size of the synthesized nanoparticles is within the nanometer range, a Zetasizer (Malvern Zetasizer) was used.

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The particle size distribution based on intensity, the average particle size (Z-average (d.nm)), and their dispersity index (PdI) are shown in Figure 1. The results showed that the average diameter of the synthesized nanoparticles was approximately 170 nm and their dispersity index was 0.132.

Fig 1. Particle size distribution of selenium nanoparticles.

3.2. Moisture content and water activity of powders

The water activity (a_w) and moisture content of powders containing *L. acidophilus* probiotic at different concentrations of AMG are shown in Table 1. The water activity of AMG powders containing *L. acidophilus* significantly decreased with higher concentration of gum. As shown in the table 1, a_w ranged from 0.31 to 0.24. A_w indicates free water (water not bound to molecules) and has a significant impact on the preservation of probiotics. According to the report by Viernstein et al., (2005), lower a_w (0.3-0.2) creates better cell viability during storage. If aw is less than 0.1, oxidized membrane lipids and reduced viability occur [12]. Values above a_w also reduce the viability of probiotics and increase the risk of contamination during storage. The a_w of AMG powders containing *L. acidophilus* significantly decreased with higher concentration of gum. Similar results were reported by Arepally et al., (2019) for Arabic gum. They observed that with an increase in the concentration of Arabic gum from 0% to 5%, the a_w decreased from 0.52 to 0.44. They attributed this decrease in a_w to the presence of high levels of carbohydrates and the branched structure of Arabic gum, which causes a strong tendency to absorb water and bind to it [13]. In most studies, the optimum viability of probiotics has been reported at a_w less than 0.2. For example, *L. paracasei* dried by spray drying showed better viability at a_w less than 0.3 [14].

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The moisture content of probiotic powders has a significant impact on the product stability as well as the viability of probiotics during the storage period and is considered as a quality parameter in cell-containing powders [15]. The results showed that the moisture content of the powders produced decreased with an increase in gum concentration. With an increase in gum concentration, the total solid content increased, thus the amount of water for evaporation decreased. Arepally et al., (2019) reported that with an increase in Arabic gum concentration up to 10%, the moisture content in probiotic powders dried by spray drying decreased [13]. The reduction in moisture content due to the addition of polysaccharides to the formulation of probiotic powders was reported by Tao et al., (2019) [16].

Table 1- Moisture percentage of AMG powders in different concentrations.

Concentration (%)	Water activity	Moisture (%)
1	0.31±0.05[a]	**7.94±1.62[a]**
5	0.26±0.01[b]	**4.63±0.02[b]**
10	0.24±0.01[c]	**2.78±0.21[c]**

3.3. Encapsulation Yield (EY)

During drying, due to the use of high temperatures in the process, viability decreases. The encapsulation yield of AMG at different concentrations is given in Table 2. The initial count of live bacteria for all concentrations was almost the same and ranged from 11.96 12.23 CFU g^{-1}. According to Table 2, with the increase in gum concentration, the microencapsulation yield has significantly increased. During drying, due to the use of high temperatures in the process, viability decreases. With the increase in AMG concentration, the microencapsulation yield has significantly increased, which could be due to the protective effect of AMG on cell viability during drying. Viability at all concentrations was higher than the minimum recommended value of 6 log CFU g^{-1}. In a study conducted by Arepally et al., (2019), *L. acidophilus* microencapsulation was performed using Arabic gum, and the results showed an increase in microencapsulation yield with an increase in Arabic gum concentration from 0% to 10%. They stated that the presence of protein in Arabic gum creates a protective layer around the bacterial cells and thus prevents their destruction [13]. Rajam et al., (2015) reported a microencapsulation yield of fructo-oligosaccharides at 70.77-72.82% [17]. The resistance of probiotics to drying depends on the type of probiotic species [18]. The high viability rate obtained in this study may be due to the matrix used for encapsulation and the concentration of wall material (10%). Previous studies have shown that a solid material concentration of about 20-30% in the feeding solution is one of the best conditions for ensuring viability [19]. The sugar present in the structure of the gum also acts as a protective agent for probiotics, which is justified by the hypothesis of water replacement. According to this hypothesis, under hydrated conditions, the configuration and integrity of proteins and cell membranes are maintained by the reaction with water molecules and the formation of hydrogen bonds. After the removal of water, the polar groups of sugars can replace water molecules [20]. The viability at all concentrations was higher than the minimum recommended amount of 6 log CFU g^{-1}. In a study, the encapsulation of *L. acidophilus* by Arabic gum showed that with an increase in Arabic gum concentration from 0% to 10%, the encapsulation efficiency increases. They stated that the presence of proteins in Arabic

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gum creates a protective layer around the bacterial cells and thus prevents their destruction [13].

Table 2. Encapsulation efficiency of AMG in different concentrations.

Concentration (%)	Initial count	Final count	Encapsulation efficiency
1	12.23	8.15	**66.63±0.5^{c}**
5	11.96	9.11	**76.17±0.7^{b}**
10	12.15	9.85	**81.6±0.9^{a}**

3.4. The viability of encapsulated *L. acidophilus* bacteria during refrigerated storage

Today, due to the increasing demand for healthy, nutritious, and beneficial foods, the food industry has focused on developing probiotic-containing foods that can maintain higher viability during the process and storage conditions [14]. However, the viability of probiotics in food is exposed to destructive factors such as the presence of antimicrobial compounds, oxygen toxicity, acidic environment, and storage temperature [21]. Figure 2 shows the reduction in the viability of encapsulated *L. acidophilus* and free cells during 4 weeks of refrigerated storage. It is clearly visible that encapsulated cells show higher viability, especially after 30 days of refrigerated storage, compared to free cells. Broeckx et al., (2017) reported that *L. rhamnosus* encapsulated by maltodextrin during a 28-week storage period at refrigeration temperature did not show more than a 1 log reduction. They explained that at refrigeration temperature, the energy kinetics are limited, and molecular movement decreases. Therefore, the metabolism of bacteria decreases. As a result of the lower metabolism, bacteria not only produce fewer residuals but also limit external destructive processes [22]. Reyes et al., (2018) named Arabic gum as the best wall material for protecting *L. acidophilus* encapsulated during storage at 4 °C compared to maltodextrin and starch. Undoubtedly, the type of encapsulating material will have a direct effect on the stability of microcapsules [23].

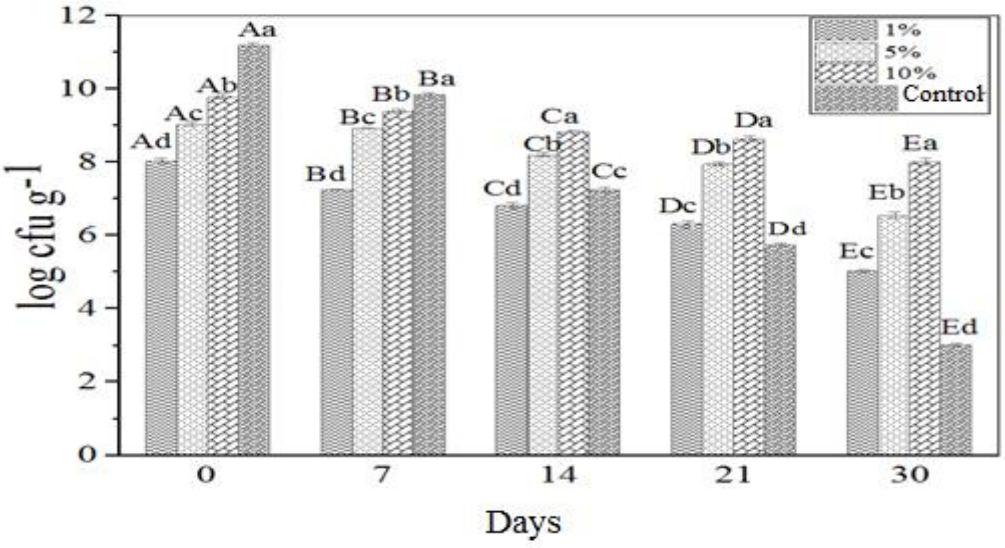

Figure 2. Viability (log CFU g^{-1}) of *L. acidophilus* as a free cell and encapsulated by AMG in refrigerated conditions.

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3.5. Evaluation of the survival rate of *L. acidophilus* encapsulated under simulated gastric conditions

Some probiotics have low resistance to unfavorable conditions of the gastrointestinal tract. To overcome these conditions, cell encapsulation by spray drying has been introduced. The survival of encapsulated and free *L. acidophilus* cells under simulated gastric conditions at pH 3 and 2 in Figure 3 is depicted. The results showed that the survival was dependent on the concentration of AMG used, so that the protective effect on the cells increased with increasing concentration. Free cells showed a significant decrease under these two conditions and therefore need protection through encapsulation. In free cells, the count of live cells decreased to 3.99 log CFU g^{-1} at pH 2 and 1.56 log CFU g^{-1} at pH 3. The use of encapsulation had a protective effect on *L.acidophilus* since the viability reduction in samples with a 10% concentration of AMG was only 0.78 log CFU g^{-1} and 0.96 log CFU g^{-1} at pH 3 and 2, respectively. Additionally, the concentration of the AMG used for encapsulation had a significant effect on viability, as the 10% concentration had a greater protective effect compared to the 1% and 5% concentrations. At the pH levels used in this study, the 5% and 10% concentrations of AMG resulted in higher cell counts after 3 h of incubation, remaining above 7 log CFU g^{-1}.

The encapsulated *L. acidophilus* viability reduction by the AMG at low pH (2 and 3) was lower compared to previous studies. This reduction may be due to different microbial species, as according to Pham et al.'s study in 2008, probiotics show differences in resistance to acid and alkaline conditions depending on the species [24]. Hashami et al. reported in 2023 that encapsulated *L. casei* cells by a pectin-gelatin mixture in simulated gastric conditions showed a 4 log reduction, while free cells registered a 7 log reduction [11]. Similar results were reported by other studies, observing a significant reduction in the survival of free cells during gastrointestinal simulation under low pH conditions. Furthermore, encapsulation methods and materials used have an impact on the viability of microcapsules in acidic environments. Therefore, the use of AMG as an encapsulating material can protect cells under acidic conditions.

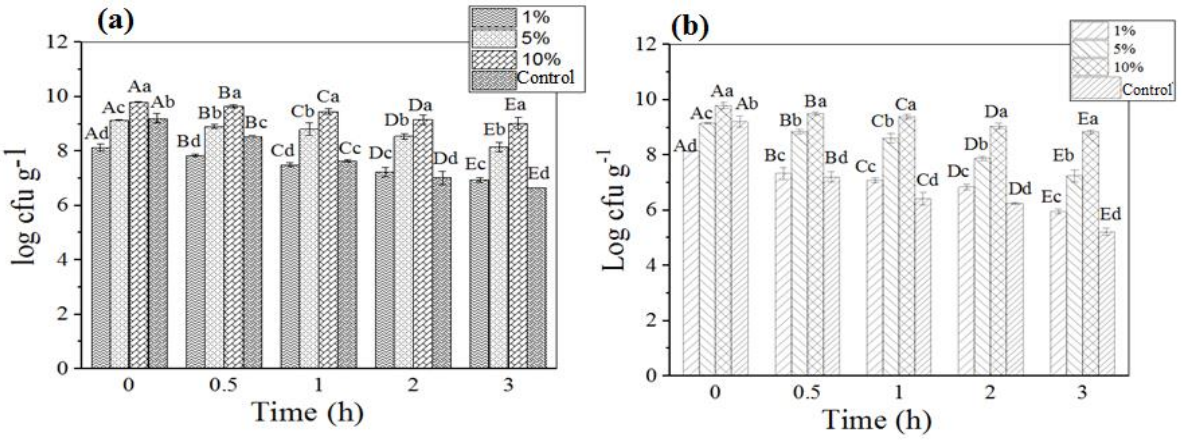

Figure 3. Viability (log CFU g^{-1}) of *L. acidophilus* as a free cell and encapsulated by AMG in simulated gastric conditions (a) pH=3 and (b) pH=2.

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3.6. Evaluation of the survival rate of encapsulated *L. acidophilus* in simulated bile salts conditions

The effect of microencapsulation on the viability of *L. acidophilus* in alkaline salt solutions (5 g L^{-1} and 10 g L^{-1}) was examined, and the results are shown in Figure 4. Another important feature of probiotic bacteria is their ability to survive in alkaline conditions created in the human small intestine to continue colonization and proliferate in the large intestine. Microencapsulation significantly ($P<0.05$) affected the viability of *L. acidophilus* cells in alkaline salt solutions even after 6 h of incubation, depending on the concentration of AMG used, so that the protective effect on the cells increased with increasing concentration. Similar results have been reported in previous studies for microencapsulated *L. casei* by cheese whey protein, where free *L. casei* cells after 6 h of incubation in 10 g L^{-1} saline solution showed a reduction equivalent to 5.96 log CFU g^{-1}, while microencapsulated cells only decreased by 2.95 log CFU g^{-1} [26].

The viability of free and microencapsulated *Bifidobacterium Bb-12* cells by spray drying with cheese whey at different bile concentrations was reported by Castro-cislaghi et al., in 2012. They found that in 5 g L^{-1} bile, the viability of microencapsulated cells after 6 h of incubation decreased by 1.71 log CFU g^{-1}, similar to the results obtained for microencapsulated *L. acidophilus* by 10% AMG concentration (1.78 log CFU g^{-1}). When the encapsulated cells were exposed to 10 g L^{-1} of bile salt for 6 h, a reduction of 2.71 log CFU g^{-1} was observed, which was higher than the results obtained for *L. acidophilus* encapsulated by 10% AMG (1.9 log CFU g^{-1}) and lower than 1% (3.93 log CFU g^{-1}) and 5% (3.15 log CFU g-1) AMG [27]. Comparison of the results of this study with other studies shows that the survival of *L. acidophilus* encapsulated by AMG in bile salt is acceptable. In addition, the survival of encapsulated cells in 5% and 10% concentrations of AMG after 6 h of incubation was higher than 6 log CFU g^{-1}.

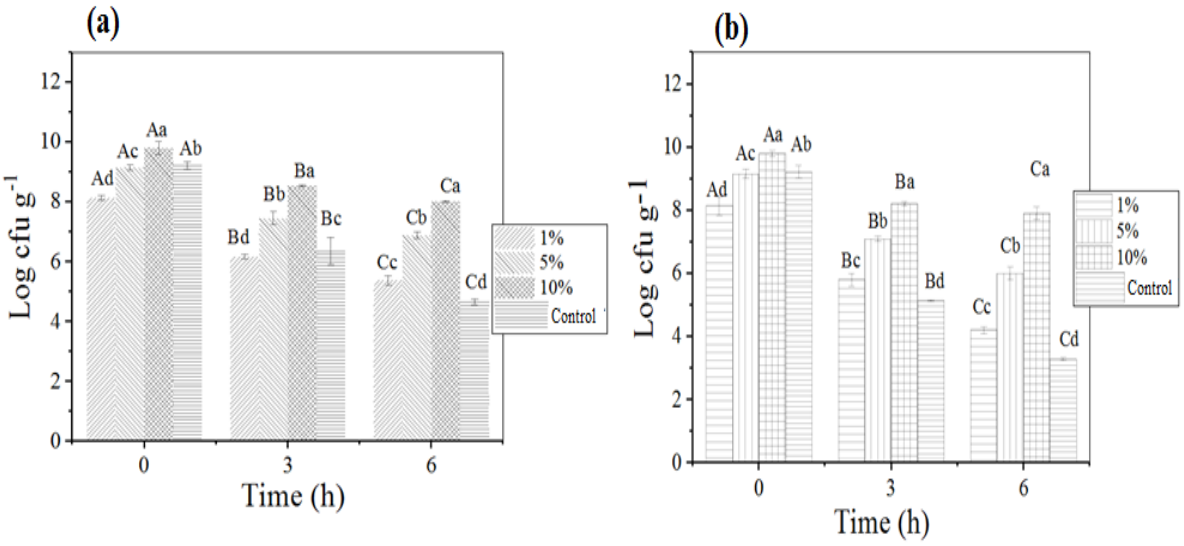

Figure 4. Viability (log CFU g^{-1}) of *L. acidophilus* as a free cell and encapsulated by AMG in simulated bile conditions (a) 0.5% and (b) 1%.

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3.7. Scanning electron microscopyy (SEM)

The dried *L. acidophilus* powders were observed by scanning electron microscope (SEM) using the spray method, as shown in Figure 5. The dried powders by the spray method had a spherical shape with a smooth surface without cracks or mechanical pressure and had the common roughness of materials produced by spray drying. The formation of roughness on the surface of atomized droplets can be attributed to the coalescence (wrinkles) during the drying process due to rapid evaporation of liquid droplets [11].

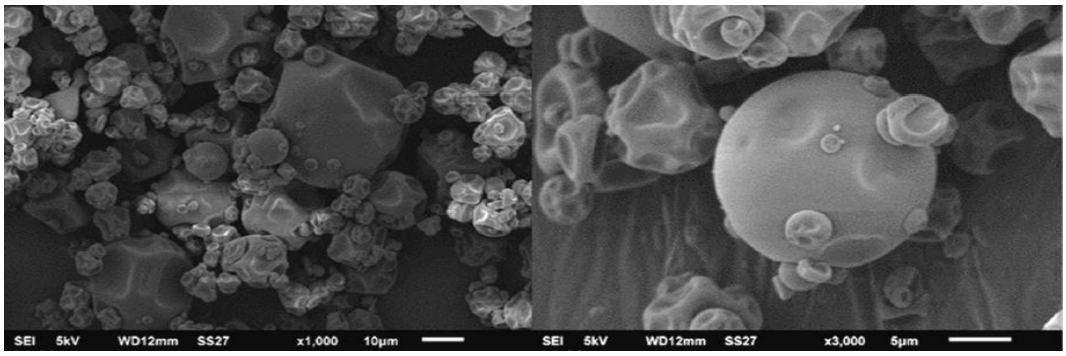

Figure 5. Scanning electron microscope (SEM) images

4. Conclusion

AMG is secreted from the *Dorema ammoniacum* plant and is found in some areas of Iran, Afghanistan, and Pakistan. AMG has shown very good encapsulation capability, which can be used in many food formulations for this purpose. This gum can be used in the pharmaceutical industry as a binder in tablets or to stabilize suspensions. These unique properties of AMG introduce it as a useful hydrocolloid.

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ریز پوشانی *لاکتوباسیلوس اسیدوفیلوس* توسط صمغ آمونیاکوم غنی شده با نانوسلنیوم: بررسی میزان زنده مانی در شرایط شبیه سازی شده دستگاه گوارش و دوره نگهداری

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چکیده

افزایش تقاضا برای هیدروکلوئیدهای با کارآیی ویژه، محققان را برای معرفی منابع جدید هیدروکلوئیدی علاقه مند کرده است. یکی از روش‌های افزایش زنده‌مانی پروبیوتیک‌ها در شرایط استرس‌زا ریز پوشانی می باشد، تا بتوان این باکتری‌ها را به تعداد کافی به بدن رساند و از اثرات سلامت بخش آنها استفاده کرد. هدف از این مطالعه بررسی امکان سنجی ریزپوشانی پروبیوتیک *ل. اسیدوفیلوس* توسط صمغ آمونیاکوم و غنی کردن آن با استفاده از نانوسلنیوم و تعیین میزان زنده مانی آن در شرایط استرس زا می باشد. صمغ آمونیاکوم با استفاده از حلال و سپس ترسیب توسط الکل از موسیلاژ حاصل از گیاه *Dorema Ammonacum* استخراج شد. محلول‌های ۱، ۵ و ۱۰٪ وزنی–حجمی از صمغ آمونیاکوم تهیه شد و سپس نانو ذرات سلنیوم سنتز شده در غلظت ۱/۵ درصد و سلول باکتری با میانگین تعداد $۱۰^{۱۱} \times ۶/۸۵$ log CFU g^{-1}، به سوسپانسیون اضافه شدند. نتایج نشان داد که با افزایش غلظت صمغ، بازده انکپسولاسیون به صورت معنی‌داری از ٦٦٪ به ۸۱٪ افزایش پیدا کرد. نرخ زنده‌مانی برای غلظت‌های ۱٪، ۵٪ و ۱۰٪ صمغ آمونیاکوم در شرایط یخچالی به ترتیب ٤۳٪، ٦۲٪، ۷۲/۳۷٪ و ۸۱/۸۳٪ بود. در شرایط شبیه سازی شده معده در سطوح pH مورد استفاده در این مطالعه در غلظت‌های ۵٪ و ۱۰٪ از صمغ آمونیاکوم شمارش سلول‌های زنده پس از ۳ ساعت انکوباسیون بالاتر از ۷ log CFU g^{-1} باقی ماند. پس از ٦ ساعت انکوباسیون در ۱۰ g L^{-1} محلول صفرایی سلول های آزاد افت ۵/۹۳ log CFU g^{-1} را نشان دادند، در حالی که برای سلول‌های ریزپوشانی شده در غلظت‌های ۱ ٪، ۵ ٪ و ۱۰ ٪ به ترتیب تنها ۳/۹۳ log CFU g^{-1}، ۳/۱۵ و ۱/۹ log CFU g^{-1}بود. نتایج نشان داد صمغ آمونیاکوم خواص انکپسوله کنندگی بسیار خوبی دارد که می‌توان از آن در بسیاری از فرمولاسیون‌های غذایی با این اهداف مورد استفاده قرار داد.

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