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Viability during refrigerated storage in selected food products and during simulated gastrointestinal conditions of individual and combined lactobacilli encapsulated in alginate or alginate-chitosan

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ABSTRACT

The viability of *Lactobacillus acidophilus* and *Lactobacillus reuteri* combined or individually encapsulated in alginate (AG) or alginate-chitosan (AG-CH), when they were added to milk, peach nectar, or blackberry jam set-style yogurt; and stored at 5 °C for 30 days was evaluated. Survival of studied encapsulated lactobacilli when exposed to simulated gastrointestinal tract (GIT) conditions was also determined. AG-CH encapsulation provided better protection than AG and improved lactobacilli survival during storage in milk, peach nectar, or blackberry jam set-style yogurt; lactobacilli counts were $\geq 10^7$ CFU/g after 30 days, except for encapsulated combined lactobacilli in peach nectar. Not significant ($p < 0.05$) changes in pH were observed when lactobacilli encapsulated in AG-CH were added to the evaluated foods. Minimal changes of pH were observed with lactobacilli encapsulated in AG ($\Delta pH < 0.49$). During successive simulated GIT conditions, AG-CH encapsulation prevented lactobacilli loss in simulated gastric juices; meanwhile, favorable releases were observed in simulated intestinal fluids. Sensory evaluation demonstrated that judges did not perceive the presence of capsules in blackberry jam set-style yogurt, but were sensed in milk and peach nectar. Encapsulation of combined lactobacilli was successful and could provide more health benefits than individually encapsulated lactobacilli to consumers. Milk and blackberry jam set-style yogurt promoted microcapsules stability and thus viability of studied lactobacilli.

1. Introduction

Several lactobacilli have been evaluated as probiotics as well as their incorporation into several food products in order to demonstrate their viability (Nagpal et al., 2012). Regular consumption of probiotics in sufficient quantities ($>10^7$ CFU/g) contributes to health benefits (FAO/WHO, 2002). A pleasant way of consuming probiotics is through food. Thus, researchers have made efforts to improve alternatives for the incorporation of probiotics in a wide variety of foods, warranting probiotic viability at the time of consumption, but more important assuring that probiotics are able to reach the colon alive. Several studies suggested probiotic protection through encapsulation with biopolymers in order to ensure their viability when incorporated to food and gastrointestinal tract (GIT) conditions (Krasaekoopt & Watcharapoka, 2014; Urbanska, Bhathena, & Prakash, 2007; Zhao, Mutukumira, Lee, Maddox, & Shu, 2012).

Encapsulation is a technology that allows for protection of sensitive components, in a homogenous or heterogenous matrix (Nazzaro, Orlando, Fratianni, & Coppola, 2012), from moisture, temperature, mechanical damage, permeability, and reactivity to pH and/or the presence of salts (Kashappa, Desai, & Park, 2005). In this technology, semipermeable microcapsules are formed using various biopolymers (Yu et al., 2010). Sodium alginate is the most widely used biopolymer for encapsulation of probiotics due to its low cost, ease of use, and because it is generally recognized as safe. Several studies have demonstrated the protective effect of alginate on the viability of different probiotics such as *Lactobacillus acidophilus* (Krasaekoopt & Watcharapoka, 2014), and *Lactobacillus reuteri* (Muthukumarasamy, Allan-Wojtas, & Holley, 2006; Zhao et al., 2012) to different stress factors. However, double encapsulations such as alginate beads coated with chitosan, have

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demonstrated better results in probiotics' viability during incorporation and storage in foods such as yogurt (Iyer, Phillips, & Kailasapathy, 2005). Furthermore, probiotic survival was better in coated than in single microcapsules when they were subjected to simulated GIT conditions. Some studies have assessed this protection for *L. acidophilus* (Urbanska et al., 2007), and *Lactobacillus casei* (Iyer et al., 2005).

Up to date, most studies have focused on individually encapsulated probiotics and evaluated their viability to various stress conditions. However, combining probiotics and encapsulating them would provide more health benefits to consumers. Dunne et al. (1999) were among the first researchers suggesting that probiotics should consist of a combination of strains (multi-strain). Moreover, if two probiotics are ingested, each probiotic that reaches the GIT may provide its beneficial effect (lowering cholesterol levels, producing antimicrobial substances such as reuterin, and stimulating the immune system, among others), thus bacteria could complement each other health effect and may even have synergistic probiotic properties. Several reports proved that multi-strain probiotic dietary treatments versus mono-strain were more effective in reducing pathogenic counts (Zoppi, Cinquetti, Benini, Bonamini, & Minelli, 2001), and reducing the mortality and enhancing growth of chickens (Jin, Ho, Abdullah, & Jalaludin, 2000). On the other hand encapsulation of combined probiotics and their addition to foods is not common. Few reports have been published regarding combined probiotics in foods, Godward and Kailasapathy (2003a, 2003b) encapsulated (by emulsification) combined *L. acidophilus* and *Bifidobacterium infantis* and then added to yogurt and Cheddar cheese; they observed good survival of encapsulated combined probiotics in yogurt.

Muthukumarasamy and Holley (2007) encapsulated *L. reuteri* and *B. longum* in alginate and incorporate them into a fermented sausage in order to evaluate their antimicrobial activity against *Escherichia coli*, during ripening of the sausage. Nowadays, lyophilized pharmaceutical products are offered with even more than three probiotics. However, more studies are needed to assess the viability of encapsulated combined probiotics added to different foods before suggesting their potential application. The aim of this study was to evaluate the viability of two lactobacilli (*L. acidophilus* and/or *L. reuteri*) added to milk, peach nectar, or blackberry jam set-style yogurt, in alginate or alginate-chitosan beads during storage at 5 °C. Furthermore, survival of studied encapsulated lactobacilli during simulated GIT conditions was also evaluated. The main contribution of this study is to prove survival of studied encapsulated lactobacilli when they are added to selected foods.

2. Materials and methods

2.1. Bacterial strains and materials

L. acidophilus NRRL-B-4495 and *L. reuteri* NRRL-B-14171 were acquired in lyophilized form from the USDA (ARS, Peoria, Illinois, USA). These strains were activated and routinely sub-cultured in MRS-broth (Difco™ BD, Sparks, Maryland, USA) under anaerobic conditions at 37 °C. For biomass production, each *Lactobacillus* was cultured in 100 mL of MRS-broth at 37 °C for 18 h under anaerobic conditions. Cells were harvested by centrifugation at 8000 × *g* for 10 min at 4 °C and washed twice with phosphate buffer (pH 7.0). The cell suspensions were subsequently subjected to microencapsulation as described later. Sodium alginate was acquired from FMC Biopolymer (Haugesund, Rogaland, Norway), sodium citrate from Jungbunzlauer Austria AG (Pernhofen, Wulzeshofen, Austria), chitosan from Sigma–Aldrich (St. Louis, Missouri, USA), and calcium chloride from RBM (Puebla, Puebla, Mexico).

2.2. Lactobacilli encapsulation

The lactobacilli-cell pellet was re-suspended in 10 mL of sterile distilled water and mixed with 90 mL of sterile sodium alginate–sodium citrate (3.0 and 0.27 g/100 mL, respectively) solution. 3 g/100 mL of alginate was used in order to increase the lactobacilli survival according with previous reports (Burgain, Gaiani, Linder, & Scher, 2011) as well as from previous experiences in our laboratory. The mixture was dropped at 5 cm from 150 mL of sterile calcium chloride (1 mol/L) solution using a syringe (needle size 0.70 × 32 mm) and left for curing (1 h) at 4 °C. Alginate beads were drained and washed with sterile distilled water, and the beads were then placed on sterile filter paper to remove water excess. Alginate beads were kept refrigerated until use, but no more than 2 h.

Alginate beads were coated with chitosan using a batch of alginate beads prepared as described above. Alginate beads were added to 100 mL of 1 g/100 mL chitosan (dissolved in 1 mL/100 mL glacial acetic acid) and stirred for 1 h at 25 °C. Beads were removed, drained, and washed with sterile distilled water, then were placed on sterile filter paper to remove water excess. Alginate beads coated with chitosan were kept refrigerated until use, but no more than 2 h.

The above described procedure was also utilized to encapsulate *L. acidophilus* combined with *L. reuteri* in alginate beads or in alginate beads coated with chitosan, in proportion of 1:1 to create the lactobacilli mixture.

Thirty beads of each batch were taken in order to determine their size. Beads were measured with a digital micrometer (Mitutoyo Corporation, Kanagawa, Japan).

2.3. Alginate beads or alginate beads coated with chitosan added to foods

Three grams of alginate beads or alginate beads coated with chitosan containing *L. acidophilus*, *L. reuteri*, or their mixture (*L. acidophilus* + *L. reuteri*), were placed in sterile glass flasks. 15 mL of commercial homogenized and pasteurized whole milk or commercial pasteurized peach nectar were added. Glass flasks were stored at 5 °C and 2 glass flasks were removed every 5 days to evaluate the viability of lactobacilli for up to 30 days.

A blackberry jam set-style yogurt was chosen for this study to evaluate the effect of blackberry jam on lactobacilli viability. Many studies have evaluated the effect of yogurt on alginate beads containing lactobacilli (Ortakci & Sert, 2012; Urbanska et al., 2007) but very few reports are available that evaluate the effect of fruit jams (Randazzo, Pitino, Licciardello, Muratore, & Caggia, 2013). For set-style yogurt preparation, commercial homogenized and pasteurized whole milk (4 L); containing 33 g/L of fat, 31 g/L of protein, 5 g/L of vitamin D, and 666 mg/L of retinol equivalents, were inoculated with 0.4 g of started yogurt mix Yo-Mix™ (Danisco Deutschland GmbH, Niebüll, Schleswig–Holstein, Germany). The inoculated milk was distributed in 90 portions of 40 mL in sterile glass bottles containing tested beads (3 g) and blackberry jam (4 g). Blackberry jam was prepared with 100 g of blackberry, 100 g of sucrose, 4 g of high methoxy pectin, and 0.5 g of cinnamon. Fruit and 50 g of sucrose were heated to boiling for 15 min. After that, the remaining sugar mixed with pectin was added and boiled for 15 min. The jam of 63 °Brix was hot packaged and stored until use. Thirty portions of blackberry jam set-style yogurt were added with *L. acidophilus* beads, 15 with alginate beads, and the rest with alginate beads coated with chitosan. The same was performed for *L. reuteri* as well as for the mixture of *L. acidophilus* and *L. reuteri*, with alginate beads or alginate beads coated with chitosan. Yogurt containers were incubated at 42 °C until a pH of 4.6 was reached. Then, containers were cooled to 5 °C for 24 h. Beads and blackberry jam were not