Food Hydrocolloids 54 (2016) 328–337

Contents lists available at ScienceDirect

Food Hydrocolloids

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

Incorporation of polysaccharides into sodium caseinate-low melting point fat microparticles improves probiotic bacterial survival during simulated gastrointestinal digestion and storage

Huan Liu [a, b], Joshua Gong [b], Denise Chabot [c], S.Shea Miller [c], Steve W. Cui [b], Jianguo Ma [a], Fang Zhong [a, **], Qi Wang [b, *]

[a] Key Laboratory of Food Colloids and Biotechnology, Ministry of Education, School of Food Science and Technology, Jiangnan University, Wuxi, 214122, PR China
[b] Guelph Food Research Centre, Agriculture and Agri–Food Canada, Guelph, Ontario, N1G 5C9, Canada
[c] ECORC, Agriculture and Agri–Food Canada, Ottawa, Ontario, K1A 0C6, Canada

ARTICLE INFO

Article history:
Received 13 July 2015
Received in revised form
15 October 2015
Accepted 19 October 2015
Available online 21 October 2015

Keywords:
Microencapsulation
Spray dry
Lactobacillus
Sodium caseinate
Storage
Water activity

ABSTRACT

In our previous study we reported that sodium caseinate (NaCas) can effectively protect probiotic bacterial cells from heat inactivation during spray drying in the presence of low melting point fat (LMF). The objective of the current research was to enhance the gastric resistance and storage properties of NaCas-LMF based probiotic microparticles by incorporating different carbohydrate polymers. Probiotic bacteria were spray dried in NaCas solutions containing LMF combined with one of: maltodextrin, pullulan, gum ghatti or gum arabic (GA). Probiotic bacteria only showed good survival (≥50%) after spray drying in microcapsules formulated with gum ghatti or gum arabic. Addition of GA and gum ghatti to NaCas showed a positive effect on the *in vitro* gastric resistance of probiotic bacteria, whereas maltodextrin and pullulan exerted a negative influence. Among the formulations tested, microparticles made from a blend of NaCas and GA showed the highest glass transition temperature (T_g), corresponding to the best survival of probiotic bacteria during storage for up to 16 weeks in a water activity range of 0.11–0.76. The presence of two T_gs in the NaCas-pullulan matrix suggested that phase separation was occurring, which could partially account for its poor protection capacity. Encapsulated probiotic bacteria in all matrices showed good release properties in the presence of pig intestinal digesta; release from microparticles was complete in less than 1 h. The current study demonstrates that NaCas-LMF combined with GA may provide effective protection to probiotic bacterial cells not only during spray drying, but also during storage and *in vitro* digestion.

1. Introduction

As defined by the Food and Agriculture Association of the United Nations (FAO) and World Health Organization (WHO), probiotics are live microorganisms which, when administered in adequate amounts, confer a health benefit to the host. In order to better exert its health benefits, a probiotic bacterium must remain viable during use. However, after ingestion of probiotics, a considerable loss of viability was reported in many tested strains when they passed through the low pH of the stomach and high bile salt conditions of the intestine (Dianawati & Shah, 2011; Ding & Shah, 2007). Through encapsulation of the bacteria in various polymer matrices, such viability losses may be minimized. In the functional food industry, spray drying is the most commonly used microencapsulation method, as it is economical, flexible and produces good quality powder products (Martín, Lara-Villoslada, Ruiz, & Morales, 2014). Although several successful formulas have been reported for encapsulation of probiotic bacteria by spray drying (Fritzen-Freire et al., 2012; Páez, Lavari, Audero, Cuatrin, Zaritzky, Reinheimer, et al., 2013; Zhao, Sun, Torley, Wang, & Niu, 2008), loss of viability of heat-sensitive strains during the spray drying process has remained a concern. Detailed discussions can be found in other previously published reviews (Cook, Tzortzis, Charalampopoulos, &

* Corresponding author.
** Corresponding author.
E-mail addresses: fzhong@jiangnan.edu.cn (F. Zhong), qi.wang@agr.gc.ca (Q. Wang).

http://dx.doi.org/10.1016/j.foodhyd.2015.10.016
0268-005X/

Khutoryanskiy, 2012; Heidebach, Först, & Kulozik, 2012; Tanzina, Avik, Ruhul, Riedl, & Lacroix, 2013). In our previous research, we reported that the addition of low melting point fat (LMF) into sodium caseinate (NaCas) particles effectively improved the survival of a heat-sensitive probiotic bacterium during spray drying (Liu et al., 2015). However, the storage properties and survival rate of encapsulated probiotic cells during gastric transition require further improvements to enhance delivery to the intestines.

Previous studies have reported that adding polysaccharides to the protein matrix could enhance both storage and gastric digestion properties of encapsulated probiotic bacteria (Borza et al., 2010; Léonard, Degraeve, Gharsallaoui, Saurel, & Oulahal, 2014; Léonard et al., 2013; Rajam, Karthik, Parthasarathi, Joseph, & Anandharamakrishnan, 2012). For example, Crittenden, Weerakkody, Sanguansri, and Augustin (2006) reported that encapsulation of *Bifidobacterium infantis* Bb-02 into particles composed of NaCas-oil-dried glucose syrup/microfluidized resistant starch significantly improved its survival in simulated gastrointestinal fluid (about 5 log CFU/g higher compared with that of non-encapsulated bacteria at pH 1.2 for 2 h) and during storage (about 6 log CFU/g higher compared with that of non-encapsulated bacteria for 5 weeks at 25 °C and 50% relative humidity). The polysaccharides most commonly used in these studies include maltodextrin, a neutral polysaccharide derived from the enzymatic hydrolysis of starch and pullulan, a linear homopolysaccharide of glucose secreted primarily by strains of the fungus *Aureobasidium pullulans* (Leathers, 2003). Research has suggested such metabolizable polymers can enhance probiotic survival under acidic conditions by providing a source of ATP for the F_0F_1-ATPase and therefore enabling proton exclusion from the cytoplasm to the environment (Borza et al., 2010; Sanders, Venema, & Kok, 1999). According to Arslan, Erbas, Tontul, and Topuz (2015) and Desmond, Ross, O'Callaghan, Fitzgerald, and Stanton (2002), during spray drying, gum arabic forms a semipermeable wall around probiotic bacteria which protects them from the adverse outside environment. Gum arabic is a natural polymer derived from Acacia trees that consists of three major fractions: arabinogalactan, arabinogalactan–protein complex and glycoprotein (Ali, Ziada, & Blunden, 2009). Gum ghatti is also a tree-derived polymer with a complex structure composed of mainly L-arabinose, D-galactose D-mannose, D-xylose and D-glucuronic acid (Al-Assaf, Phillips, & Amar, 2009). Both gum arabic and gum ghatti are weak polyelectrolytes due to the existence of carboxylic groups. It is known that carboxyl groups have the ability to form self-complementary dimer synthons (i.e. the pairing of OH···O hydrogen bonds), which enhances the strength of the hydrogen bonding considerably (Barth, Weckesser, Lin, Dmitriev, & Kern, 2003). In the current study, we hypothesized that addition of gum arabic or gum ghatti to NaCas microparticles could offer additional protection to probiotic bacteria during the gastric digestion and storage as compared to neutral polysaccharides. Therefore, the purpose of this study was to investigate the effects of incorporation of gum arabic and gum ghatti into NaCas-LMF microparticles on the storage properties and gastric resistance of probiotic bacteria as compared to the two neutral polysaccharides pullulan and maltodextran.

2. Materials and methods

2.1. Materials

Sodium caseinate, maltodextrin (dextrose equivalent value = 16.5–19.5), gum ghatti and gum arabic (reagent grade) were purchased from Sigma–Aldrich Chemical Co., Ltd (St. Louis, MO, USA). Pullulan was obtained from Hayashibara Co., Ltd (Okayama, Japan). Low melting point fat (LMF), a multi-purpose bakery shortening based on palm oil and fractions of palm oil, was obtained from IOI Loders Croklaan Inc. (SansTransTM 39, IL, USA). All glassware used in this study was sterilized at 121 °C for 15 min.

2.2. Bacterial cell preparation

Lactobacillus zeae LB1 is an isolate from chicken intestine (Wang, Wang, Gong, Yu, Pacan, Niu, et al., 2011) that has been shown to reduce the population of enteric pathogens in animals (Yang, Brisbin, Yu, Wang, Yin, Zhang, et al., 2014; Yin et al., 2014). This isolate was cultured in de Man Rogosa Sharpe (MRS) broth or agar (Beckton Dickinson and Company Sparks, MD, USA) at 37 °C under anaerobic atmosphere (85% N_2, 10% CO_2, and 5% H_2). The activation was conducted by incubation on MRS agar for 24 h. After two successive subcultures in MRS broth from a single colony on agar, a fresh culture was obtained through 1% (v/v) inoculation and 12 h growth in MRS broth at 37 °C. The bacterial cells were harvested in the early stationary phase by centrifugation (Sorvall™ RC 6 Plus, Thermo Scientific Inc., MA, USA) at 4000 × g for 20 min at 4 °C and washed twice in sterile 0.85% (w/v) sodium chloride solution. The pellet was re-suspended in the saline solution to obtain a suspension that contained approximately 10^{10} colony-forming units (CFU)/mL. The bacterial cell suspension was then stored at 4 °C and used the same day.

2.3. Microencapsulation of bacterial cells

LMF was preheated at 50 °C in a water bath to dissolve all crystals. NaCas, maltodextrin, pullulan, gum arabic and gum ghatti solutions (10%, w/w) were prepared and mixed well in different ratios (NaCas: maltodextrin/pullulan/gum arabic/gum ghatti = 1:0, 3:1, 1:1, 1:3, and 0:1, w/w). LMF was then added into each of the solutions except for the maltodextrin and pullulan only sample (10% (w/w), 40 °C). The mixtures were first mixed using a blender (Polytron® PT 10-35 GT-D, Kinematica Corporation, Switzerland) at 6000 rpm for 1 min (40 °C), then recirculated through a high pressure homogenizer (Nano DeBEE, B.E.E. International Inc., MA, USA) at 3000 psi 3 times (40 °C). After holding the prepared emulsion at 0 °C overnight, a suspension of isolate LB1 was dispersed into the emulsions and stirred at 100 rpm on a stirrer for 10 min at 0 °C. The final solutions (10^9 CFU/g dry coating material) were then spray dried using a laboratory scale spray dryer (ADL 310, Yamato Scientific America Inc., CA, USA). A constant inlet temperature of 170 °C and outlet temperature of 80 °C and a flow rate of 5 mL/min were used. For reference purposes, free cells dispersed in sterile 0.85% (w/v) sodium chloride solution were spray dried under the same conditions. Dried powder samples were then collected from the base of cyclone and stored in tightly sealed sterile bottles at 4 °C.

2.4. Simulated gastrointestinal digestion and release test

For *in vitro* digestion experiments, simulated gastric fluid (SGF) consisting of 0.32% (w/w) pepsin and 0.2% (w/w) NaCl was prepared and the pH adjusted to 2.0 using 1 M HCl. Simulated intestinal fluid (SIF) consisting of 0.1% (w/w) pancreatin and 0.08% (w/w) bile salts was prepared in phosphate buffer (0.2 M, pH = 7.0). The bacterial cells were harvested as described above and diluted in sterile 0.85% (w/v) sodium chloride solution to approximately 10^9 CFU/mL. Microparticles (0.1 g) or free cells (0.1 mL) were then added to test tubes containing 9.9 mL of pre-warmed (37 °C), freshly prepared and filter sterilized SGF. The samples were vortexed and incubated at 37 °C for 30, 60, 90, and 120 min. To measure the survival rate of encapsulated LB1 cells, an aliquot of 1 mL was withdrawn and added into 9 mL PBS and homogenized for