Food Chemistry 216 (2017) 97–105

Contents lists available at ScienceDirect

Food Chemistry

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

Effective *Lactobacillus plantarum* and *Bifidobacterium infantis* encapsulation with chia seed (*Salvia hispanica* L.) and flaxseed (*Linum usitatissimum* L.) mucilage and soluble protein by spray drying

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ARTICLE INFO

Article history:
Received 26 April 2016
Received in revised form 5 August 2016
Accepted 8 August 2016
Available online 9 August 2016

Keywords:
Microencapsulation
Spray drying
Probiotics
Flaxseed
Chia seeds

ABSTRACT

Mucilage (M) and soluble protein (SP) extracted from chia seed and flaxseed were used as encapsulating material for two probiotic bacteria: *Bifidobacterium infantis* and *Lactobacillus plantarum* by spray drying. Probiotic survival and viability after spray drying and during storage were evaluated. *B. infantis* and *L. plantarum* displayed high survival (⩾98%) after encapsulation with mixtures of maltodextrin (MD) combined with M and SP from flaxseed (MD:FM:FSP – 7.5:0.2:7.5%, w/w/w) and chia seed (MD:CM:CSP – 7.5:0.6:7.5%, w/w/w), respectively. These ternary blends protected the probiotics and enhanced their resistance to simulated gastric juice and bile solution. Probiotics encapsulated with the ternary blends incorporated in instant juice powder exhibited high viability (>9 Log_{10}CFU/g) after 45 days refrigerated storage. Encapsulation with the ternary blends reduced particle size of the probiotic powders thereby offering additional functional benefits. Our results reveal that chia seed and flaxseed are excellent sources of probiotic encapsulating agents.

1. Introduction

Probiotics are defined as "live microorganisms that, when administered in adequate amounts confer a health benefit on the host" (FAO/WHO, 2002). Several medical conditions, including diarrhea, irritable bowel syndrome, cancer, depressed immune function, infant allergies, diabetes mellitus, lactose intolerance, among others, can be treated with probiotics. They can also inhibit the development and/or displacement of pathogenic bacteria in the gut (Sharma & Devi, 2014). Species in the *Lactobacillus* and *Bifidobacterium* genera are the most studied probiotic bacteria, and some of them are used by the food industry in fermented and non-fermented food products (Felicio et al., 2016; Homayouni, Azizi, Ehsani, Yarmand, & Razavi, 2008), in supplements, nutraceutical and pharmaceutical products. *L. plantarum* strains are commonly found in fermented vegetable food; some produce antimicrobial peptides (Wen, Philip, & Ajam, 2016) and antiallergenic proteins (Song, Oh, & Lim, 2016). Bifidobacteria are found in human and infant intestinal tract; some species, including *B. infantis*, are potent anti-depressants, antianxiety agents, and exert anti-nociceptive effects on irritable bowel syndrome (Wang & Kasper, 2014).

Microencapsulation improves probiotic survival and viability according to several studies (Bustamante, Villarroel, Rubilar, & Shene, 2015; Fritzen-Freire et al., 2012; Homayouni et al., 2008). It is often performed by spray drying due to its low energy requirements and cost, high process yield, ready scalability, and low end product moisture content ensuring its storage stability (Corcoran, Ross, Fitzgerald, & Stanton, 2004; Lian, Hsiao, & Chou, 2002). Nevertheless, the elevated temperatures used in spray drying and the low moisture content can be stressful to cells affecting their survival during drying (Boza, Barbin, & Scamparini, 2004). The survival and viability of probiotics can be improved for a given strain by determining the effects of culture age and conditions for pre-adapting the culture, optimizing spray drying conditions, and solid composition of the encapsulating solution (Corcoran et al., 2004; Fritzen-Freire et al., 2012; Rajam, Karthik, Parthasarathi, Joseph, & Anandharamakrishnan, 2012; Simpson, Stanton, Fitzgerald, & Ross, 2005).

Abbreviations: CM, chia seed mucilage; CSP, chia seed soluble protein; FM, flaxseed mucilage; FSP, flaxseed soluble protein; M, mucilage; MD, maltodextrin; SEM, scanning electron microscope; SP, soluble protein.

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http://dx.doi.org/10.1016/j.foodchem.2016.08.019
0308-8146/

The composition of the encapsulating solution is relevant because it can define the level of protection against oxygen, acid pH, and high salt concentration. It can also minimize the heat treatment effects to some extent, improving survival during drying and viability during storage. Several encapsulating agents (maltodextrin, protein, reconstituted skim milk, polysaccharides, among others) have been used to protect probiotics from thermal and dehydration damage during spray drying, maintain their stability during storage, and their viability under gastrointestinal conditions of the host (Lapsiri, Bhandari, & Wanchaitanawong, 2012; Rajam et al., 2012). Today, there is interest in identifying new encapsulating agents with similar or better physico-chemical properties (Rodríguez-Huezo et al., 2007) and bioactive characteristics such as prebiotic (Gibson, Probert, Van Loo, Rastall, & Roberfroid, 2004) activity, that is ingredients that selectively stimulate probiotic bacteria in the gastrointestinal system (Fritzen-Freire et al., 2012).

Flaxseed (*Linum usitatissimum* L.) and chia (*Salvia hispanica* L.) seeds are sources of bioactive phenolic compounds, polyunsaturated fatty acids, dietary fiber and proteins (Oomah, 2003; Sandoval-Oliveros & Paredes-López, 2013). Flaxseed mucilage (FM) is a mixture of neutral polysaccharides consisting of rhamnogalacturonan I and arabinoxylan with the latter exhibiting prebiotic properties (Naran, Chen, & Carpita, 2008). The intake of FM reduces blood glucose and cholesterol in diabetics (Oomah, 2003) and may suppress postprandial lipemia (Kristensen et al., 2013). On the other hand, flaxseed protein intake controls blood glucose by stimulating insulin secretion (Nuttall, Mooradian, Gannon, Billintong, & Krezowski, 1984).

Chia seeds can be considered a functional food because it beneficially affects cardiovascular diseases, inflammatory and nervous system disorders, intestinal transit regulation, and blood cholesterol and triglyceride levels (Muñoz, Cobos, Diaz, & Aguilera, 2013). Chia seeds have been used as nutritional supplements as well as in the manufacture of bars, breakfast cereals and cookies (Muñoz, Aguilera, Rodriguez-Turienzo, Cobos, & Diaz, 2012), while chia seeds flour has been used for the formulation of gluten-free bread with high nutritional value (Costantini et al., 2014). Globulins (mostly 11S and 7S) are the major proteins in chia seeds (Sandoval-Oliveros & Paredes-López, 2013). 7S globulins exhibit emulsifying properties and 11S globulins have antihypertensive activity (Barba de la Rosa, Herrera, Utsumi, & Paredes-López, 1996; Plietz et al., 1986).

The use of soluble fiber and proteins derived from both seeds (flaxseed and chia) is limited in the food industry despite their beneficial functional and bioactive properties. Besides, at least for chia there are no studies evaluating the seed as source of ingredients for encapsulating bacteria through spray drying, their effects on cell survival after spray drying, and cell viability during storage of the encapsulated cells.

This investigation aims at evaluating the feasibility to increase *B. infantis* ATCC 15679 and *L. plantarum* ATCC 8014 survival after spray drying using mucilage and soluble protein fractions from chia seeds or flaxseed as encapsulating materials. The viability of encapsulated *B. infantis* and *L. plantarum* were determined during storage at 4 °C and during simulated juice and bile incubation. We also evaluated the viability of the encapsulated probiotics incorporated in a commercial instant juice powder during storage at 4 °C.

2. Materials and methods

2.1. Probiotic strains, chemicals, and culture mediums

B. infantis ATCC 15679 and *L. plantarum* ATCC 8014 were from American Type Culture Collection (Rockville, MD, USA) and *L. acidophilus* La-05 (La-05®) from Chr. Hansen, (Hónsholm, Denmark). Food grade maltodextrin (MD) with a dextrose equivalent of 15 was purchased from PRINAL®. Flaxseed and chia seeds were purchased from the local market. Pepsin (0.7 FIP-U/mg) was obtained from Merck (Darmstadt, Germany) and bile bovine from Sigma (St Louis, MO, USA). Lactobacilli broth (MRS broth, de Man, Rogosa, & Sharpe, 1960) and agar were from Difco (USA). GasPak™ anaerobiosis generator system was purchased from BD (USA). All chemicals were of analytical grade.

2.2. Extraction of FM and flaxseed soluble protein (FSP)

The FM was extracted according to Bustamante et al. (2015). Briefly, seeds were extracted with hot distilled water (90–95 °C, pH 5.0, 30 min under agitation) at 1:10 (w/v) ratio. The extraction was repeated three times. The extract was spread on a tray and dried (60 °C) in an air convection heat oven. Mucilage-free seeds were dried (60 °C), milled and passed through a 0.5 mm mesh. The flour was defatted with hexane twice (ratio 1:10, w/v) in a shaker (160 rpm, 10 °C, 8 h). The defatted flour was recovered by filtration (Whatman® N° 1), the solvent evaporated at room temperature, and the solvent-free flour stored at 4 °C until protein extraction.

FSP was extracted from the defatted flour with 0.10 M NaCl in 0.10 M Tris buffer at pH 8.6 (ratio 1:16, w/v) (Li-Chan & Ma, 2002) in a shaker (160 rpm, 4 °C, 16 h). The extract was passed through a double layer of cheesecloth and centrifuged (7000×*g*, 4 °C, 20 min). The supernatant was dialyzed (MWCO 6000–8000, Spectra/Por, Spectrum Laboratories, Inc., CA, USA) against distilled water for 2 days at 4 °C, dried (60 °C), milled and stored at −20 °C until use.

2.3. Extraction of chia seed mucilage and soluble protein

The CM extraction was carried out according to Muñoz, Cobos, Diaz, and Aguilera (2012) with some modifications. Seeds were extracted with hot distilled water (80 °C, pH 6.0, 2 h) at 1:40 (w/v) ratio. The extraction was repeated twice and the extract was spread on a tray and dried (60 °C) in an air convection heat oven. Mucilage-free seeds were dried (60 °C), milled and passed through a 0.5 mm mesh. The flour was defatted with hexane twice (ratio 1:10, w/v) in a shaker (160 rpm, 10 °C, 8 h). The defatted flour was recovered by filtration (Whatman® N° 1) and the solvent was evaporated at room temperature; the solvent-less flour was stored at 4 °C until protein extraction.

Chia seed protein was extracted from defatted flour according to Sandoval-Oliveros and Paredes-López (2013) with some modification. The defatted flour was mixed with distilled water at 1:10 (w/v) ratio, the suspension stirred (4 °C, 4 h) and centrifuged (7000×*g*, 4 °C, 40 min). The pellet was re-suspended in 50 mM Tris-HCl, pH 8, containing 0.5 M NaCl. The extract was passed through a double layer of cheesecloth and centrifuged. The supernatant was dialyzed (MWCO 6000–8000, Spectra/Por, Spectrum Laboratories, Inc., CA, USA) against distilled water for 2 days at 4 °C, dried (60 °C), milled and stored at −20 °C until use.

2.4. Bacterial culture preparation

Bacteria were sub-cultured twice with 5% (v/v) inoculum in 5 ml of MRS broth (37 °C, 12 h). For *B. infantis*, MRS broth was supplemented with L-cysteine-HCl (0.05%, w/v) and incubated under anaerobic conditions (GasPak™ anaerobiosis generator system). The cells for the spray drying assays were grown in 400 ml of MRS broth inoculated (5%, v/v) with the grown culture and incubated under the same conditions as described for the inoculum. Cells in late-log phase were harvested by centrifugation (6000×*g*, 4 °C, 6 min); the pellet washed with sterile distilled water and