Survival of alginate-prebiotic microencapsulated *Lactobacillus acidophilus* in mayonnaise sauce

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Abstract:

BACKGROUND: Nowadays, Microencapsulation of probiotic bacteria is the newest method for increasing the survival ability of probiotics in Food products. The *Lactobacillus acidophilus* as a probiotic bacterium has more beneficial effects and the low survival of this bacterium under food processing conditions and adverse environment have been studied by many researchers. **OBJECTIVES:** In this study, *Lactobacillus acidophilus* was added to mayonnaise sauce as either free cells or encapsulated and the survival of probiotic was evaluated during storage for 30 days at 4^{o}C. **METHODS:** The emulsion method was performed for the microencapsulation process of *Lactobacillus acidophilus*. A morphological characteristic of capsules was indicated by optical microscope and scanning electron microscope. **RESULTS:** The microencapsulation with calcium alginate-resistant starch significantly affected ($p<0.05$) the survival of *Lactobacillus acidophilus* compared to free state in mayonnaise sauce. No differences were detectable in the morphological of capsules with resistant starch by scanning electron microscope and Sensory properties of mayonnaise sauce were improved by the addition of encapsulated *Lactobacillus acidophilus*. **CONCLUSIONS:** The microencapsulation significantly increased the survival of *Lactobacillus acidophilus*.

Introduction

The probiotics are defined as "viable bacteria that show a highly beneficial effect on the health of the host upon ingestion by improving the properties of its indigenous microflora" (Rokka and Rantamäki, 2010). Because of their healthy propertiesthere has been an increased interest in the role of probiotic bacteria as a functional food in human health, and probiotics are increasingly incorporated in non-dairy food products (Holzapfel and Schillinger, 2002). The microorganisms most frequently used are the lactic acid bacteria (lactobacilli and bifidobacteria) as they occupy a central role in the gut flora (Rolfe, 2000). As the lactic acid bacteria play an important role in the human intestinal flora, it will more easily colonise the gut (Ishibashi and Shimamura, 1993). Consequently, it is important to know what their properties are to increase their effects on health and improve the manufacture of the food probiotic products (Andersson et al., 2001).

Prebiotics stimulate the growth of probiotics (Klaenhammer et al., 2012). The encapsulation of both prebiotics and probiotics may enhance the probiotics' survival to hard conditions (Chavarri et al., 2010). Thus, it has been proven that the addition of Hi-Maize starch increased the viability of probiotics, and the addition of prebiotics improved the probiotics' resistance when inoculated to the functional food (Sultana et al., 2000).

International Dairy Federation require that products claimed to be 'probiotic products' contain a minimum of 107 viable probiotic bacteria per gram of product (Ahmadi et al., 2012). However, many products fail to meet these standards when they are consumed. This is due to death of probiotics cells in food products during storage, low pH and water activity (Fahimdanesh et al, 2012; Zanjani et al., 2012). Consequently the microencapsulation process ensuring the stability of probiotics in foods remains strong, which leads to the development of immobilized cell technology to produce probiotics with increased cell resistance to environmental stress factors (Doleyres and Lacroix, 2005).

Encapsulation of probiotics may be defined as a process to entrap bacteria within hydrocolloids, thereby producing particles with diameters of a few micrometers to a few millimeters (Kailasapathy, 2002; Chandramouli et al., 2004). The carrier material of encapsulates used in food products or processes should be food grade and able to form a barrier for the active agent and its surroundings (Zuidam and Shimoni, 2010). Alginate is the most widely used encapsulation matrix for various food-grade and non-food compounds (Wang et al., 1999). Alginates are naturally derived linear copolymers of 1, 4-linked D-mannuronic acid (M) and L-guluronic acid (G) residues (Webber and Shull, 2004). Extrusion and emulsion techniques can be applied to generate calcium alginate microspheres. Low toxicity, good biocompatibility, and controllable biodegradability, coupled with wide applicability, make alginate a good candidate not only for conventional and novel drug delivery systems but also as a biologically active agent (Prabaharan and Mano, 2005).

In the emulsion technique, a small volume of cell-polymer suspension (discontinuous phase) is added to a large volume of a vegetable oil (continuous phase) (Krasaekoopt et al., 2003). The relative viability of the encapsulated microbial depends on operating parameters, such as inner phase volume ratio and the median diameter of the oil droplets (Manojlovic et al., 2010).

Probiotics have been inoculated to various food products such as iranian white brined cheese (Mirzaei et al., 2012), yoghurt and freeze-dried yoghurt (Capela et al., 2006), ice cream (Cruz et al., 2009), frozen desserts (Sheu et al., 1993), and dark chocolates (Nebesny et al., 2006). Supplementation of mayonnaise sauce with probiotics micro-organisms, such as *Lactobacillus acidophilus* may provide additional health benefits. (Fahimdanesh et al., 2012); however, there is no report in the literature on the mayonnaise sauce containing micro-encapsulated *Lactobacillus acidophilus* and resistant starch as a filler material. The objective of this study was to determine the viability of free and encapsulated *Lactobacillus acidophilus* cells during storage of product at 4°C for a period of 30 days.

Materials and methods

Preparation of *Lactobacillus acidophilus*: *Lactobacillus acidophilus* PTCC1643 (Persian Type Collection Culture) was obtained from Iran Scientific and Industrial Organization. The cultures were inoculated in MRS broth (de Man-Rogasa-Sharpe) for 48 h under anaerobic conditions at 37°C, and the probiotic biomass in late-log phase was harvested by centrifuging at 3500 rpm for 15 min at 4 ^{o}C. The cultures were then washed twice by sterile saline solution (0.9 %) and used in the microencapsulation process.

Microencapsulation of *Lactobacillus acidophilus*: All glassware and solutions used in the protocols were sterilized at 121°C for 15 min. Alginate-starch microcapsules were produced using a modified encapsulation method reported by Fahimdanesh et al. (2012) and Zanjani et al. (2012). A 2% sodium alginate (Sigma, 71238) mixture in distilled water was prepared containing 2% resistant starch (Hi-maize 260 national starch UK) and 0.1% probiotic cultures were transferred into the solution and stirred for 10 min. The mixture was added to 200 mL vegetable oil containing 0.2% tween 80 and mixed (400 rpm for 20 min, Heydolph Stirrer, Germany) until it was fully emulsified. Alginate-starch microcapsules were prepared by adding 200 mL calcium chloride 0.1 M into a mixture. The mixture was allowed to stand 30 min to separate prepared calcium alginate microcapsules in the bottom of the beaker at the calcium chloride layer. The oil layer was drained and microcapsules were collected by washing with 0.9% saline and stored at 4^{o}C.

Preparation of mayonnaise sauce: Mayonnaise sauces were procured from a local supplier (Mahram Industries Co., Qazvin, Iran). The *Lactobacillus acidophilus* cultures were added as free or encapsulated cultures and stored for 30 days.

Size and shape of capsules: The size and shape of microcapsules were measured by optical microscopy (Master sizer Malvern 2000 UK) and scanning electron microscope (SEM), respectively. The diameters of 110 randomly selected microcapsules were determined by using measurement software (Leica Qwin 550). In this study, observations were made using the scanning electron microscope (LEO 440 I, England) at an accelerating voltage of 15 kV.

Release of entrapped bacteria: Freshly prepared, the microcapsules containing probiotic bacteria (1 g) were liquefied in 9 mL phosphate buffer (0.1 M) solution at pH 7 by gently shaking at room temperature for 15 min. *L. acidophilus* was enumerated on MRS agar (Merck, KGaA Germany) in triplicate.

Determination of pH: The pH value of Mayonnaise sauce samples was measured using a Digital pH-meter (744, Metrohm, Switzerland). The pH value was determined according to the standard method of the Institute of Standard and Industrial Research of Iran (ISIRI number 2454, 2001).

Sensory evaluation: Sensory evaluation of mayonnaise sauce was carried out by a trained panel of 20 judges and the samples were evaluated using a sensory rating of 1-5 for the Color and 1-5 for Texture and 1-10 for Taste (Fahimdanesh et al., 2012). For each one of these attributes, the average response was reported.

Statistical analysis: All data were presented as mean values of at least three replicates. Statistical analysis of the results was carried out by SPSS 20 (SPSS Inc., Chicago, IL) software. Data were analyzed by analysis of variance (ANOVA) using the General Linear Model (GLM) procedure. Sensory evaluations were analyzed by means of the non-parametric Friedman test; significance was declared at $p<0.05$.

Results

Optical microscopy and scanning electron microscope showed that the microcapsules were globular in shape (Figures 1,2) and starch particle around the capsules was detectable.

pH changes: The pH changes in the control and experimental mayonnaise sauce during storage at 4^{o}C for a period of 30 days is shown in Table 1.

Survival of free and encapsulated *Lactobacillus acidophilus*: Graph 1 shows the bacterial counts (*L.acidophilus*) in mayonnaise sauce. In the case of free *L.acidophilus*, the cell numbers dropped substantially (about 5 log numbers) by 30 days of storage at 4^{o}C, while the encapsulated *L.acidophilus* showed a decrease of 3 log after 30 days.

Sensory evaluations: The sensory scores of the mayonnaise sauce samples are given in Table 2.

Discussion

Physical properties of the microcapsules: All capsules were spherical in shape (Figures 1,2). However, no significant difference in capsule shapes was observed. This finding is in agreement with Fahimdanesh et al. (2012) who reported that physical properties of the microcapsules were spherical. The mean value of microcapsules was 180 μm.

Table 1. pH changes. (a)Control mayonnaise sauce without probiotic.

Storage (days)	Control[a]	Mayonnaise sauce with *L.acidophilus*	
		free	encapsulated
0	4.1	4.1	4.1
10	4.07	4.05	4.08
20	4.04	4.05	4.05
30	4.02	4.02	4.02

Table 2. Sensory properties. A: Mayonnaise sauce with free *L.acidophilus*, B: Mayonnaise sauce with encapsulated *L.acidophilus*, C: Mayonnaise sauce without probiotic (control).

Samples	Color (1-5)	Body (1-5)	Taste (1-10)	Total acceptability (1-20)
A	3.70	4.05	8.05	15.80
B	4.75	4.60	8.35	17.7
C	3.85	3.85	8.15	15.85

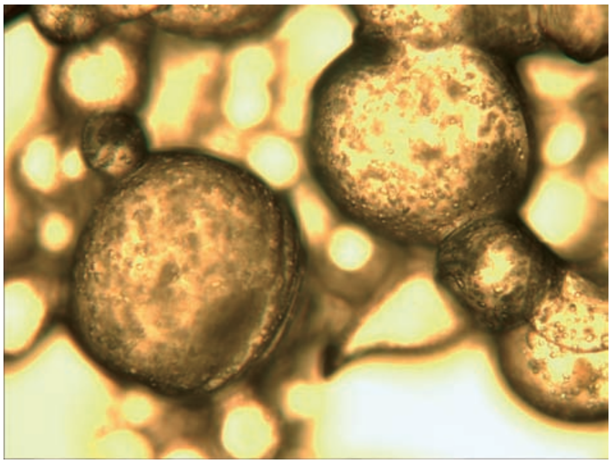


Figure 1. Optical microscope image of *Lactobacillus acidophilus* capsule at 40× magnification.

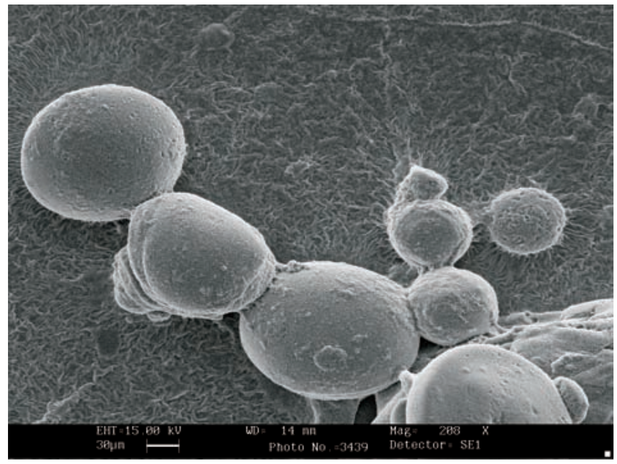
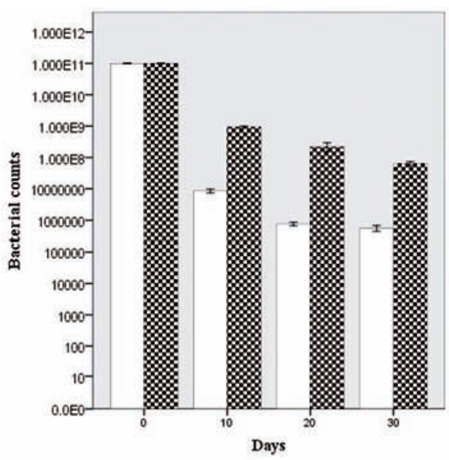


Figure 2. Scanning electron micrograph and surface morphology of alginate-starch microcapsules.



Graph 1. Survival of free and encapsulated *Lactobacillus acidophilus* at 4°C during 30 days storage.

□ free ▩ Alginate-Starch

Furthermore, the results showed that resistant starch grains were present in the cavities. Many researchers have shown that millimeter size (more than 1 mm) gives a sandy texture to product (Arnaud et al., 1992; Hansen et al., 2002). Zanjani et al. (2012) reported that the emulsion technique gives a smooth texture when the Microcapsules are incorporated into products. This finding is in agreement with those of Hansen et al. (2002) and Sultana et al. (2000).

pH changes: The pH changes for encapsulated cultures attained 4.02 (Table 1). This may be a result of nutrients and slow release of metabolites across the alginate-starch shell of the microcapsules (Sultana et al., 2000; Fahimdanesh et al., 2012). The pH of samples containing free *L.acidophilus* decreased the same as control. Many studies have shown that the concentration of acetic acid and low pH of mayonnaise sauce could decrease the survival of free probiotics. However, there were no significant differences between free and control samples (Collins, 1985; Lock and Board, 1994; Khalil and Mansour, 1998; Fahimdanesh et al., 2012).

Survival of free and encapsulated *Lactobacillus acidophilus*: The loss of *L.acidophilus* cells showed significant differences ($p<0.05$) between the free and encapsulated states in mayonnaise sauce at the end of 30 days storage (Graph 1). Fahimdanesh et al. (2012) reported that microencapsulation with resistant starch improved the counts of l.casei and B.bifidum in acidic foods. A reduction in the viable cells of culture bacteria in mayonnaise 16 weeks has been reported (Khalil and Mansour, 1998). Previous findings suggested that the decline in bacterial cells, as a result of pH, is likely due to the bactericidal activity of acetic acid (vinegar), leading to the eventual death of cells (Collins, 1985; Lock and Board, 1995; Khalil and Mansour, 1998; Fahimdanesh et al., 2012). The resistant starch could give a higher viability of *L.acidophilus* (105 to 106/g) in acidic condition. This finding is in agreement with those of Hussein and Kebary (1999), Sultana et al. (2000), Krasaekoopt et al. (2003), Homayouni et al. (2008) and Zanjani et al. (2012). Data from the study done by Ding and Shah (2008) also showed greater losses in viability of free *L.acidophilus* than encapsulated *L.acidophilus* in orange juice (pH 2.81) after four weeks.

Sensory evaluations: The points allocated for taste showed that the addition of free and encapsulated *L.acidophilus* had no effect on sensory properties of mayonnaise sauce (Table 2). It was expected that addition of resistant starch could slightly affect the taste of the Mayonnaise sauces. However, the panelist could not identify the differences in taste between Mayonnaise sauces with encapsulated *L.acidophilus* from controls and samples containing free bacteria. Total evaluation in terms of colour, texture of all samples showed that the addition of free and encapsulated *L.acidophilus* had a significant effect ($p<0.05$) on sensory properties of probiotic mayonnaise sauce. Fahimdanesh et al. (2012) reported that exopolysaccharide production by lactobacilli may improve the texture of samples. Furthermore, many researchers have shown that oxidation of oil during storage time could affect the color of Mayonnaise sauce (Li Hsieh and Regenstein, 1991; Khalil and Mansour, 1998; Fahimdanesh et al., 2012).

This study indicates that using a prebiotic starch with alginate for encapsulation was beneficial as it enhanced the survival of the *Lactobacillus acidophilus* in mayonnaise sauce during the 30 day storage. The study showed that microencapsulation technique can significantly improve the survival of *L.acidophilus* in mayonnaise sauce. No significant differences in microcapsule shapes were detectable by a SEM and optical microscopy. Furthermore, microencapsulation with alginate-starch may improve sensory evaluation of the mayonnaise sauce. Further works are needed to evaluate the protection effect of microencapsulation on the probiotic survival in the gastrointestinal tract.

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زنده مانی لاکتوباسیلوس اسیدوفیلوس ریزپوشانی شده با آلژینات و پری بیوتیک در سس مایونز

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چکیده

زمینه مطالعه: امروزه ریزپوشانی سلول های پروبیوتیکی یکی از جدیدترین روش ها به منظور افزایش قابلیت زنده مانی پروبیوتیک‌ها در فرایندهای مواد غذایی است. لاکتوباسیلوس اسیدوفیلوس به عنوان یک باکتری پروبیوتیک دارای اثرات سلامت بخش زیادی می باشد و قابلیت زیستی اندک این باکتری در فراورده های غذایی و شرایط نامناسب همواره توسط پژوهشگران مورد مطالعه قرار گرفته شده است. **هدف:** در این مطالعه، لاکتوباسیلوس اسیدوفیلوس به حالت آزاد و ریزپوشانی شده به سس مایونز افزوده شد و زنده مانی آن در طی ۳۰ روز نگهداری در دمای $4\ ^{o}C$ مورد ارزیابی قرار گرفت. **روش کار:** لاکتوباسیلوس اسیدوفیلوس (PTCC 1643) به روش امولسیون بوسیله آلژینات کلسیم و نشاسته مقاوم ذرت ریزپوشانی شد و زنده مانی، pH و ویژگی های حسی سس مایونز ارزیابی شد و اندازه و شکل کپسول ها تشکیل شده با میکروسکوپ نوری مورد بررسی قرار گرفت. **نتایج:** ریزپوشانی با آلژینات کلسیم و نشاسته مقاوم در افزایش زنده مانی لاکتوباسیلوس اسیدوفیلوس، تاثیر معنی داری ($p<۰/۰۵$) نسبت به حالت آزاد در سس مایونز داشت. تفاوت قابل ملاحظه ایی از نظر ساختار و شکل کپسول ها با نشاسته مقاوم توسط میکروسکوپ نوری و میکروسکوپ الکترونی مشاهده نشد و ریزپوشانی لاکتوباسیلوس اسیدوفیلوس ویژگی های حسی محصول را بهبود داد. **نتیجه گیری نهایی:** ریزپوشانی، زنده مانی لاکتوباسیلوس اسیدوفیلوس را در سس مایونز افزایش می دهد.

واژه های کلیدی: میکروسکوپ الکترونی، پری بیوتیک، ریزپوشانی، نشاسته، لاکتوباسیلوس اسیدوفیلوس.

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