African Journal of Microbiology Research Vol. 6(40), pp. 6853-6858, 20 October, 2012
Available online at http://www.academicjournals.org/ajmr
DOI: 10.5897/AJMR12.1240
ISSN 1996-0808 ©2012 Academic Journals

Full Length Research Paper

Effect of microencapsulation plus resistant starch on survival of *Lactobacillus casei* and *Bifidobacterium bifidum* in mayonnaise sauce

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Accepted 24 August, 2012

Two types of probiotics (*Lactobacillus casei* and *Bifidobacterium bifidum*) were added to mayonnaise sauce as either free cells or encapsulated with resistant starch. The survival of *L. casei* and *B. bifidum* was evaluated during the product storage for 30 days at 4°C. The viability of *B. bifidum* cells disappeared in the free state after 10 days; however, free *L. casei* approximately decreased 6 log cycles in a number of cells after 30 days storage at 4°C. When the *L. casei* and *B. bifidum* were encapsulated in calcium alginate capsules with Hi-Maize starch, the viable cell numbers were decreased to 1.3×10^7 and 9×10^5 CFU/g, respectively. In general, the results showed that encapsulation with resistant starch can significantly enhance the survival of probiotic bacteria in mayonnaise sauce during storage. No differences were detectable in the morphology of capsules by scanning electron microscopy and optical microscopy. Sensory qualities of mayonnaise sauce were improved by the addition of encapsulated probiotic bacteria.

Key words: Mayonnaise sauce, microencapsulation, probiotic, *Lactobacillus casei, Bifidobacterium bifidum,* resistant starch, survival.

INTRODUCTION

Probiotic bacteria are live microorganisms which, when inoculated in adequate amounts bestow health benefit on humans (Fuller, 1989; Lee and Salminen, 1995; Homayouni et al., 2008). Probiotic bacteria such as lactobacillus and bifodobacterium strains have been reported to play therapeutic role by lowering cholesterol, preventing cancer, alleviation of constipation and reduction of lactose intolerance (Modler et al., 1990; Guerin et al., 2003). Encapsulation is a new method to promote survival of probiotics during storage or adverse conditions (Kailasapathy, 2002; Krasaekoopt et al., 2003; Muthukumarasamy and Holley, 2006).

Calcium alginate capsules entrapment of probiotics is the most used microencapsulation device for several reasons: Alginate as a natural polymer (obtained from brown alga) has been used in various probiotic encapsulations; furthermore, it is a simple and a low cost way to immobilize cells. Alginate, a natural polymer and non-toxic to humans, can be safely used in products (Smidsrod and Skjak-Braek, 1990; Hansen et al., 2002; Allan-Wojtas et al., 2008). Probiotics are microencapsulated gently and consequently high yield are observed (Khalil and Mansour, 1998). The addition of Prebiotic compounds, such as starch, and oligo-saccahrides promotes the survival of Probiotic bacteria (Modler et al., 1990; Mituoka, 1992; Sultana et al., 2000; Dembczynski and Jankowski, 2002; Homayouni et al., 2008; Donthidi et al., 2010; Mirzaei et al., 2012). A limiting factor in survival of probiotic bacteria has been reported as low pH (3.6 to 4.6) of mayonnaise sauce owing to the concentration of acetic acid (Radford and Board, 1993; Khalil and Mansour, 1998). It has

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been proven that microencapsulated probiotics survive better in acid conditions (Khalil and Mansour, 1998; Kebary and Hussein, 1999; Mokarram et al., 2009; Nazzaro et al., 2009; Chávarri et al., 2010; Brinques and Ayub, 2011; Su et al., 2011). Alginate microcapsules can be prepared by emulsion and extrusion techniques. In emulsion technique, the size of capsules is much smaller than extrusion (Kailasapathy, 2002; Chandramouli et al., 2004).

Probiotics have been inoculated to various food products such as sausage (Muthukumarasamy and Holley, 2006), chocolate (Nebesny et al., 2006; Possemiers et al., 2010), ice cream (Homayouni et al., 2008), juices (Luckow and Delahunty, 2004), and cream-filled cake (Zanjani et al., 2012). It was reported that mayonnaise sauce might be a suitable carrier for probiotics microorganisms due to its high water activity (Khalil and Mansour, 1998); however, survival of free and microencapsulated *Lactobacillus casei* and *Bifidobacterium bifidum* in mayonnaise sauce containing resistant starch as a filler material has not yet been reported.

The aim of this study is inoculation of probiotics as a free and microencapsulated form with resistant starch in mayonnaise sauce and evaluation of microencapsulation on survival and sensory properties of mayonnaise sauce during refrigerated storage (4°C).

MATERIALS AND METHODS

Preparation of probiotics

L. casei PTCC 1608 (Persian Type Collection Culture) and *B. bifidum* PTCC 1644 were purchased from Iran Scientific and Industrial Organization. Pure freeze dried cultures were inoculated in MRS broth (de Man-Rogasa-Sharpe) for 24 h under aerobic and anaerobic conditions at 37°C, respectively and biomasses were then harvested by centrifuging at 4000 rpm for 10 min at 4°C. The cultures were then washed twice by sterile saline solution (0.9%) and used in the microencapsulation process.

Microencapsulation of bacteria

All glassware and solutions used in the protocols were sterilized at 121°C for 15 min. The encapsulation way for making alginate capsules was a modified version of methods basically reported by Sheu and Marshall (1993) and Sultana et al. (2000). 2 g of resistant starch (Hi-maize 260 national starch UK) were added to 100 ml distilled water and boiled until it formed a gel, then 2 g of sodium alginate (Sigma 71238) were added until they dissolved completely. After cooling, 0.1% probiotic cultures were transferred into the solution and stirred for 5 min. The final mixture was suspended in 200 ml vegetable oil containing 0.2% tween 80 and mix (350 rpm for 20 min, Heydolph Stirrer, Germany) until they appeared creamy. Alginate capsules were prepared by adding 200 ml calcium chloride 0.1 M into a mixture, the phase separation of oil/water emulsion occurred. The mixture was allowed to stand for 30 min, to separate prepared calcium alginate capsules in the bottom of the calcium chloride layer. The oil layer was drained and capsules were collected by centrifuging in 350 rcf for 15 min. The whole procedure was stored at 4°C.

Preparation of mayonnaise sauce

Mayonnaise sauces (Mahram Industries Co., Qazvin, Iran) were purchased from Iranian supermarkets. Mayonnaise sauce samples were inoculated by free and microencapsulated bacteria separately and stored for 30 days at 4°C until analysis.

Size and shape of capsules

The mean diameter of capsules was measured by optical microscopy (Master sizer Malvern 2000 UK). The diameters of 100 randomly selected capsules were measured by using measurement software (Leica Qwin 550). The morphology of the capsules was observed using scanning electron microscope (SEM). In this study, the capsules were examined with scanning electron microscope (LEO 440 I, England) at an accelerating voltage of 10 kV.

Release of entrapped bacteria

The capsules containing probiotic bacteria were released by phosphate buffer (pH 7.0, 0.1 M) reported by Sheu and Marshall (1993) and Sultana et al. (2000). 1 g of capsules was transferred in 9 ml buffer. The solution was vortexed on a shaker for 15 min vigorously (IKA-MS2, Minishaker, USA) until bacteria released from capsules completely. Total bacterial counts were enumerated on MRS agar (Merck, KGaA Germany). All experiments were carried out in triplicate.

Determination of pH

The pH value of mayonnaise sauce samples was determined using a Digital pH-meter (744, Metrohm, Switzerland). The pH value was measured according to the standard method of Institute of Standard and Industrial Research of Iran (ISIRI number 2454, 2001). In this study, the pH-meter was calibrated using standard pH 4.0 and pH 7.0 buffer solutions.

Sensory evaluation

Sensory evaluation of mayonnaise sauce was conducted after 30 days of refrigerated storage. A panel consisting of 20 panelists evaluated the mayonnaise sauce samples using a sensory rating of 1 to 5 for the color and appearance, 1 to 5 for body and texture, and 1 to 10 for flavor and taste (Homayouni et al., 2006).

Statistical analysis

All statistical analyses were carried out by SPSS 20 (SPSS Inc., Chicago, IL) software. Analysis of variance by the general linear model (GLM) procedure and mean differences ($P < 0.05$) between treatments were analyzed by Duncan's multiple range test.

RESULTS AND DISCUSSION

Size and morphology of microcapsules

The morphology of capsules was measured by SEM and optical microscopy. All capsules were spherical in shape, and starch particle were present on the surface of the capsules (Figures 1 and 2). This finding is in agreement

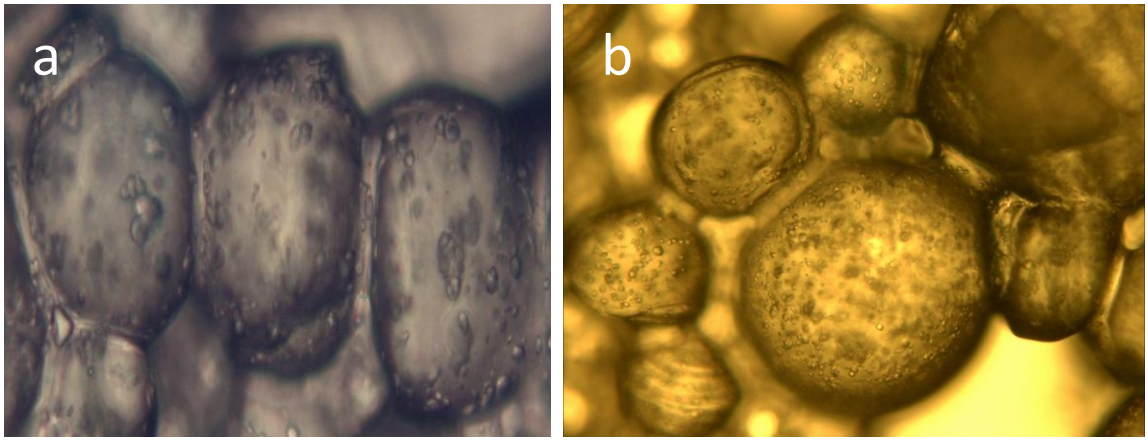

Figure 1. Optical microscope image of coated alginate–resistant starch capsule (a and b) at 40× magnification.

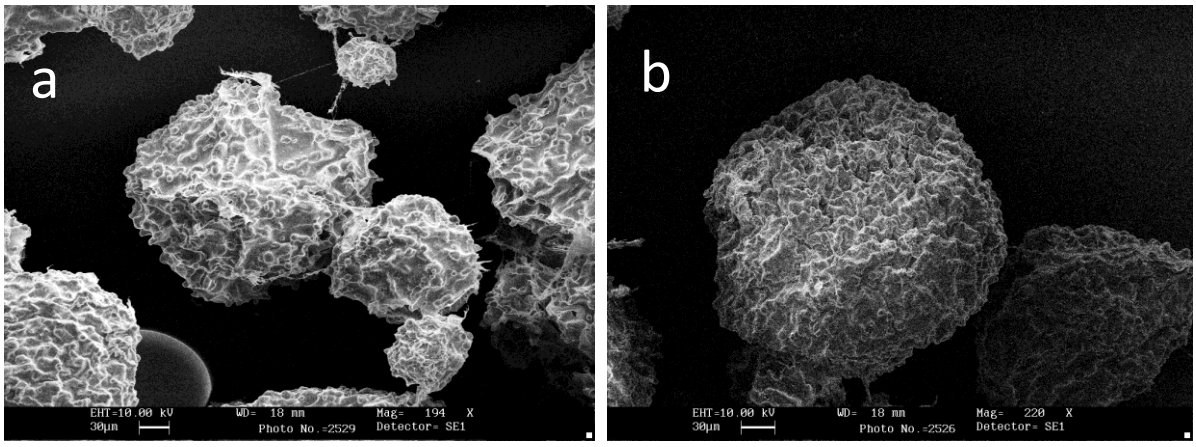

Figure 2. Scanning electron photomicrograph of microcapsules (a and b).

with Sultana et al. (2000), which reports that the shape of the microcapsules was spherical, and resistant starch granules filled the cavities of alginate matrix and assisted to support the structure of microcapsules. The mean diameter of microcapsules was 160 µm. Furthermore, the results showed that this diameter was desirable for mayonnaise sauce and delivered soft texture to product. Several reports have shown that larger capsules (more than 1 mm) give sandy texture to product (Arnaud et al., 1992; Hansen et al., 2002). Similar shapes of microcapsules were also shown by many researchers (Sultana et al., 2000; Mokarram et al., 2009; Mirzaei et al., 2012; Zanjani et al., 2012).

pH changes during mayonnaise sauce storage

The pH value of mayonnaise sauce was not affected ($P > 0.05$) during storage at 4°C for a period of 30 days (Table 1). The pH of control samples reached 4.02 at the end of 30 days storage and the final pH of mayonnaise sauce samples with free probiotics decreased same as control. This may be due to low survival of free probiotics in lower pH (4.6), thus there were no significant differences between free and control samples (Collins, 1985; Lock and Board, 1995; Khalil and Mansour, 1998; Mokarram et al., 2009; Nazzaro et al., 2009; Chávarri et al., 2010; Brinques and Ayub, 2011; Su et al., 2011). The pH of samples containing encapsulated cells of *L. casei* and *B. bifidum* attained 4.02 and 4.01, respectively. Many studies have shown that microencapsulation of probiotic bacteria could be slow absorption of nutrients and decelerating release of metabolites across the alginate shell of capsules (Sultana et al., 2000; Homayouni et al., 2008).

Survival of free and encapsulated bacteria in mayonnaise sauce

In this study, we used free or encapsulated *L. casei* and *B. bifidum* with resistant starch for the first time in

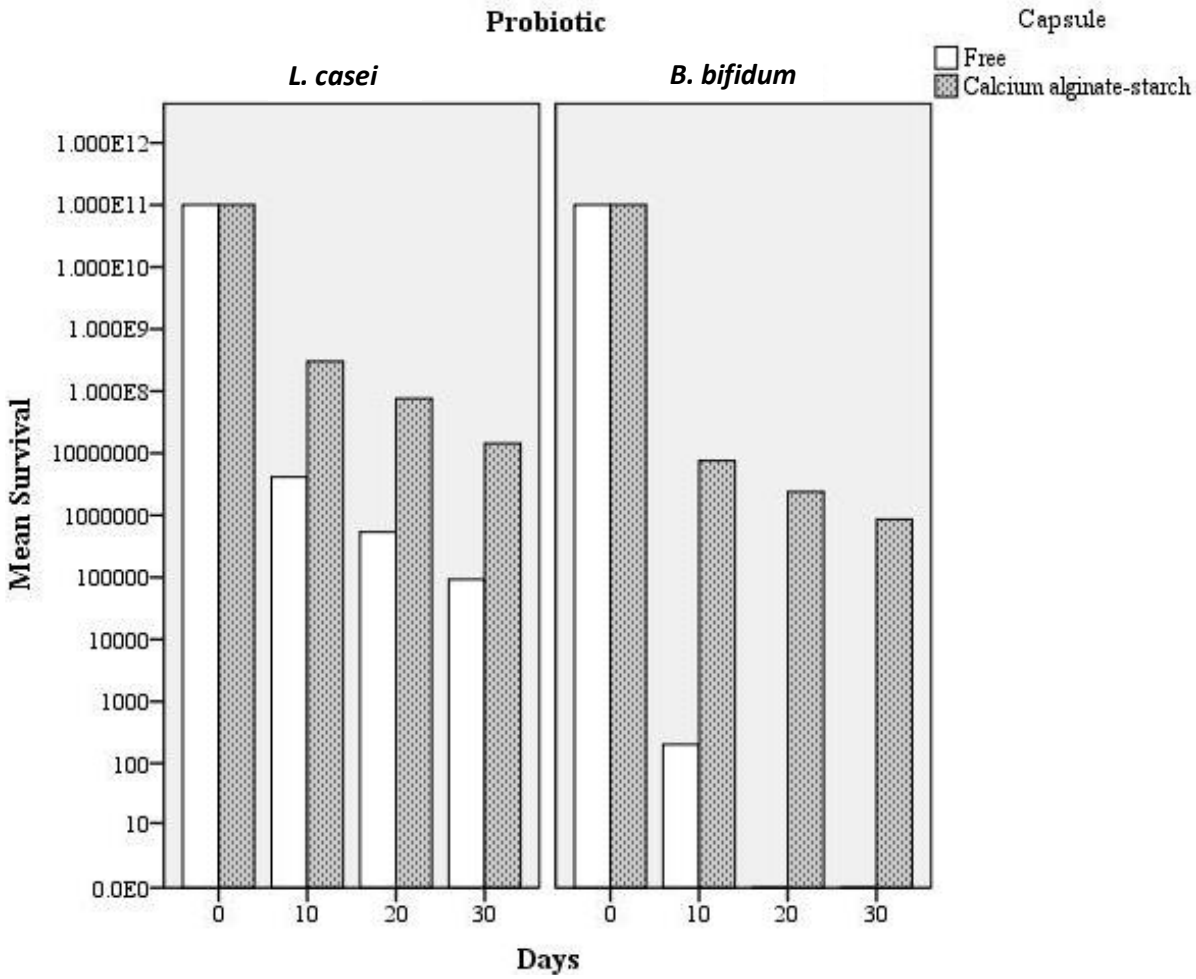

Figure 3. Survival of free and encapsulated *L. casei* and *B. bifidum* incorporated into mayonnaise sauce during storage.

Table 1. pH changes of mayonnaise sauce during storage.

Storage (days)	Control[a]	Mayonnaise sauce with free probiotics		Mayonnaise sauce with encapsulated probiotics	
		A[b]	B[b]	A[b]	B[b]
0	4.10	4.10	4.10	4.10	4.10
10	4.07	4.06	4.06	4.07	4.08
20	4.04	4.04	4.03	4.05	4.06
30	4.02	4.02	4.02	4.02	4.01

[a]Control mayonnaise sauce without probiotic; [b] A— *Lactobacillus casei*, B— *Bifidobacterium bifidum*.

mayonnaise sauce. The mayonnaise sauce was evaluated through storage of 30 days at 4°C for monitoring the survival carefully (Figure 3). On the one hand, the free *L. casei*, approximately decreased 6 log cycles in number of cells, but on the other hand the *B. bifidum* cells were sensitive to lower pH (4.6) and did not survive after 10 days in the free state (Figure 3). These decreases of *L. casei* and *B. bifidum* in the free state might be credited to the bactericide activity of acetic acid in mayonnaise sauce (Collins, 1985; Lock and Board, 1995; Khalil and Mansour, 1998). The survival of encapsulated *L. casei* and *B. bifidum* with resistant starch declined slightly after 30 days to 1.3×10^7 and 9×10^5 CFU/g, respectively. The results showed that there were significant differences ($P < 0.05$) between the free and encapsulated probiotics in mayonnaise sauce at the end of 30 days refrigerated storage. We have shown the positive effect of alginate capsules with resistant starch

Table 2. Sensory properties of mayonnaise sauce.

Samples	Color and appearance (1-5)	Body and texture (1-5)	Flavor and taste (1-10)	Total acceptability (1-20)
A	4.50[a]	4.52[a]	9.50[a]	18.52[a]
B	4.55[b]	4.53[c]	9.50[ab]	18.58[c]
C	4.57[c]	4.53[c]	9.50[ab]	18.60[d]
D	4.59[d]	4.54[d]	9.51[b]	18.64[e]
E	4.50[a]	4.53[b]	9.50[ab]	18.53[b]

[a–d]Means in the same column followed by different letters were significantly different ($P < 0.05$). A: Mayonnaise sauce with free *L. casei*, B: Mayonnaise sauce with free *B. bifidum*, C: Mayonnaise sauce with encapsulated *L. casei*, D: Mayonnaise sauce with encapsulated *B. bifidum*, E: Mayonnaise sauce without probiotic (control).

during storage time. Many studies have shown that incorporation of resistant starch into the alginate mix could increase the survival of bacteria due to curbing the diffusion of calcium ions outside of capsules (Sultana et al., 2000; Kailasapathy, 2006). Sultana et al. (2000) reported that encapsulation with resistant starch could help the protection of bacterial cells in adverse condition. Furthermore, microencapsulation with resistant starch may improve viable cells of bacteria in acidic foods. This finding is in agreement with those of Kebary and Hussein (1999), Sultana et al. (2000), Krasaekoopt et al. (2003), Homayouni et al. (2008) and Mirzaei et al. (2012). The hi-maize starch could give a higher survival ability of probiotics (10^5 to 10^6/g) in acidic environment of mayonnaise sauce.

Sensory evaluation of mayonnaise sauce

The sensory scores of 30 days mayonnaise sauces samples are given in Table 2. The results showed that there were no significant differences ($P > 0.05$) in the flavor and taste of the mayonnaise sauce samples. It was expected that addition of resistant starch to alginate capsule could slightly flavor the mayonnaise sauces. However, the panelist could not identify the differences in flavor between mayonnaise sauces with encapsulated probiotics from controls and samples containing free cells. The points allocated for color and appearance, and body and texture showed that the addition of free and encapsulated probiotics had significant effect ($P < 0.05$) on sensory properties of probiotic mayonnaise sauce. Khalil and Mansour (1998) reported that the low color scores of mayonnaise samples containing free cells or control phase of probiotics might be due to oxidation rancidity of oil during storage. Furthermore, Li Hsieh and Regenstein (1991) reported that formation of secondary oxidation products could affect the color and appearance of mayonnaise during storage time. The production of exopolysaccharide by bifidobacteria and lactobacilli may improve the body and texture of mayonnaise samples. This finding is in agreement with those of Cerning et al. (1986), Roberts et al. (1995), Khalil and Mansour (1998) and Kailasapathy (2006).

Conclusion

This study indicates that microencapsulation with resistant starch enhanced the survival of *L. casei* and *B. bifidum* compared to free cells in mayonnaise sauce during 30 days storage, and provided a good protection of bacteria cells from the bactericide activity of acetic acid in mayonnaise sauce. It may also be noted *L. casei* and *B. bifidum* have a different response to the acidic environment. No significant differences in capsule shapes were detectable by a SEM. Furthermore, microencapsulation with resistant starch may improve sensory evaluation of the mayonnaise sauce. Further work should focus on microencapsulation processes such as spray-drying and centrifugal extrusion.

REFERENCES

Allan-Wojtas P, Hansen LT, Paulson AT (2008). Microstructural studies of probiotic bacteria-loaded alginate microcapsules using standard electron microscopy techniques and anhydrous fixation. LWT-Food Sci. Technol. 41:101-108.

Arnaud JP, Lacroix C, Choplin L (1992). Effect of agitation rate on cell release rate and metabolism during continuous fermentation with entrapped growing. Biotechnol. Tech. 6(3):265-270.

Brinques GB, Ayub MAZ (2011). Effect of microencapsulation on survival of *Lactobacillus plantarum* in simulated gastrointestinal conditions, refrigeration, and yogurt. J. Food Eng. 103(2):123-128.

Cerning J, Bouillanne C, Desmazeaud MJ, Landon M (1986). Isolation and characterization of exocellular polysaccharide produced by *Lactobacillus bulgaricus*. Biotechnol. Lett. 8(9):625-628.

Chandramouli V, Kailasapathy K, Peiris P, Jones M (2004). An improved method of microencapsulation and its evaluation to protect *Lactobacillus* spp. In simulated gastric conditions. J. Microb. Methods 56(1):27-35.

Chávarri M, Marañón I, Ares R, Ibáñez FC, Marzo F, Villarán MdC (2010). Microencapsulation of a probiotic and prebiotic in alginate-chitosan capsules improves survival in simulated gastro-intestinal conditions. Int. J. Food microbiol. 142(1–2):185-189.

Collins MA (1985). Effects of pH and acidulate type on the survival of some food poisoning bacteria in mayonnaise. M. A. N. 3:215-221.

Dembczynski R, Jankowski T (2002). Growth characteristics and acidifying activity of *Lactobacillus rhamnosus* in alginate/starch liquid-core capsules. Enzyme Microb. Tech. 31(1–2):111-115.

Donthidi AR, Tester RF, Aidoo KE (2010). Effect of lecithin and starch

on alginate-encapsulated probiotic bacteria. J. Microencapsul. 27(1):67-77.
Fuller R (1989). Probiotics in man and animals. J. Appl. Bacteriol. 66:365-378.
Guerin D, Vuillemard JC, Subirade M (2003). Protection of bifidobacteria encapsulated in polysaccharide-protein gel beads against gastric juice and bile. J. Food Protect. 66:2076-2084.
Hansen LT, Allan-Wojtas PM, Jin YL, Paulson AT (2002). Survival of Ca-alginate microencapsulated *Bifidobacterium* spp. in milk and simulated gastrointestinal conditions. Food Microbiol. 19(1):35-45.
Homayouni A, Azizi A, Ehsani MR, Yarmand MS, Razavi SH (2008). Effect of microencapsulation and resistant starch on the probiotic survival and sensory properties of synbiotic ice cream. Food Chem. 111(1):50-55.
Homayouni A, Ehsani MR, Azizi A, Yarmand MS, Razavi SH (2006). A review on the method of increasing probiotic survival in functional dairy foods. In Proceedings of the 9th Iranian nutrition congress, pp. 288-297.
Kailasapathy K (2002). Microencapsulation of probiotic bacteria: technology and potential applications. Curr. Issues Intest. Microbiol. 3(2):39-48.
Kailasapathy K (2006). Survival of free and encapsulated probiotic bacteria and their effect on the sensory properties of yoghurt. LWT-Food Sci. Technol. 39(10):1221-1227.
Kebary KMK, Hussein AS (1999). Improving viability of bifidobacteria by microentrapment and their effect on some pathogenic bacteria in stirred yoghurt. AAlim. 28(2):110-132.
Khalil AH, Mansour EH (1998). Alginate Encapsulated Bifidobacteria Survival in Mayonnaise. J. Food Sci. 63(4):702-705.
Krasaekoopt W, Bhandari B, Deeth H (2003). Evaluation of encapsulation techniques of probiotics for yoghurt. Int. Dairy J. 13(1):3-13.
Lee YK, Salminen S (1995). The coming of age of probiotics. Trends Food Sci. Tech. 6(7):241-245.
Li Hsieh YT, Regenstein JM (1991). Factors Affecting Quality of Fish Oil Mayonnaise. J. Food Sci. 56(5):1298-1301.
Lock JL, Board RG (1995). The fate of *Salmonella enteritidis* PT4 in home-made mayonnaise prepared from artificially inoculated eggs. Food Microbiol. 12(0):181-186.
Luckow T, Delahunty C (2004). Which juice is 'healthier'? A consumer study of probiotic non-dairy juice drinks. Food Qual. Prefer. 15(7–8):751-759.
Mirzaei H, Pourjafar H, Homayouni A (2012). Effect of calcium alginate and resistant starch microencapsulation on the survival rate of Lactobacillus acidophilus La5 and sensory properties in Iranian white brined cheese. Food Chem. 132(4):1966-1970.
Mituoka T (1992). The human gastrointestinal tract. In: Wood, B.J.B. (Ed.), The Lactic Acid Bacteria. The Lactic Acid Bacteria in Health and Disease, Elsevier, London, 1:69-114.
Modler HW, McKellar RC, Yaguchi M (1990). Bifidobacteria and bifidogenic factors. Can. Inst. Food Sci. Tech. J. 23:29-41.
Mokarram RR, Mortazavi SA, Najafi MBH, Shahidi F (2009). The influence of multi stage alginate coating on survivability of potential probiotic bacteria in simulated gastric and intestinal juice. Food Res. Int. 42(8):1040-1045.
Muthukumarasamy P, Holley RA (2006). Microbiological and sensory quality of dry fermented sausages containing alginate-microencapsulated *Lactobacillus reuteri*. Int. J. Food Microbiol. 111(2):164-169.
Nazzaro F, Fratianni F, Coppola R, Sada A, Orlando P (2009). Fermentative ability of alginate-prebiotic encapsulated *Lactobacillus acidophilus* and survival under simulated gastrointestinal conditions. J. Funct. Foods. 1(3):319-323.
Nebesny E, Zyzelewicz D, Motyl I, Libudzisz Z (2006). Dark chocolates supplemented with Lactobacillus strains. Eur. Food Res. Technol. 225(1): 33-42.
Possemiers S, Marzorati M, Verstraete W, Van de Wiele T (2010). Bacteria and chocolate: A successful combination for probiotic delivery. Int. J. Food Microbiol. 141(1–2):97-103.
Radford SA, Board RG (1993). Review: Fate of pathogens in home-made mayonnaise and related products. Food Microbiol. 10:269-278.
Roberts CM, Fett WF, Osman SF, Wijey C, O'Connor JV, Hoover DG (1995). Exopolysaccharide production by *Bifidobacterium longum* BB-79. J. Appl. Microbiol. 78(5):463-468.
Sheu TY, Marshall RT, Heymant H (1993). Improving survival of culture bacteria in frozen desserts by microentrapment. J. Dairy Sci. 76:1902-1907.
Smidsrod O, Skjak-Braek G (1990). Alginate as immobilization matrix for cells. Trends Biotechnol. 8(3):71-78.
Su R, Zhu XL, Fan DD, Mi Y, Yang CY, Jia X (2011). Encapsulation of probiotic *Bifidobacterium longum* BIOMA 5920 with alginate–human-like collagen and evaluation of survival in simulated gastrointestinal conditions. Int. J. Biol. Macromol. 49(5):979-984.
Sultana K, Godward G, Reynolds N, Arumugaswamy R, Peiris P, Kailasapathy K (2000). Encapsulation of probiotic bacteria with alginate–starch and evaluation of survival in simulated gastrointestinal conditions and in yoghurt. Int. J. Food Microbiol. 62(1–2):47-55.
Zanjani MAK, Tarzi BG, Sharifan A, Mohammadi N, Bakhoda H, Madanipour MM (2012). Microencapsulation of *Lactobacillus casei* with calcium alginate-resistant starch and evaluation of survival and sensory properties in cream-filled cake. Afr. J. Microbiol. Res. 6(26):5511-5517.