Food Chemistry 239 (2018) 287–294

Contents lists available at ScienceDirect

Food Chemistry

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

Production of RS4 from rice starch and its utilization as an encapsulating agent for targeted delivery of probiotics

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ARTICLE INFO

Article history:
Received 16 December 2016
Received in revised form 3 May 2017
Accepted 20 June 2017
Available online 21 June 2017

Keywords:
Probiotics
RS4
Microencapsulation
Emulsification
Targeted delivery

ABSTRACT

The research reported in this article is based on the hypothesis that crosslinking of starch can make it a potential wall material for targeted delivery of probiotics by altering its digestion. Three probiotic strains namely *Lactobacillus casei*, *Lactobacillus brevis* and *Lactobacillus plantarum* were microencapsulated with resistant starch. Encapsulation yield (%) of resistant starch microspheres was in the range of 43.01–48.46. The average diameter of resistant starch microparticles was in the range of 45.53–49.29 μm. Fourier transform infrared (FT-IR) spectroscopy of microcapsules showed peaks in the region of 900–1300 cm^{-1} and 2918–2925 cm^{-1} which corresponds to the presence of bacteria. Differential Scanning Calorimeter (DSC) showed better thermal stability of resistant starch microcapsules. Microencapsulated probiotics survived well in simulated gastrointestinal conditions and adverse heat conditions. The viability of the microcapsulated lactobacilli also remained high (>7 log cfu g^{-1}) for 2 months at 4 °C. The results revealed that resistant starch is the potential new delivery carrier for oral administration of probiotics.

1. Introduction

Probiotics are defined as live microorganisms, which confer a health benefit on the host when administered in adequate amounts (FAO/WHO, 2001; Homayouni, 2009). They can provide beneficial effects on the human body by keeping the healthy gut microbiota, inhibiting the growth of pathogenic bacteria, relieving constipation, stimulating the immune system, synthesizing vitamins and antimicrobial agents, and improving the absorption of calcium (Rokka & Rantamaki, 2010; Homayouni Rad, Torab, Ghalibaf, Norouzi, & Mehrabany, 2013). However, in order for probiotics to exert these beneficial effects, their high viability should be preserved in the gut after their passing through the upper gastrointestinal tract. The acidic conditions of the stomach and the bile salts secreted into the duodenum are the main obstacles for the survival of the ingested bacteria (DeCastro-Cislaghi, Silva, Fritzen-Freire, Lorenz, & Anna, 2012). Due to numerous health benefits of probiotics, it is urgent to develop new methods to preserve their viability. Moreover, for development of functional foods having adequate amounts of viable cells survival of probiotics during processing and storage of food is also essential (Homayouni, Azizi, Javadi, Mahdipour, & Ejtahed, 2012).

Microencapsulation is a promising technique for bacterial cell protection and several studies have investigated the protective role of this technique against adverse conditions to which probiotics can be exposed (De Castro-Cislaghi, Silva, Fritzen-Freire, Lorenz, & Anna, 2012; Sultana et al., 2000). Recently microencapsulation has also been found to be a useful technique for stabilization of probiotics in functional food applications (Sathyabama, Kumar, Devi, Vijayabharathi, & Priyadharisini, 2014). Several encapsulation technologies, such as emulsion, extrusion, spray drying and coacervation have been reported (Heidebach, Forst, & Kulozik, 2012). However considering the cost, simplicity and gentle formulation conditions for retention of cell viability, emulsification technique is widely used (Homayouni, Azizi, Ehsani, Yarmand, & Razavi, 2008; Homayouni, Ehsani, Azizi, Yarmand, & Razavi, 2007). Emulsion technology has a potential for large-scale production of the microspheres in shorter time (Takei, Yoshida, Hatate, Shiomori, & Kiyoyama, 2009), which is essential for commercial application. For emulsification technology, polysaccharide aqueous solution is dispersed in oil phase to form W/O emulsion, and $CaCl_2$ solution is then added with continuous stirring for emulsification and encapsulation of probiotics (Mokarram, Mortazavi, Najafi, & Shahidi, 2009).

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http://dx.doi.org/10.1016/j.foodchem.2017.06.110
0308-8146/

The most common wall materials used for encapsulation are food grade biopolymers, such as alginate, cyclodextrin, chitosan, xanthan gum, whey proteins, gelatin and starch (Homayouni et al., 2014; Wani et al., 2016). Starch has often been used in combination with alginate as an encapsulant for probiotics (Mirzaei, Pourjafar, & Homayouni, 2012; Sultana et al., 2000). A majority of these reports used high-amylose starch to take advantage of its prebiotic properties, besides its non-specific encapsulant capability.

Resistant starch (RS) is that portion of starch, which escapes digestion in the small intestine and thus may be fermented in the colon (Ashwar, Gani, Shah, Wani, & Masoodi, 2015). The ability of resistant starch to escape digestion in the small intestine reflects its potential as a wall material for targeted delivery of probiotics into the colon (Ashwar et al., 2015). Because of these health benefits of resistant starch, different techniques have been employed for its preparation. These include hydrothermal treatments, annealing, partial gelatinization and recrystallization, autoclaving, pullulanase debranching, temperature-cycled retrogradation, phosphorylation, hydroxypropylation, acetylation, oxidation, and citric acid modification (Ashwar, Gani, Shah, & Masoodi, 2017; Ashwar et al., 2016). Sang, Seib, Herrera, Prakash, and Shi (2010) reported production of resistant starch (RS4) by the phosphorylation of starch. Chuang, Panyoyai, Katopo, Shanks, and Kasapis (2016) reported that the interactions of calcium ions with the phosphate and hydroxyl groups of starch produce dense structures. The dense structures can be used as an encapsulating material for targeted delivery of probiotics.

To our knowledge no data on characterization of resistant starch type 4 (RS4) as an encapsulating agent and prebiotic for targeted delivery of probiotics has been published. This work was therefore intended to develop RS4, which was then used to encapsulate three probiotic bacterial cultures namely *Lactobacillus plantarum*, *Lactobacillus casei*, and *Lactobacillus brevis*. The microstructure and thermal stability of microcapsules, survival of encapsulated probiotics in simulated gastrointestinal conditions and their viability during storage were also investigated with the objective of producing enough viable probiotic bacteria for potential application in biotechnological or food industries.

2. Materials and methods

2.1. Materials

The probiotic cultures viz. *Lactobacillus brevis* (MTCC 01), *Lactobacillus casei* (MTCC 297) and *Lactobacillus plantarum* (MTCC 021) were procured from National Dairy Research Institute, Karnal, India (NDRI). These probiotic cultures were activated in sterile MRS broth (HiMedia Laboratories Pvt Ltd, Mumbai, India) and incubated at 37 °C for 24 h. Afterwards, the cells were harvested as per the method of Rajam, Karthik, Parthasarathi, Joseph, and Anandharamakrishnan (2012). All the chemical reagents used in the present study were of analytical grade.

2.2. Extraction of starch

Rice starch was extracted according to the alkali steeping method described by Ashwar et al. (2016). The extracted starch was analyzed for moisture (925.10), protein (920.87), fat (920.85) and ash (923.03) according to the methods of AOAC (1990).

2.3. Preparation of cross-linked phosphorylated rice starch

Phosphorylated rice starch was prepared according to the method described by Woo and Seib (2002).

2.4. Resistant starch content

Resistant starch content was determined using the Megazyme Assay Kit (Megazyme International, Wicklow, Ireland), following the approved AACC method 32–40 (AACC, 2000). Briefly 100 mg sample and 4 mL of enzyme mixture (pancreatic α-amylase and amyloglucosidase) were added to each test tube, mixture vortexed and then incubated in a shaking water bath for 16 h at 37 °C (200 strokes/min) to hydrolyze digestible starch. At the end of the incubation period suspension was mixed with 4 mL absolute ethanol and vortexed to deactivate the enzymes and RS was recovered as a pellet by centrifugation (1500*g*, 10 min). Pellet was washed with 50% ethanol twice to remove the digested starch. The sediment was dissolved in 2 mL of 2 M KOH by vigorously stirring for 20 min in an ice bath. This solution was neutralized with 8 mL sodium acetate buffer (1.2 M). Solution was mixed with amyloglucosidase (0.1 mL, 3300 U/mL) and then incubated in a water bath at 50 °C for 30 min, then the samples were centrifuged at 3000*g* for 10 min. Three mL of glucose-oxidase–peroxidase-aminoantipyrine (GOPOD) was added to aliquots (0.1 mL) of the supernatant, and the mixture was incubated at 50 °C for 20 min. Absorbance was measured using a spectrophotometer at 510 nm. Resistant starch was calculated as the amount of glucose × 0.9. Each sample was analyzed in triplicate.

2.5. Encapsulation

A slightly modified method of Sultana et al. (2000) was used. The slurry was prepared by mixing 2 g/100 mL of resistant starch and 1 mL of the three cultures separately. The mixture was dropped into oil in the ratio of 1:1. To this mixture 0.02 mL/100 mL of Tween 80 was added. After that the mixture was homogenized at 1500 rpm for 10 min till it was emulsified and appeared creamy. Then the mixture was dropped into 0.1 mol/L calcium chloride solution. The beads were left for 30 min, separated by centrifugation and washed with 0.9 g/100 mL saline containing 5 mL/100 mL glycerol, freeze dried and stored at 4 °C.

2.6. Confirmational study by using ATR-FTIR

Spectra of the samples were recorded using FTIR spectrometer system (Cary 630 FTIR, Agilent Technologies, USA), coupled to an ATR accessory. Analysis was carried out at room temperature, and spectra were acquired in the range of 400–4000 cm^{-1} at a resolution of 4 cm^{-1}, using Resolution Pro software version 2.5.5 (Agilent Technologies, USA).

2.7. Thermal analysis

The thermal characteristics of RS4 microcapsules like melting temperature and enthalpy of melting were studied with a Differential Scanning Calorimeter (DSC-1STAR^e System, Mettler-Toledo). The 3.5 mg sample was weighed into platinum pan and distilled water (8.0 μL) was added. The pan was kept at room temperature for one hour before analysis. The samples were heated at 10 °C/min from 20 to 200 °C. An empty platinum pan was used as a reference.

2.8. Morphological characterization of the microcapsules

Morphology of the microcapsules was investigated using a scanning electron microscope (SEM) (Hitachi S-300H-Tokyo, Japan). The freeze-dried microcapsules were placed on an adhesive tape attached to a circular aluminum specimen stub. After coating vertically with gold- palladium, the samples were photographed at an accelerator potential of 5 kV.