LWT - Food Science and Technology 73 (2016) 191–196

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

The effect of prebiotics on the viability of encapsulated probiotic bacteria

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ARTICLE INFO

Article history:
Received 2 February 2016
Received in revised form
4 June 2016
Accepted 6 June 2016
Available online 7 June 2016

Keywords:
Alginate
Plantago psyllium
Inulin
Encapsulation
Lactobacillus

ABSTRACT

Probiotics are affected by several factors decreasing their action on the gastrointestinal system. Therefore, is necessary to protect them, using encapsulation techniques for incorporating into various food products. The aim of this work was to evaluate the effect of three natural prebiotics (potato starch (PS), *Plantago psyllium* (PSY) and Inulin (INU)) co-encapsulated with alginate, on viability of *Lactobacillus casei* Shirota (Lc) and two strains of *Lactobacillus plantarum* (Lp33 and Lp17). Physicochemical properties, prebiotic consumption, encapsulation yield and the viability of encapsulated microorganisms during storage at controlled conditions were analysed. The findings revealed a higher encapsulation yield when PSY (94% for Lp17) and INU (78% in Lp33) were used in co-encapsulation with alginate. Capsules produced were spherical and exhibited good flow properties, and the viability during storage was higher at 4 °C for PSY capsules, which also offered the best protection in gastrointestinal conditions.

1. Introduction

New eating habits and trends in disease prevention through diet have forced the creation of functional foods capable of alleviating or preventing gastrointestinal and cardiovascular diseases or even cancer. Many of these advances have been achieved due to the addition of probiotics in foods and the addition of some soluble fibre, known as prebiotics (Oliveira, Perego, Oliveira, & Coverti, 2011). Probiotics are those microorganisms that, when consumed in adequate amounts, confer healthy benefits to consumers (10^6–10^7 CFU/g or mL) (FAO/WHO, 2002). These bacteria are mainly consumed in dairy products. However, there are certain factors that affect viability, such as pH and the lactic and acetic acid, hydrogen peroxide and dissolved oxygen content inside the product (Picot & Lacroix, 2004). For these reasons, the food industry has opted for the development and application of technologies, such as encapsulation, to protect microorganisms before and after consumption in order to ensure their impact on the consumer.

Nevertheless, encapsulation does not ensure the total viability of microorganisms, necessitating in some case the incorporation of natural prebiotics to improve protection and the viability of microorganisms. The encapsulation of prebiotics by complex coacervation is an encapsulation method that allows the addition of one or two polymers within the same capsule (Krasaekoopt, Bhandari, & Deeth, 2004; Iyer & Kailasapathy, 2005). The technique is also characterized by resistance to stomach acidity and fermentation by intestinal bacteria, thus offering health benefits inside the digestive system. Prebiotics are used as an energy source and as metabolic substrates and micronutrients (Chávarri, Marañón, Ares, & Ibáñez, 2010).

The combination of probiotics and prebiotics is known as a symbiotic combination and is used in food products to take advantage the synergic effects of probiotics and prebiotics (Al-Sheraji et al., 2013). Different types of starch, including modified starches, from diverse botanical sources have been used to protect probiotics (Avila-Reyes, Garcia-Suarez, Jimenéz, San Martín-Gonzalez, & Bello-Perez, 2014). Native starches with a small granule size, such as native rice starch containing high levels of slowly digestible starch and resistant starch, are considered as prebiotic (Fuentes-Zaragoza et al., 2011). Other prebiotics used in foods are inulin, fructans and their enzymatic hydrolysates (Lotfipour, Mirzaeei, & Maghsoodi, 2012) as obtained from European chicory (*Cichorium intybus*), which is a soluble fibre with a different polymerization degree, which often reaches the intestine almost intact where it is fermented by bacteria in the intestinal walls and colon. *Plantago psyllium* is composed of a main chain of xylose and highly

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http://dx.doi.org/10.1016/j.lwt.2016.06.021
0023-6438/

branched side chains of xylose and arabinose. It is known as a water-soluble fibre from whole and mature seeds as well as the shells and epithelia of the genre *Plantago*. Its prebiotic effect has been reported in different studies as the *Plantago* fibre, after consumption goes directly to intestine where it is fermented by bacteria promoting their activities and development (Lotfipour et al., 2012). The application of *Plantago psyllium* in encapsulation and its prebiotic benefits have not been fully explored (Mirzaeei, Rezaei, & Rashidi, 2012). There are reports of encapsulated probiotics with and without prebiotic addition and increased viability in those capsules enriched with prebiotics such as inulin or modified starches (Guimarães, Vendramini, dos Santos, Ferreira, & Lemos, 2013). Prebiotics, such as resistant starch, inulin and fructooligosaccharide in the drying medium can accumulate within the cells and reduce the osmotic difference between the internal and external environment, in addition to reaching the large intestine without modification, and are available to be metabolized by microorganisms as lactobacillus and bifidobacteria (Schwab, Vogel, & Gänzle, 2007).

Therefore, the objective of this study was to evaluate the effect of the addition of natural prebiotics (potato starch, *Plantago psyllium* or inulin), on physicochemical properties, yield encapsulation and cell viability during gastric and intestinal simulation tests and during storage at 4 and 22 °C of *Lactobacillus casei* Shirota and two species of *Lactobacillus plantarum* (Lp17 and Lp33) co-encapsulated with alginate.

2. Material and methods

2.1. Material

For the prebiotics, potato starch extracted directly from natural potato (PS), *Plantago psyllium* (PSY) fibre (Solgar, England) and chicory inulin (INU) (Alfa Aesar, USA) were utilized. All water used in this work was distilled and sterilized.

2.2. Bacterial strains

The different bacteria used in this study (*Lactobacillus casei* Shirota (Lc), *Lactobacillus plantarum* 33 (Lp33) and 17.2b (Lp17)) were provided by the Laboratory of Microbial Food Technology belonging to the Chemistry and Biological Technology Institute of Nova de Lisboa University, Portugal.

2.3. Inoculum standardization

Before use, the bacteria were reactivated three times to standardize their concentration. They were first incubated with 5 mL of MRS broth at 37 °C for 24 h and an inoculum concentration of 1% v/v (Ding & Shah, 2007). Later, two reactivations of 16 h under the same conditions of incubation and concentration were made. The last reactivation was used in the recovery process of encapsulation. Bacteria were harvested by centrifugation (Eppendorf 5430R, Germany) at 2935 × *g* for 10 min at 4 °C and re-suspended in phosphate buffer saline (PBS), prior to being added in the emulsion (Picot & Lacroix, 2004; Iyer & Kailasapathy, 2005).

2.4. Prebiotics consumption

MRS medium was modified by replacing glucose as a carbon source and by replacing it, in the same ratio (20 g L^{-1}), with PS, INU or PSY. Then, the medium was homogenized for 5 min using a homogenizer (IKA Ultra Turrax T25 basic, USA) with a speed of 22,000 rpm. Three modified media were sterilized at 121 °C for 15 min before inoculation with the bacteria at a concentration of 1% v/v. The optical density was monitored every 2 h for a 24 h period using a UV-visible spectrophotometer (Jasco SSE-343 V530, Japan) and a wavelength of 600 nm (Zhou, Martins, Groboillot, Champagne, & Neufeld, 1998).

2.5. Capsule preparation

The methodology of Krasaekoopt, Bhandari, and Deeth (2004) was modified. A total 100 g of emulsion was prepared, mixing 1% of prebiotics (PS, PSY or INU) and 85% of distilled water, previously sterilized. Thereafter, were added 10% of edible oil and 20 mL of culture suspended in PBS. This mixture was stirred at 10,000 U/min for 15 min on a magnetic stirrer plate (P-Selecta Agimatic-HS, Spain). Subsequently, 2% of sodium alginate (sterilized under ultraviolet light for 15 min) was added, and the mixture was stirred again for 30 min at 600 u/min. All percentages used were in w/v relation with respect to the 100 g of emulsion prepared. Then, the emulsion was injected using a peristaltic pump (Watson Marlow 502S, England) with a power of 1–2% (1.2 rpm) through a hose with a diameter of 3 mm and a needle with a diameter of 0.1 mm. The suspension was dropped into 1 L of sterile calcium chloride ($CaCl_2$, 2% w/v) with a flow rate of 1 g/min from a height of 20 cm. Prepared capsules were crosslinked in the $CaCl_2$ for 30 min. They were then washed with sterile distilled water and dried on filter paper at 40 °C for 4 h in an air oven (Guimarães et al., 2013).

2.6. Characterization of capsules

The diameter of capsules was measured using photographs taken by an inverted microscope (Motic AE31) with a 5 megapixel camera (CMOS, Moticam 5) and a 4× objective. The moisture content (% wet basis) of the capsules was determined by oven drying at 102 ± 2 °C until the capsules reached a constant weight (AOAC, 1995). The hygroscopicity of the microcapsules was determined according to the procedure described by Fritzen-Freire et al. (2012). The water activity was measured at 25 °C using Aqualab 4 TE (Decagon 142 Devices, Pullman, WA, USA).

2.7. Flow properties of the capsules

The loose bulk density and tapped bulk density were determined by pouring 2 g of the sample into an empty measuring cylinder. The cylinder was slightly tapped and the volume was read directly to calculate the loose bulk density. The tapped bulk density was calculated by vortexing the measuring cylinder containing the sample for 2 min in order to reach a constant volume (Quispe-Condori, Saldaña, & Temelli, 2011), and the flowability of the microcapsules was evaluated in terms of the Haussner ratio as described by Lebrun et al. (2012).

2.8. Encapsulation efficiency

The encapsulation efficiency was determined by the release of the encapsulated bacteria by dissolution of the calcium alginate wall into a sodium citrate solution of 1% adjusted to pH 6 and by steeping the capsules for 15 min at 37 °C and homogenizing them using a Stomacher instrument (Seward Circulator 400, England) at 230 rpm for 3 min. Viability counts were made on MRS agar at the end of 48 h of incubation at 37 °C (Krasaekoopt et al., 2004).

2.9. The survival of free and encapsulated bacteria in simulated gastrointestinal conditions

Simulated gastric juice and simulated intestinal juice (SGJ) were used according to the method described by Valero-Cases and Frutos