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Microencapsulation of functional strains by high pressure homogenization for a potential use in fermented milk

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ABSTRACT

This study was aimed to evaluate the potential of high pressure homogenization for the microencapsulation of two probiotic lactic acid bacteria, *Lactobacillus paracasei* A13 and *Lactobacillus salivarius* subsp. *salivarius* CET 4063 to produce functional fermented milks. Microcapsules of the considered functional microorganisms were obtained by HPH treatments at 50 MPa in the presence of sodium alginate and vegetable oil. The microencapsulated microorganisms were then inoculated as adjuncts to produce fermented milks. As controls were used fermented milks in which the two probiotic lactobacilli were inoculated without encapsulation. The viability of the strains was monitored during almost 2 months of refrigerated storage. The survival of lactic acid bacteria after the gastric-duodenal simulated test was determined. Fermented milk texture parameters, the presence of *exo*-polysaccharides and the production of volatile molecules were also evaluated over storage. The microcapsules, for both the considered probiotic strains, were homogeneous and with a size < 100 μM and therefore did not adversely affect the sensory properties of the fermented milks. The encapsulation decreased the hyperacidity phenomena generally related to the inclusion of probiotic microorganisms in fermented milks. The lower acidity of the products due to the microencapsulation was fundamental for the improvement of the viability of the starter culture and the sensory characteristics of the products. The microencapsulation conditions increased the resistance to the simulated digestion processes, although the strain *Lb. paracasei* A13 generally showed a higher resistance to the gastric barrier respect to *Lb. salivarius* CECT 4063. By contrast, the data obtained showed a reduction of EPS production by the microencapsulation. The volatile profiles showed specific profiles in relation to the probiotic strain used and microencapsulation process. In conclusion, the results of this study underlined the applicative potential of HPH microencapsulation of probiotic microorganisms to produce fermented milk with improved functionality and with enhanced sensory properties.

1. Introduction

The development of a suitable technology for the maintenance of probiotic viability is a key step for the industrial production of functional foods, since, during the fermentation process or during the product storage, probiotic microorganisms could lose their viability (Sarao & Arora, 2017). In general, the viability of probiotic strains added to a food products, especially to a fermented milk, is affected by several factors such as strain sensitivity to process factors (low pH, oxygen, fermentation temperature), food matrix composition (water activity, pH, presence of natural antimicrobials, nutrient availability) and packaging and storage conditions (i.e. refrigeration temperature). Moreover, the gastrointestinal tract (GIT) conditions can affect the viability of the probiotic bacteria during the passage (Barbosa & Teixeira, 2017). In fact, the low pH of the stomach and the presence of bile salts in small intestine can further contribute the loss of viability. On the other hand, it is well established that to confer a functional effect within the body, a probiotic food should contain an adequate number of viable probiotic bacteria (> 7 log CFU/g of food) (Espitia, Batista, Azeredo, & Otoni, 2016). Many attempts have been performed by many researchers to maintain high viability of probiotic strains in food products. For example, Patrignani et al. (2009) and Patrignani, Lanciotti, Mathara, Guerzoni, and Holzapfel (2006) increased the viability of functional strain isolated from Masai milk by the strain selection and the optimization of the milk formulation. Burns, Patrignani, Serrazanetti, Vinderola, Reinheimer, Lanciotti, R., and

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Guerzoni (2008) and Patrignani et al. (2009) used high pressure homogenization (HPH), applied to milk (ranging between 60 and 100 MPa), to increase the availability of free amino acids end free fatty acids for the maintenance of *Lb. paracasei* A13 and *Lb. acidophilus* 08 viability in probiotic fermented milks and cheeses. Muramalla and Aryana (2011) and Tabanelli et al. (2013) demonstrated that sublethal HPH treatment (performed at 50 MPa) improved functional properties of probiotic bacteria (such as hydrophobicity, auto-aggregation and resistance to biological stresses) and preserved their viability during dairy product refrigerated storage. With the same purpose, some authors investigated other non-thermal technologies, applied to probiotic strains at sublethal level, such as Pulsed Electric Field (PEF) and High Hydrostatic Pressure (HPP) (Cueva, 2009; da Cruz, Faria, A, Saad, André Bolini, Sant'Ana, & Cristianini, 2010; Patrignani, Lanciotti, & Guerzoni, 2011). Other evidences highlighted the use of growth factors for the preservation of high probiotic strain cell loads in dairy products during the storage (Prasanna, Bell, Grandison, & Charalampopoulos, 2012).

Recently, the literature data pointed out the use of polymers such as pectin, alginate, carrageenan, chitosan, whey, gelatin and lipids for microencapsulation of bacteria with positive effects in protection of probiotic cells during storage condition and GI environment (Ariful, Yun, Choi, & Cho, 2010). Although the most used entrapping techniques are the extrusion, freeze and spray drying, Ding and Shah (2009) proposed the use of a microfluidizer device with four different settings, based on all possible combinations between number of passes (10 or 20) and pressure (69 MPa or 138 MPa) for the encapsulation of probiotic bacteria starting from an emulsion of sodium alginate and vegetable oil. These process conditions gave microcapsules having a diameter < 100 μm, contrarily to the emulsions obtained by some stirrer conditions (Ding & Shah, 2009). However, the final microcapsule dimensions are related to the pressure applied and to the cell surface properties of bacteria (Burgain et al., 2014).

Thus, the main aim of this research was to evaluate the potential of microencapsulation, carried out by HPH, by using a lab scale equipment, of two probiotic bacteria, *Lactobacillus paracasei* A13 and *Lactobacillus salivarius* CET 4063, for the production of functional fermented milks characterized by high viability of the functional strains. More specifically, the two probiotic bacteria were encapsulated by HPH at 50 MPa using 5 passes, starting from a sodium alginate-vegetable oil emulsion, and used as adjuncts for the production of functional fermented milks. As controls, the products obtained by the same microorganisms without encapsulation were used. All the fermented milks were stored at 4 °C and the viability of fermentation starter and probiotic cells, encapsulated or not, was checked during the refrigerated storage and during a stomach duodenum passage simulation performed after 20 and 34 days of product storage. Moreover, the fermented milks were characterized 24 h after coagulation for their textural feature and EPS presence while their volatile molecule profiles were performed during the refrigerated storage up to 34 days.

2. Material and methods

2.1. Bacterial strains

The strains used in this experimental work were *Lactobacillus paracasei* A13, originally isolated from Argentinean fermented milks, *Lactobacillus salivarius* CET 4063, *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*, obtained from Sacco srl (Italy). Stock cultures of lactobacilli *Lb. paracasei* and *Lb. salivarius* were maintained at −70 °C in de Man, Rogosa and Sharpe (MRS) broth (Oxoid, Basingstoke, United Kingdom), while for *Str. thermophilus* and *Lb. delbrueckii* subsp. *bulgaricus* were provided in lyophilized form in mix by Sacco. Fresh cultures of each strain were obtained from frozen stocks by two consecutive transfers in MRS broth (Oxoid, Basingstoke, United Kingdom) using a 1% (*v*/v) inoculum and incubated at 37 °C in aerobic conditions for overnight. The starter cultures were obtained in skim milk (Oxoid, Basingstoke, United Kingdom) inoculating the medium and incubating at 42 °C.

2.2. Production of microcapsules of Lactobacillus salivarius CET 4063 and Lactobacillus paracasei A13

Functional strains were cultivated in overnight at 37 °C in MRS broth (1 L). In order to obtain a final concentration of 10^{10} CFU/mL, each strain was centrifuged and the pellet suspended in 100 mL of ringer solution. The strain cell loads were evaluated, after proper serial dilutions, onto MRS for *Lb. salivarius* and MRS-LP (Litium Chlorine 0.2% and Sodium Propionate 0.3%) for *Lb. paracasei* A13. Twenty five millilitres of each functional strain, grown in MRS at 37 °C for 24 h, at a concentration of 10^{10} CFU/mL were added into 100 mL of 3% sodium alginate, previously sterilized in autoclave. The mixture was added of 1 mL di Tween 80 and then 200 mL of vegetable oil and gently mixed. Following, it was processed by high pressure homogenization using a Panda homogenizer (Gea, Parma, Italy), provided of a thermal exchanger, at 50 MPa for 5 cycles. This treatment was chosen after different previous trials performed to select the best parameters (in terms of level of pressure and number of passes) to guarantee a good capsule size and high viability of the functional strains. After the last homogenization cycle, sterile calcium chloride solution (0.1 M) was gently added to the emulsion until its breaking. The capsules were maintained at refrigerated conditions for overnight and then collected by centrifugation and let at 4 °C until their use.

2.3. Morphological analysis of the obtained microcapsules

Microcapsule morphology was investigated using scanning electron microscope (SEM) according to the method proposed by De Prisco, Maresca, Ongeng, and Mauriello (2015).

2.4. Enumeration of encapsulated functional strains and encapsulation yield

For each encapsulated strain, 1 g of microcapsules was diluted with 9 mL of phosphate buffer (pH 7) to permit the cell release from alginate. The tubes were left in agitation for 30 min and following serial dilutions were performed and plated onto MRS for *Lb. salivarius* and MRS-LP (Litium Chlorine 0.2% and Sodium Propionate 0.3%) for *Lb. paracasei* A13. To calculate the encapsulation yield, the formula $(N/N_0) * 100$ was used where N is the number of viable cells released from the microcapsules and N_0 is the number of viable cells in the cell concentrate for microencapsulation.

2.5. Preparation of fermented milks

The production of fermented milks was carried out in lab conditions. Milk, treated at 105 °C for 7 min, was splitted in 100 mL containers and each one was inoculated with starter cultures (*Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*) at level of 6 log CFU/mL while the functional strains, encapsulated or not, were inoculated at level of at least 7 log CFU/mL. The inoculated milks were incubated at 42 °C until the reaching of pH 4.6. After that, the fermented milks were stored at refrigeration temperature (4 °C) for 56 days. For each fermented milk, 30 repetitions were performed.

2.6. Reduction of pH

The reduction of pH during fermentation and its evolution in fermented milks during refrigerated storage was monitored using pH meter Basic 20 (Crison Instruments, Modena, Italy).