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Survival of *Lactobacillus Acidophilus* as Probiotic Bacteria using Chitosan Nanoparticles

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ABSTRACT

Chitosan was used for nanoencapsulation of *Lactobacillus acidophilus* as probiotic bacteria. *In vitro* experiments were conducted with the objective of investigating the survival of the bacterial cells in gastro-intestinal conditions. The results demonstrated that the size of chitosan nanoparticles noticeably increased by increasing chitosan concentration from 0.05 to 0.5 g/mL. Encapsulation of the cells caused a decrease at the leakage of probiotic bacteria when compared to free bacteria. However, the number of probiotic cells reduced from 3.27 to 3.23 log CFU/mL in gestural acid condition in contrast to free cells which they approximately dropped from 3.3 to 3 log CFU/mL after 120 min. Good probiotic viability and stability was also obtained by nanocapsulation of *Lactobacillus acidophilus* in intestinal juice. At this condition, the initial capsulated cells numbers were 3.28 log CFU/mL which after placing in biliary salt condition for 120 min, it reached to 3.23 log CFU/mL. On the other hand, the free bacteria cells reduced from 3.3 to 2.97 log CFU/mL in intestinal environment. Overall, nanocapsulation of probiotic bacteria plays a pivotal role in enhancing the viability and survival of them against gastro- intestinal environmental conditions.

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1. INTRODUCTION

In definition, probiotics are living microbial food supplements which after being received in adequate amounts can improve the intestinal microbial balance for the host [1]. There has been an increasing interest in the use of probiotic bacteria in food industries for human health. The ability of the organism to survive in the host and also in the products influences on the probiotic effects. The popularity of probiotic is constantly growing and various food products are marketed. It has been reported that probiotic intake has some advantages such as reduction in occurrence of diarrhea and number of colon cancer cells [2], alleviation of symptoms of lactose malabsorption and possible anticarcinogenic activity, controlling intestinal infection and serum cholesterol concentrations, nutritional enhancement and improvement in lactose intolerance [3].

Health benefits of probiotics may be because of prevention of their adhesion to the intestine, enrichment of the immune system, production of bacteriocins and competition with pathogens [4]. In order to provide a beneficial effect, a recommended minimum level for probiotics in food products is 10^6 to10^7 colony-forming units CFU/mL at the time of consumption [5, 6]. *Lactobacillus acidophilus* is one of the most effective forms of probiotic bacteria. Research has shown that it can be incorporated in fermented foods because of its beneficial effects [7]. Adaptation to the host intestinal environment and being viable and biologically active at the target site in the host is essential for *Lactobacillus acidophilus* as probiotics to be efficacious [8]. In addition, they must be remained stable throughout processing, storage and products shelf-life while delivering by food, particularly dairy products [9].

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Several factors influence on the colonization and survival of probiotic bacteria, including gastric pH, digestive enzymes and bile acids [10]. However, it must be noted that it is a big challenge that the bacteria may not survive in sufficient numbers in the gastrointestinal tract and traditional fermented dairy products [11]. Hence, providing probiotic living cells with a physical barrier against adverse environmental conditions is of vital importance which nowadays has received considerable interest [12]. Several approaches have attempted to increase the viability and stability of probiotic bacteria in the human gastrointestinal tract such as use of micronutrients and encapsulation process [13]. Encapsulation is the process of forming a constant coating around an inner matrix which is completely limited within the capsule wall as a core of encapsulated material [14]. It tends to maintain better cell viability and stability in spite of the acidity into the stomach, preventing their direct exposure to unfavorable environment, but permits diffusion of nutrients in and out of the matrix; encapsulation also stabilizes the cells. Encapsulation also potentially has a tendency to enhance the cells viability during production, storage and handling, protects the beneficial bacteria from destruction by stomach acid and provides controlled and constant delivery of cells in the gut. Encapsulation can be done with both forms of micro and nano [15]. Several researches have been done for microencapsulation of probiotic bacteria which include spray drying, emulsion, extrusion and phase separation [16]. However, study on nanocapsulation is a new research interest today.

Protecting probiotic bacteria against harsh environmental conditions such as freezing and those encountered during gastric juice passage is a critical action [17]. In recent years, development of a wide spectrum of nanometer range particles has been carried out by researchers. Nanocapsules are defined as vesicular systems in which probiotic bacteria are held in a cavity surrounded by a polymer film [18]. Small size and use of biodegradable materials are two main advantages of using nanoencapsulation for probiotic capsulation. Because of small size, probiotic bacteria have easy accessibility in the body and efficient uptake by a variety of cell types. They can also transport to inflammatory sites, epithelium (e.g., intestinal tract and liver), tumors, or penetrate micro capillaries [18]. However, some studies show that the number of capsules in nano size pass through intestinal epithelium are more than capsules in micro size [19]. Another benefit of using nanocapsule is the direct and continuous delivery of probiotic bacteria to the gut. They have been found to have prolonged circulation in blood as well as targeting of the cells to specific sites. Therefore, viable amount of probiotic bacteria grant beneficial effects onto the host [14].

Probiotic encapsulation is an exciting approach in biopharmacy and food industry which is designed to increase viability of bacterial living cells in the intestinal tract by giving protection to the sensitive bacterial microbes. As mentioned before, this technology will be more effective if being carried out in nano form instead of micro form.

Some factors must be noted for selecting biomaterials to encapsulate probiotics which are (a) physicochemical properties, (b) toxicology test (c), manufacturing and sterilization processes [14]. Among these parameters, the choice of the biomaterial and the physico-chemical properties of the capsules are crucial because it determines the encapsulation efficiency and the viability and stability of capsule.

Natural and synthetic polymers can be used for probiotics encapsulation [14]. These biomaterials must be biodegradable and biocompatible because they are in straight contact with the living cells. Several studies reported for encapsulation of probiotic bacteria are based on alginates [20], cellulose acetate phthalate, whey protein, gelatin and starch, vegetable gum and kappa-carrageenan [14, 21].

Chitosan is an interesting natural polysaccharide which has received much attention for use in pharmaceutical research and in industry as a carrier for drug delivery and as a biomedical material [22-24]. It is an appropriate candidate for encapsulating probiotics due to its simplicity, non-toxicity, coagulation ability, soft tissue–compatibility, immune stimulating activity and low cost [25].

It is also an excellent biocompatible, biodegradable and bioadhesive material. Chitosan has a long shelf-life and can be obtained by a very mild ionotropic gelation procedure [26]. It can be isolated by partial deacetylation of chitin from crustacean shells, the membranes of fungi and insect cuticles [4]. It has a positive charge and the primary amino groups which provide special properties for it. The distribution of free amino and N-acetyl groups makes it soluble in acidic pH. However, chitosan is water insoluble at a pH higher than 5.4. In addition, these free amine groups which are available for cross-linking lead to cationic nature allowing for ionic cross-linking with multivalent anions [24]. Considering these properties, chitosan seems to have a notable potential in the development of probiotics encapsulation. However, in the literature, the studies in which researchers discuss on nanocapsulation of probiotics are rare [22, 23].

Regarding the advantages of using nanocapsuls, the present research is focused on nanocapsulation of *Lactobacillus acidophilus* as probiotic cells by chitosan. The effect of various chitosan concentrations on the size of formed capsules was examined. The probiotic bacteria loading was also evaluated by considering the chitosan concentration. Finally, the viability of probiotic

cells in simulated stomach and intestinal conditions was studied.

2. MATERIALS AND METHODES

2. 1. Materials Chitosan from shrimp shell was purchased from Sigma (St. Louis, USA). Deacetyl degree was a minimum of 85%. Tripolyphosphate sodium (TPP) was also purchased from Sigma. Acetic acid and cultured MRS environment were provided from Merck (Darmstadt, Germany). *Lactobacillus acidophilus* (1643 PTCC) was obtained in lyophilized form from Iranian Research Organization for Science and Technology (IROST). All other reagents and solvents were of analytical grade and used without further purification.

2. 2. Preparation of Probiotics Freeze-dried bacterial cells were added to 5mL MRS broth and incubated at 37 °C for 24h under aerobic condition. Then, the cultures were transferred into 95 ml MRS broth and incubated under the same conditions. The cells were harvested by centrifuging at 4500 rpm for 5 min at 4 °C. After that, the cells were washed twice with sterile solution of peptone (0.1%). In order to dilute bacteria, they were kept in saline solution. For avoiding of bacteria precipitation in normal saline solution and for minimizing the size of solved bacteria, the solution containing the bacteria was sonicated for 15 min. Tween 80 was also added to the solution for better homogenization [20].

2. 3. Encapsulation Process of Probiotics Chitosan nanoparticles were prepared with ionic gelation of chitosan with tripolyphosphate (TPP) anions [22, 27]. Briefly, different concentrations of chitosan (0.05, 0.1, 0.2, 0.3, 0.4 and 0.5 mg/mL) were dissolved in acetic acid solution. A solution of TPP at 0.1 mg/mL was also prepared. Then, 10 mL of TPP solution was added dropwise under constant stirring to 20 mL chitosan solution. A milky color suspension was formed spontaneously at the mentioned condition which indicated the preparation of chitosan nanoparticles. The probiotic cells loaded nanoparticles were also prepared with incorporation of TPP solution into chitosan solution containing approximately 1 mL of bacteria diluted in previous step with normal saline [26]. Briefly, bacterial solution and a constant volume of saline normal (1 mL) were mixed with chitosan solution under sonication to reduce the size of bacteria for being encapsulated in nanoparticle. After stirring of the solution about 2 h, TPP solution was added to the mixture. The effect of different parameters such as chitosan concentration on encapsulation and loading efficiency was evaluated. On the other hand, viability of probiotic bacteria in simulated gastro- intestinal system was evaluated.

2. 4. Survival Assay and Numeration of Encapsulated Bacteria For determining the bacteria viability, approximately 1 g of produced probiotic-loaded nanoparticles was dispersed in 99 mL of sodium citrate 0.01 w/v (pH=6) for 1 min by a magnetic agitator. Then, the solution was kept at room temperature for 10 min to destroy the capsule's wall. Consequently, the bacterial cells were released in medium for enumerating. The samples were diluted to obtain appropriate concentrations of bacteria and were plated in MRS agar environment. After that, the plates were incubated 72 h at 37 °C in aerobic condition. Finally, the encapsulated bacteria which were viable were enumerated as CFU/mL.

2. 5. Morphology and Size of Nanoparticles Scanning electron microscopy (SEM, Phillips XL30, Holland) was used to provide morphological information on chitosan nanoparticle. The average particle size were determined by Zetasizer (Malvern Instruments, UK), based on the dynamic light scattering (DLS) technique.

2. 6. Selecting the in Vitro Conditions for Cells Release It is crucial to take into account two conditions when probiotics are encapsulated. First, the protective material of probiotics must be reliable in media which simulate the gastric fluid, and then it should be confirmed that the encapsulated probiotic bacteria are released in media which simulate the intestinal fluid. In the literature, there is no standard protocol for establishing the *in vitro* conditions to simulate the stomach or the intestinal [14]. Various factors must be considered to simulate the stomach or the intestinal condition to reflect reality as much as possible in humans. Some of these factors are type of medium and its composition, exposure time and pH values.

2. 6. 1. Simulation of the Stomach Conditions According to Pinto et al. [28], an electrolyte solution containing 6.2 g/L NaCl, 2.2 g/L KCl, 0.22 g/L $CaCl_2$ and 1.2 g/L $NaHCO_3$ (pH 2.5) was used to simulate artificial stomach solution. Lysozyme was added to a final concentration of 100 ppm. Then, after adding 0.3% pepsin (Fluka Biochemika, Steinheim, Germany), the solution was incubated for 5 min at 37 °C. For doing experiments, the capsulated probiotic bacteria were placed in the prepared artificial stomach solution. Sodium citrate (1%) was also used to solve the cell wall during agitation. Enumerating of probiotic bacteria was done using MRS agar cultural environment at 37 °C.

2. 6. 2. Simulation of the Intestinal Conditions The artificial intestinal syrup was simulated according to Pinto et al. [28]. The solution contained 6.4 g/L $NaHCO_3$, 0.239 g/L KCl, 1.28 g/L NaCl, 0.5% bile salts (Oxgall, Merck, Darmstadt, Germany) and 0.1% pancreatin (Fluka Biochemika, Steinheim, Germany) [29]. The solution pH was adjusted to 7.2. Then, the capsulated bacteria were transferred to solution. After solving the cell wall during agitation, the samples were spread-plated onto MRS agar to determine the CFU/mL.

2. 7. Statistical Analysis Statistical analysis was done using the SPSS version 16. A one-way analysis of variance (ANOVA) was applied to establish the significance of differences in the chitosan concentrations. The significance level was 5%.

3. RESULTS and DISCUSSION

3. 1. Determination of Nanoparticle Size at Various Chitosan Concentrations The effect of various chitosan concentrations on the size of formed nanoparticles is depicted in Figure 1. Various chitosan concentrations of 0.05, 0.1, 0.2, 0.3 mg/mL with 0.1 % TPP were considered. The minimum particle size was achieved at the chitosan concentration of 0.05 mg/ml. In addition, an increase at chitosan concentration resulted in the larger particle size. Therefore, it was revealed that the chitosan concentration affected the formation of nanoparticles.

According to Figure 1, adding a constant volume of TPP to the chitosan solution resulted in nanoparticles formation ranging from 120 to 338 nm. Considering the results obtained from Zetasizer instrument, there was a meaningful difference in the size of nanoparticles produced at various chitosan concentrations. In the research carried out by Chandramouli et al. [30], chitosan nanoparticles ranging from 100 to 250 nm were produced at various chitosan and TPP concentrations using gelation process. In their study, the weight ratio of chitosan to TPP and also the effect of solution pH were evaluated. They showed that the size of nanoparticles increased with an increase in chitosan concentrations.

3. 2. Determination of the Size of Probiotic Bacteria-loaded Nanoparticles at Various Chitosan Concentrations Bacterial solution and a constant volume of saline normal (1mL) were mixed with 30 mL of chitosan solution under sonication. Chitosan concentrations were considered as 0.05, 0.1, 0.2, 0.3, 0.4, 0.5 mg/mL. After stirring the solution for about 2 h, TPP solution was added to the mixture. Figure 2 shows that the size of bacteria-loaded nanoparticles dramatically increases by increasing the chitosan concentration. The results obtained by Zetasizer also revealed that the bacteria-loaded nanoparticles are larger than the chitosan-TPP ones, possibly due to larger size of the probiotic bacteria. In addition, a study was done by Mohammadpour Doniqi et al. [23] in which the effect of different factors on encapsulation process was evaluated. In their research, the cationic nanoparticles with gelation ionic method from chitosan and TPP were produced. The experimental results showed that an increase in chitosan concentrations to 0.5 mg/mL resulted in an increase in size of bacteria- loaded nanoparticles.

3. 3. Evaluating of SEM for Chitosan Nanoparticles Figure 2 shows the SEM image of the smallest particle achieved with chitosan concentration of 0.05%. As seen, spherical, distinct and regular shape of particle was obtained through SEM image. It reveals that the obtained nanoparticle has a size of about 120 nm and is pure. In the study done by Hasanzadeh Kafshgari et al. [31], chitosan nanoparticles consisting of hydropropyle cyclodexterin were prepared by ionic gelation method.

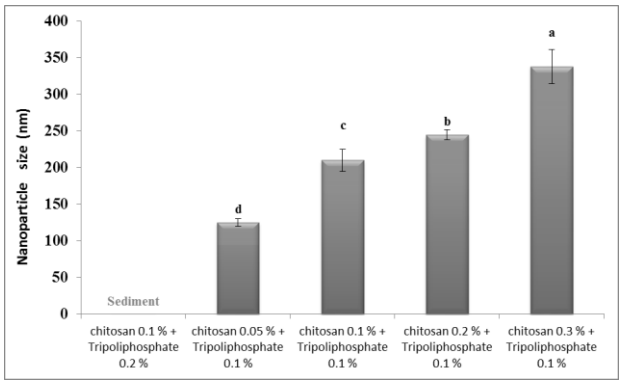


Figure1. Average size of nanoparticles formed regarding to various chitosan concentrations. (Values of columns indicated by different letters are significantly different (p>0.05))

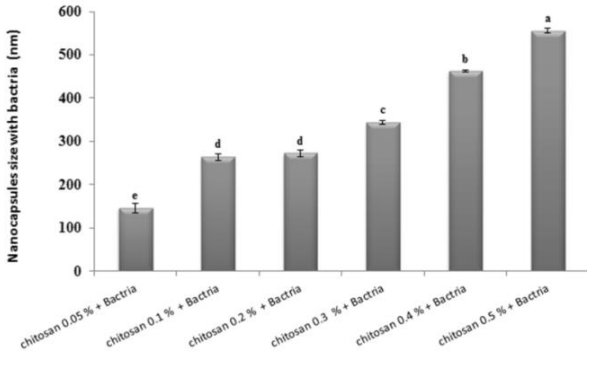


Figure 2. Average size of bacteria-loaded nanoparticles at various chitosan concentrations (Values of columns indicated by different letters are significantly different (p>0.05))

In their research, they showed that the produced nanoparticles had symmetric and spherical shape.

3. 4. Determination of Probiotic Loading on Chitosan Nanoparticles The effect of chitosan concentration on probiotic loading on nanoparticle was examined. The obtained results (Figure 4) revealed that by increasing the chitosan concentrations, the amount of bacteria encapsulated in chitosan decreased. The highest bacterial cell loading (3.278 Log CFU/mL) was achieved for nanoparticles with chitosan concentration of 0.05 mg/mL. It has been described that lower concentration of chitosan causes a decrease in the liquid phase resistance against dispersion, resulting in smaller nanoparticles and further stimulating probiotic encapsulation [32]. As described in section 2.4, the enumeration of cell numbers was done in triplicate for each sample and then, the average values were presented. Figure 5 (a)-(c) show the probiotic loading on nanoparticles with chitosan concentrations of 0.05, 0.1 and 0.2 mg/mL and nanocapsule size of 146, 264.5 and 272.4 nm, respectively.

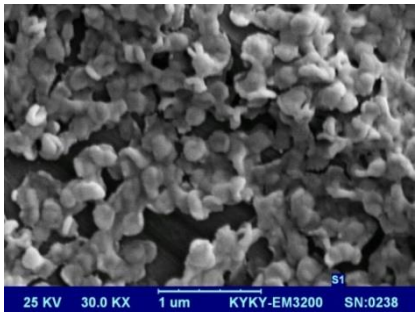


Figure 3. SEM image of the smallest obtained nanoparticles (0.05 mg/mL chitosan)

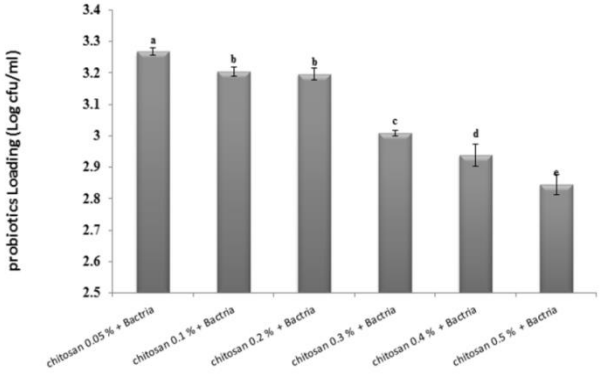
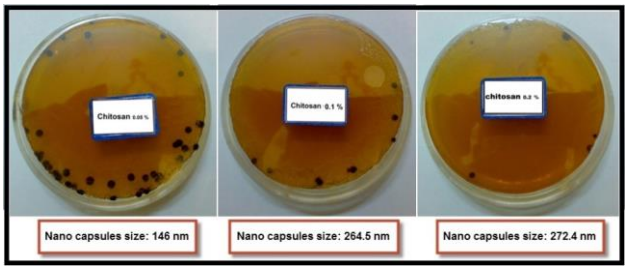


Figure 4. The logarithmic mean of probiotic loading on nanochitosan with respect to various chitosan concentrations (Values of columns indicated by different letters are significantly different ($p>0.05$))



(1) (2) (3)

Figure 5. Size and probiotic-loading for nanoparticles with chitosan concentrations of (1) 0.05 mg/ml, (2) 0.1 mg/ml and (3) 0.2 mg/ml

In the study carried out by Wu et al. [33], the ammonium glycyrrhizinate-loaded chitosan nanocapsules were prepared by ionic gelation of chitosan with TPP. The effect of chitosan concentration on the physicochemical properties of nanocapsules was evaluated. The results showed that an increase in glycyrrhizin ammonium and chitosan concentration resulted in reduction on encapsulation efficiency.

3. 5. Survival of Free and Encapsulated *Lactobacillus acidophilus* in Gestural Acid Conditions Survival of free and encapsulated cells of probiotic bacteria in acidic stomach condition (pH =2) is depicted in Table 1. The viability of cells was monitored at time duration of 0 to 120 min with a time interval of 30 min.

The results reported for free and capsulated bacteria revealed that reduction on logarithmic mean of free cells is more significant in contrast with the capsulated bacteria. In gastric condition, the initial cell number of free bacteria was 3.3 Log CFU/mL. However, it has a sharp reduction during 90 min. The survived free bacteria after 90 min were approximately 3.1 Log CFU/mL. The number of free cells after passing 120 min of encapsulation process reaches to 3 Log CFU/mL. In contrast, the cell number of encapsulated bacteria varied from 3.27 to 3.23 CFU/mL during 120 min. Consequently, encapsulation process increases the viability of bacteria in the gastric acid condition. The same results were obtained by Chandramouli et al. [30]. It was shown that only capsulated probiotics can live in gastric conditions. In addition, Zanjani et al. [15] found that surviving speed of *Bifidobacteria* in alginate particles containing chitosan was more than those in alginate particles.

3. 6. Survival of Free and Encapsulated *Lactobacillus acidophilus* in Intestinal Conditions The survival of the free and encapsulated probiotic cells in intestinal syrup was evaluated. The experiments were conducted in intestinal condition at time duration of 0 to 120 min with interval of 30 min (Table 2).

TABLE 1. Survival of the free and encapsulated cells versus time (chitosan concentration of 0.05 mg/mL) in the acidic conditions of the stomach

Test	Time (min)				
	0	30	60	90	120
Free cells (Log CFU/mL)	3.30 ± 0.011^{a}	3.267 ± 0.012^{b}	3.204 ± 0.013^{c}	3.11 ± 0.017^{d}	3.097 ± 0.017^{d}
Encapsulated cells (Log CFU/mL)	3.278 ± 0.01^{a}	3.269 ± 0.02^{ab}	3.255 ± 0.02^{b}	3.230 ± 0.03^{c}	3.230 ± 0.013^{c}

Values in each column with the same letter did not differ significantly ($P < 0.05$)

TABLE 2. Logarithmic mean of free and encapsulated cells at various times in the stomach-intestinal bile salt

Test	Time (min)				
	0	30	60	90	120
Free cells (Log CFU/mL)	3.30 ± 0.011^{a}	3.24 ± 0.012^{b}	3.211 ± 0.012^{bc}	3.097 ± 0.013^{d}	2.977 ± 0.012^{d}
Encapsulated cells (Log CFU/mL)	3.278 ± 0.01^{a}	3.242 ± 0.012^{b}	3.230 ± 0.02^{c}	3.217 ± 0.017^{d}	3.230 ± 0.022^{c}

Values in each column with the same letter did not differ significantly ($P < 0.05$)

The number of free cells remarkably decreased from 3.3 to 2.97 Log CFU/mL during 120 min. In contrast, the number of capsulated cells had no significant reduction during the tested time. At first the number of capsulated cells was 3.28 Log CFU/mL which after placing in biliary salt condition for 120 min, it reached to 3.23 Log CFU/mL. Capsulation of probiotic bacteria with chitosan was able to improve the resistance of cells in intestinal environment; hence, increased the life time of cells. These results are in agreement with the findings of Zhao et al. [34] who proved that capsulation with chitosan is the best protection for biliary salt solution. They stated that it is an ionic balance reaction in attracting biliary salt by particles; so, the spread of biliary salt in particles can be limited. Thus, capsulated probiotics are protected in reaction to biliary salt.

4. CONCLUSION

The present research showed that encapsulation of *Lactobacillus acidophilus* as probiotic bacteria using chitosan nanoparticles enhance its stability and viability. In addition, encapsulation considerably improves the bacterial survival in simulated gastric and intestinal environment. The results showed that an increase at chitosan concentrations resulted to the larger size of nanoparticles. The survival of encapsulated probiotic bacteria was also higher than that of free cells. The results obtained by *in vitro* experiments demonstrated that chitosan is a good material for probiotics encapsulation in stomach and biliary salts. In conclusion, nanoencapsulation can be considered to be a promising outlook for introducing of viable probiotic bacteria in foods and maintaining their survival during simulated gastric and intestinal juice.

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6. REFERENCES

1. Galdeano, C.M. and Perdigon, G., "Role of viability of probiotic strains in their persistence in the gut and in mucosal immune stimulation", ***Journal of Applied Microbiology***, Vol. 97, No. 4, (2004), 673-681.
2. Fitton, N. and Thomas, J.S., "Gastrointestinal dysfunction", ***Surgery (Oxford)***, Vol. 27, No. 11, (2009), 492-495.
3. Sanders, M.E., "Considerations for use of probiotic bacteria to modulate human health", ***The Journal of Nutrition***, Vol. 130, No. 2, (2000), 384S-390S.
4. Vivek, K., "Use of encapsulated probiotics in dairy based foods", ***International Journal of Food, Agriculture and Veterinary Sciences***, Vol. 3, No. 1, (2013), 188-199.
5. Mortazavian, A., Razavi, S.H., Ehsani, M.R. and Sohrabvandi, S., "Principles and methods of microencapsulation of probiotic microorganisms", ***Iranian Journal of Biotechnology***, Vol. 5, No. 1, (2007), 1-18.
6. Shah, N.P., "Functional cultures and health benefits", ***International Dairy Journal***, Vol. 17, No. 11, (2007), 1262-1277.
7. Saranya, S. and Hemashenpagam, N., "Antagonistic activity and antibiotic sensitivity of lactic acid bacteria from fermented dairy products", ***Advances in Applied Science Research***, Vol. 2, No. 4, (2011), 528-534.
8. Conway, P.L., "Selection criteria for probiotic microorganisms", ***Asia Pacific Journal of Clinical Nutrition***, Vol. 5, No., (1996), 10-14.
9. Ross, R., Fitzgerald, G., Collins, K. and Stanton, C., "Cheese delivering biocultures--probiotic cheese", ***Australian Journal of Dairy Technology***, Vol. 57, No. 2, (2002), 71.

10. Reid, G., Sanders, M., Gaskins, H.R., Gibson, G.R., Mercenier, A., Rastall, R., Roberfroid, M., Rowland, I., Cherbut, C. and Klaenhammer, T.R., "New scientific paradigms for probiotics and prebiotics", ***Journal of Clinical Gastroenterology***, Vol. 37, No. 2, (2003), 105-118.
11. Lourens-Hattingh, A. and Viljoen, B.C., "Yogurt as probiotic carrier food", ***International Dairy Journal***, Vol. 11, No. 1, (2001), 1-17.
12. Favaro-Trindade, C., Heinemann, R. and Pedroso, D., "Developments in probiotic encapsulation", ***CAB Rev***, Vol. 6, (2011), 1-8.
13. Chen, M.J. and Chen, K.N., "Applications of probiotic encapsulation in dairy products", ***Encapsulation and Controlled Release Technologies in Food Systems***, (2007), 83-112.
14. Gbassi, G.K. and Vandamme, T., "Probiotic encapsulation technology: From microencapsulation to release into the gut", ***Pharmaceutics***, Vol. 4, No. 1, (2012), 149-163.
15. Zanjani, M.A.K., Tarzi, B.G., Sharifan, A. and Mohammadi, N., "Microencapsulation of probiotics by calcium alginate-gelatinized starch with chitosan coating and evaluation of survival in simulated human gastro-intestinal condition", ***Iranian Journal of Pharmaceutical Research: IJPR***, Vol. 13, No. 3, (2014), 843.
16. Sultana, K., Godward, G., Reynolds, N., Arumugaswamy, R., Peiris, P. and Kailasapathy, K., "Encapsulation of probiotic bacteria with alginate–starch and evaluation of survival in simulated gastrointestinal conditions and in yoghurt", ***International Journal of Food Microbiology***, Vol. 62, No. 1, (2000), 47-55.
17. Boh, B., "Developements et applications industrielles des microcapsules", ***Microencapsulation: Des Sciences Aux Technologies. Paris: Lavoisier***, (2007), 9-22.
18. Singh, R. and Lillard, J.W., "Nanoparticle-based targeted drug delivery", ***Experimental and Molecular Pathology***, Vol. 86, No. 3, (2009), 215-223.
19. Desai, M.P., Labhasetwar, V., Amidon, G.L. and Levy, R.J., "Gastrointestinal uptake of biodegradable microparticles: Effect of particle size", ***Pharmaceutical Research***, Vol. 13, No. 12, (1996), 1838-1845.
20. Yeung, T.W., Üçok, E.F., Tiani, K.A., McClements, D.J. and Sela, D.A., "Microencapsulation in alginate and chitosan microgels to enhance viability of bifidobacterium longum for oral delivery", ***Frontiers in Microbiology***, Vol. 7, (2016).
21. Anal, A.K. and Singh, H., "Recent advances in microencapsulation of probiotics for industrial applications and targeted delivery", ***Trends in Food Science & Technology***, Vol. 18, No. 5, (2007), 240-251.
22. Kouchak, M. and Azarpanah, A., "Preparation and in vitro evaluation of chitosan nanoparticles containing diclofenac using the ion-gelation method", ***Jundishapur Journal of Natural Pharmaceutical Products***, Vol. 10, No. 2, (2015).
23. Mohammadpour Dounighi, N., Eskandari, R., Avadi, M., Zolfagharian, H., Mir Mohammad Sadeghi, A. and Rezayat, M., "Preparation and in vitro characterization of chitosan nanoparticles containing mesobuthus eupeus scorpion venom as an antigen delivery system", ***Journal of Venomous Animals and Toxins Including Tropical Diseases***, Vol. 18, No. 1, (2012), 44-52.
24. Gilania, S., Najafpour, G., Moghadamniab, A. and Kamaruddinc, A., "Kinetics and isotherm studies of the immobilized lipase on chitosan support".
25. Kim, J., Muhammad, N., Jhun, B.H. and Yoo, J.-W., "Probiotic delivery systems: A brief overview", ***Journal of Pharmaceutical Investigation***, Vol. 46, No. 4, (2016), 377-386.
26. Mohammadpourdounighi, N., Behfar, A., Ezabadi, A., Zolfagharian, H. and Heydari, M., "Preparation of chitosan nanoparticles containing naja naja oxiana snake venom", ***Nanomedicine: Nanotechnology, Biology and Medicine***, Vol. 6, No. 1, (2010), 137-143.
27. Werle, M., Takeuchi, H. and Bernkop-Schnürch, A., "Modified chitosans for oral drug delivery", ***Journal of Pharmaceutical Sciences***, Vol. 98, No. 5, (2009), 1643-1656.
28. Pinto, M.G.V., Franz, C.M., Schillinger, U. and Holzapfel, W.H., "Lactobacillus spp. With in vitro probiotic properties from human faeces and traditional fermented products", ***International Journal of Food Microbiology***, Vol. 109, No. 3, (2006), 205-214.
29. Fernandez, M., Boris, S. and Barbes, C., "Probiotic properties of human lactobacilli strains to be used in the gastrointestinal tract", ***Journal of Applied Microbiology***, Vol. 94, No. 3, (2003), 449-455.
30. Chandramouli, V., Kailasapathy, K., Peiris, P. and Jones, M., "An improved method of microencapsulation and its evaluation to protect lactobacillus spp. In simulated gastric conditions", ***Journal of Microbiological Methods***, Vol. 56, No. 1, (2004), 27-35.
31. Kafshgari, M.H., Khorram, M., Khodadoost, M. and Khavari, S., "Reinforcement of chitosan nanoparticles obtained by an ionic cross-linking process", ***Iran Polym J***, Vol. 20, No. 5, (2011), 445-456.
32. Zhang, H.-l., Wu, S.-h., Tao, Y., Zang, L.-q. and Su, Z.-q., "Preparation and characterization of water-soluble chitosan nanoparticles as protein delivery system", ***Journal of Nanomaterials***, Vol. 2010, (2010), 1.
33. Wu, Y., Yang, W., Wang, C., Hu, J. and Fu, S., "Chitosan nanoparticles as a novel delivery system for ammonium glycyrrhizinate", ***International Journal of Pharmaceutics***, Vol. 295, No. 1, (2005), 235-245.
34. Zhao, L.-M., Shi, L.-E., Zhang, Z.-L., Chen, J.-M., Shi, D.-D., Yang, J. and Tang, Z.-X., "Preparation and application of chitosan nanoparticles and nanofibers", ***Brazilian Journal of Chemical Engineering***, Vol. 28, No. 3, (2011), 353-362.

Survival of *Lactobacillus Acidophilus* as Probiotic Bacteria using Chitosan Nanoparticles

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چکیده

کیتوزان برای نانوکپسولاسیون لاکتوباسیلوس اسیدوفیلوس به عنوان باکتری های پروبیوتیک استفاده شد. آزمایش های داخل آزمایشگاهی با هدف بررسی بقای سلول های باکتری ها در شرایط دستگاه گوارش انجام شد. نتایج نشان داد که اندازه نانوذرات کیتوزان به طرز محسوسی با افزایش غلظت کیتوزان از ۰/۰۵ تا ۰/۵ گرم / میلی لیتر افزایش می یابد. انکپسولاسیون سلول ها باعث کاهش در کمبود باکتری های پروبیوتیک در مقایسه با باکتری های آزاد شد. با این حال، تعداد سلول های پروبیوتیک از ۳/۲۷ تا ۳/۲۳ log CFU/mL در شرایط اسید اشاری در مقایسه با سلول های آزاد کاهش می یابد که آنها تقریبا از ۳/۳ تا ۳ log CFU/mL پس از ۱۲۰ دقیقه کاهش می یابند. زنده ماندن و ثبات خوب پروبیوتیک نیز با استفاده از نانوکپسولاسیون لاکتوباسیلوس اسیدوفیلوس در شیره روده به دست آمد. در این شرایط، تعداد اولیه سلول های کپسوله شده ۳/۲۸ log CFU/mL است که پس از قرار گرفتن در شرایط نمک صفراوی برای ۱۲۰ دقیقه، آن به ۳/۲ log CFU/mL رسید. از سوی دیگر، سلول های باکتری آزاد از ۳/۳ تا ۲/۹۷ log CFU/mL در محیط روده کاهش یافت. به طور کلی، نانوکپسولاسیون باکتری های پروبیوتیک، نقش اساسی در افزایش دوام و بقای آنها در برابر شرایط محیطی گوارشی ایفا می کند.

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