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Freeze-dried alginate-silica microparticles as carriers of probiotic bacteria in apple juice and beer

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ABSTRACT

Herein the survivability of free and encapsulated L*actobacillus. rhamnosus* GG (LGG) in alginate or in alginate-silica microcarriers was investigated upon storage in apple juice and 5vt% beer at 4 °C. This study showed that the encapsulation approach utilized herein limits the release of the bacteria over time into the beverages, with core-shell alginate-silica beads being more efficient than alginate beads alone. Additionally, the statistical analyses showed a $p < 0.001$ for apple juice and $p < 0.005$ for beer when comparing the viability of alginate *vs* silica-coated beads at the 1 week time point in both, the filtrate and inside the carriers. This is of particular importance for the further delivery of bacteria to the intestines, to which encapsulation generally appears to be beneficial during the gastrointestinal passage.

1. Introduction

The attention on probiotic consumption has increased over the years as a result of relevant findings highlighting the importance of maintaining the gut homeostasis (Kostic, Xavier, & Gevers, 2014; Maynard, Elson, Hatton, & Weaver, 2012; Tremaroli & Bäckhed, 2012; Woting & Blaut, 2016). Food matrices containing beneficial bacteria as probiotic delivery systems are therefore inserted in this stimulating domain of functional foods research (Falco, Falkman, Risbo, Cárdenas, & Medronho, 2017; Krasaekoopt & Watcharapoka, 2014; Singh et al., 2017). Dairy products containing these bacteria are known to dominate the market still up to date (Haffner, Diab, & Pasc, 2016a). However, the global population status when it comes to cholesterol restricted diets, the rise of vegetarianism or more strikingly, the increase of lactose intolerance (Granato, Branco, Nazzaro, Cruz, & Faria, 2010; Suez et al., 2014), incites the development of non-dairy novelties. In recent times, beverages made of fruits, vegetables and cereals have been investigated (Perricone, Bevilacqua, Altieri, Sinigaglia, & Corbo, 2015). As a matter of fact, the beverages niche belongs to one of the fastest growing markets in the world along with snack bars (Sutton, 2007). As a proof of their potential, Tropicana®, GoodBelly® and ProViva® are examples of companies that launched beverages containing free probiotics on the American and Swedish markets (Fig. 1).

Fruit and vegetable juices are indeed promising probiotic carriers due to their essential nutrients content along with their appeal to a niche of consumers who already care about healthier habits (Barbosa & Teixeira, 2016). Additionally, the correlation of fruits extracts with benefits for certain health conditions reinforces their positive interest. For instance, aqueous extracts of kiwifruit and avocado have shown high anti-inflammatory activity in Crohn's disease assays. The non-aqueous extracts of both fruits along with blueberry and broccoli have shown a certain potentiality as well (Sutton, 2007).

Another study investigated the intake of nine micronutrients (*i.e.* vitamin E, calcium, folate, retinol, β-carotene, riboflavin, pantothenic acid, biotin and nicotin acid) on genome damage and repair (Fenech, 2004). These compounds are often present in fruits and vegetables, fact that emphasizes the idea of coupling juices with probiotics. The challenge can be still considerable since bacteriocins and low pH are main factors affecting bacteria survivability in the case of fruits or vegetables.

On a rather unusual tone, beer could also be a food matrix of interest for probiotic delivery (Sohrabvandi, Razavi, Mousavi, & Mortazavian, 2010). As a popular beverage consumed worldwide, it opens up the benefits of probiotics consumption to a wider public. The studies on beer as a probiotic carrier are scarce however, with the main reason lying on the eventual detrimental effects of the alcohol on the bacterial strains (Sohrabvandi et al., 2010).

Choosing robust strains accordingly and microencapsulating them prior to the addition to the beverage are two key factors for the successful delivery of viable probiotics into the intestines. In terms of bacteria already in the juice market, *Lactobacillus plantarum* 299v has been commercializing by Probi®. There are suggestions that this specific strain improves symptoms of the irritable bowel syndrome (Ducrotté, Sawant, & Jayanthi, 2012; Niedzielin, Kordecki, & Birkenfeld, 2001; Nobaek, Johansson, Molin, Ahrné, & Jeppsson, 2000).

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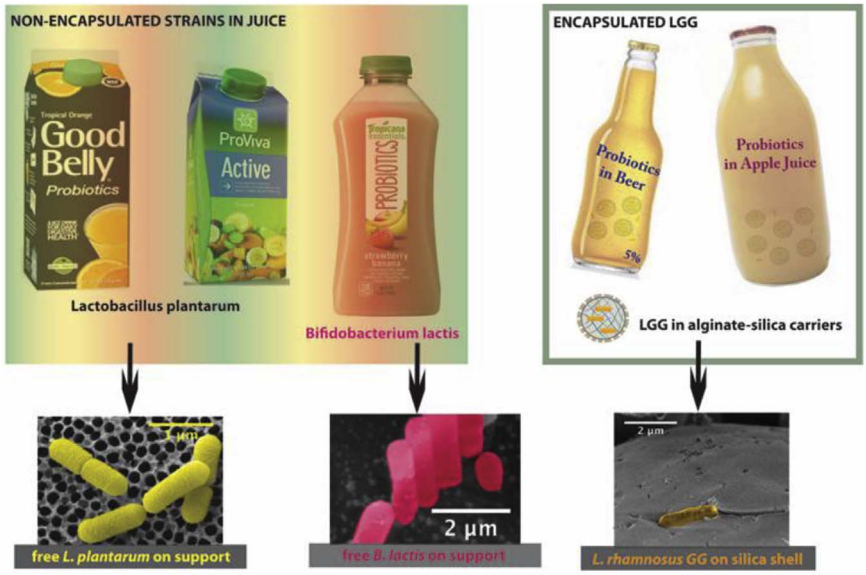

Fig. 1. (left) Non-encapsulated strains (*L. plantarum and B. lactis*) are present in juices sold currently on the American and Swedish markets; (right) the concept of encapsulated LGG in beer and apple juice. *L. plantarum and B. lactis* SEM micrographs were adapted. (Ingham, Beerthuyzen, & van Hylckama Vlieg, 2008; Ruas-Madiedo, Gueimonde, Arigoni, de los Reyes-Gavilan, & Margolles, 2009).

In the light of such context, entrapped *Lactobacillus rhamnosus* GG (LGG) in alginate and silica-coated alginate carriers was added to apple juice and beer as chosen food matrices. The encapsulation strategy was built around the fact that alginate results in a rather flexible network compatible with cells proliferation. Yet, the polymer protection is not sufficient to prevent bacteria leakage on one hand and to insure the cells protection inside the beads from the external acidic conditions in a second hand. A silica shell reinforces the system while remaining compatible with both bacteria and human oral intake. With reference to silica, it has been used in dry powdered foods and commercially available probiotic sachets such as Lactibiane (Pileje *SAS*) as an anti-caking agent.

The ultimate focus of the current study was to investigate the viability of free and encapsulated LGG in both carriers (alginate and alginate-silica) after 3 h and one-week in apple juice and beer with a 5 vt % of alcohol content as a proof-of-concept.

2. Materials and methods

2.1. Preparation of freeze-dried core-shell microcapsules

Culture growth and alginate solution: The stock solutions of *Lactobacillus rhamnosus* GG (LMG 18243 from BCCM) were prepared in De Man, Rogosa and Sharpe broth (MRS, Fisher), mixed with glycerol (50/50 vol%) and kept at −80 °C. The LGG culture was obtained from a pre-inoculum and it was incubated for 24 h at 35 °C. The bacteria pellet was harvested with 20 mL of 0.9 wt% NaCl (4500 rpm, 5 min, 21 °C). The supernatant was discarded and 20 mL of 1 wt% sodium alginate (Algin Texturas, Spain) in 0.2 mm filtered 10 mmol/L HCl–Tris pH 8 containing 10 wt% of sucrose (Sigma) was added to the pellet, which was vortexed until obtaining a homogeneous LGG alginate slurry.

Electrospraying: The in-house assembled electrospraying device possesses a variable high-voltage with a 0–30 kV power supply. The LGG alginate slurry was contained in a sterile plastic syringe connected to a stainless steel sterile needle (23G, BD). The anode was connected to the needle and the ground electrode to the aluminium plate holding the collector dish. Negative voltage was applied to the needle and the aluminium plate holding the collector dish was connected to the ground. Solution in the collector dish was also connected to the ground. The distance between the needle tip and the surface of the collecting solution was set at 3.5 cm, the voltage 7.5 kV and the flow 15.0 mL h^{-1}. The alginate beads formed immediately once in contact with 1.5 wt% $CaCl_2$ solution (Roth), previously filtered onto a 0.2 µm sieve. They were kept in the Ca^{2+} solution for at least 1 h before further manipulation.

Mineralization: This step was already described in our previous work (Haffner et al., 2016b). In brief, a volume of 2.5 mL of alginate beads prepared via electrospraying was filtered through a sterile 100 µm sieve and rinsed with 50 ml of autoclaved 0.9 wt% NaCl. The beads were transferred into a sterile conical where 10 mL of 1.5 wt% Tween 40 (Alfa Aesar) was dropped over while vortexing. 100 µL of (3-aminopropyl)trimethoxysilane (APTMS, Acros) was added to the solution and vortexed at 20 Hz for 1 min. In sequence, 100 µL of tetramethyl orthosilicate (TMOS, Acros) was added in the same way and vortexed for another 1 min. The silica-coated alginate beads were filtered through a sterile 100 µm sieve and rinsed with 50 ml of autoclaved 0.9 wt% NaCl. The material was labelled silica-coated.

Freeze-drying: The alginate or silica-coated beads were transferred to fresh sterile conical tubes and plunged in liquid nitrogen for about 5 min. The tubes were placed in a freeze-drier (Cryodos-80, Telstar) for about 20 h reaching −85 °C and 0.2 mbar.

2.2. Bacterial viability

Addition of freeze-dried carriers in beer and apple juice: 10 mL of apple juice (Tropicana Pure premium, 100% apple juice, pasteurized) or 10 mL of beer (Heineken, 5 vt% alcohol content, pasteurized) were added over a batch of 1-day freeze-dried alginate beads (36 mg) or 1-day freeze-dried silica-coated beads (47 mg) and shaked up and down few times. In the case of the control, a pellet of fresh LGG (100 µL of LGG in 10 mL of MRS, 24 h culture at 35 °C) was harvest with 10 mL of 0.9 wt% autoclaved NaCl, centrifuged (4500 rpm, 5 min, 21 °C) and the pellet was lyophilized for about 20 h. The pellet was sequentially re-suspended in 10 mL of autoclaved 0.9 wt% NaCl. 100 µL of the re-suspended pellet was spiked over the 10 mL of apple juice or beer. The conical tubes were kept immobile at 4 °C for 3 h and one-week before the investigations on the release of LGG into the filtrate or its maintenance in the beads. The experiment was done with two biological replicates.

LGG in the filtrate: Alginate and silica-coated beads were filter with a sterile 100 µL sieve and the filtrate was serial diluted and agar plated. These values represent the released bacteria from the carriers.

LGG in the beads: The filtered beads were crushed with a sterile disposable spatula in a first step. 5 mL of autoclaved 0.9 wt% NaCl was added to the tubes and further crushed with an Ultraturrax (3000 rpm, 1 min). The Ultraturrax probe was sterilized before every use under a