Food Hydrocolloids 70 (2017) 345–355

Contents lists available at ScienceDirect

Food Hydrocolloids

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

Stability of *Lactobacillus rhamnosus* GG incorporated in edible films: Impact of anionic biopolymers and whey protein concentrate

Christos Soukoulis [a], Solmaz Behboudi-Jobbehdar [b], William Macnaughtan [b], Christopher Parmenter [c], Ian D. Fisk [b, *]

[a] Environmental Research and Innovation, Luxembourg Institute of Science and Technology (LIST), 5. Avenue des Hauts-Fourneaux, L-4362, Esch sur Alzette, Luxembourg
[b] Division of Food Sciences, School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough, LE12 5RD, Leicestershire, United Kingdom
[c] Nottingham Nanotechnology and Nanoscience Centre, University of Nottingham, University Park, Nottingham, NG7 2RD, United Kingdom

ARTICLE INFO

Article history:
Received 6 April 2017
Accepted 10 April 2017
Available online 12 April 2017

Keywords:
Probiotic
Edible film
Alginate
Pectin
Carrageenan
Dairy protein

ABSTRACT

The incorporation of probiotics and bioactive compounds, via plasticised thin-layered hydrocolloids, within food products has recently shown potential to functionalise and improve the health credentials of processed food. In this study, choice of polymer and the inclusion of whey protein isolate was evaluated for their ability to stabalise live probiotic organisms. Edible films based on low (LSA) and high (HSA) viscosity sodium alginate, low esterified amidated pectin (PEC), kappa-carrageenan/locust bean gum (κ-CAR/LBG) and gelatine (GEL) in the presence or absence of whey protein concentrate (WPC) were shown to be feasible carriers for the delivery of *L. rhamnosus* GG. Losses of *L. rhamnosus* GG throughout the drying process ranged from 0.87 to 3.06 log CFU/g for the systems without WPC, losses were significantly reduced to 0 to 1.17 log CFU/g in the presence of WPC. Storage stability (over 25d) of *L. rhamnosus* GG at both tested temperatures (4 and 25 °C), in descending order, was κ-CAR/LBG > HSA > GEL > LSA = PEC. In addition, supplementation of film forming agents with WPC led to a 1.8- to 6.5-fold increase in shelf-life at 4 °C (calculated on the WHO/FAO minimum requirements of 6 logCFU/g), and 1.6–4.3-fold increase at 25 °C. Furthermore probiotic films based on HSA/WPC and κ-CAR/LBG/WPC blends had both acceptable mechanical and barrier properties.

1. Introduction

According to the FAO/WHO probiotics are "viable microorganisms which when administered in adequate amounts ($>10^6$–10^7 CFU/g of ingested product) may confer health benefits to the human host". Reported health-associated benefits of probiotics include modulation of the gastrointestinal system, reduction in rotavirus and antibiotic induced diarrhoea, stimulation of the immune system and reduction of lactose intolerance and irritable bowel symptoms (Saad, Delattre, Urdaci, Schmitter, & Bressollier, 2013). Due to the sensitivity of probiotics to common processing conditions such as heat treatment, low pH environments, high osmotic pressure and high redox potentials, the design of effective physicochemical barriers to stabilise the organisms is essential to their full commercial exploitation in a wide range of food categories (Burgain, Gaiani, Linder, & Scher, 2011; Jankovic, Sybesma, Phothirath, Ananta, & Mercenier, 2010; Meng, Stanton, Fitzgerald, Daly, & Ross, 2008). Anhydrobiotics technology i.e. the encapsulation of living cells in low moisture (glassy) matrices fabricated via spray or freeze drying, remains to date the most popular approach to ensure maximal viability of probiotics (Behboudi-Jobbehdar, Soukoulis, Yonekura, & Fisk, 2013; Burgain et al., 2011; Meng et al., 2008; Soukoulis, Behboudi-Jobbehdar, Yonekura, Parmenter, & Fisk, 2014a; Tripathi & Giri, 2014). Nevertheless, the use of edible films (plasticised thin layered biopolymer structures) to embed viable probiotic cells is increasingly being studied (Gialamas, Zinoviadou, Biliaderis, & Koutsoumanis, 2010; Kanmani & Lim, 2013; López de Lacey, López-Caballero, & Montero, 2014; López de Lacey, López-Caballero, Gómez-Estaca, Gómez-Guillén, & Montero, 2012; Romano et al., 2014; Soukoulis, Behboudi-Jobbehdar, Yonekura, Parmenter, & Fisk, 2014c; Soukoulis, Singh, Macnaughtan, Parmenter, & Fisk, 2016). Edible films have the

* Corresponding author.
E-mail address: Ian.Fisk@nottingham.ac.uk (I.D. Fisk).

http://dx.doi.org/10.1016/j.foodhyd.2017.04.014

potential to stabilise food structures at multiple scale lengths whilst creating bespoke structures (enhanced mechanical properties, prolonged shelf-life, maintenance of structural integrity) and be used to deliver nutritional enhancements through probiotic inclusion. On the downside, inclusion of plasticisers may increase the lethality of entrapped bacterial cells due to osmolysis, inability to completely repress the cellular metabolic activity and increased exposure to oxygen, but are essential for the formation of edible films. To overcome this, the inclusion of compounds that scavenge free radicals, promote cells adhesion properties and suppress the matrix's glass transition temperature are often proposed (Burgain, Gaiani, Francius, et al., 2013). Edible films could offer significant benefits for intermediate moisture foods (IMF) when compared to conventional dehydrated microcarriers, this is mainly due to their ability to retain their physical state and biological activity throughout IMF storage, where dehydrated microcarriers, as opposed to edible films, in most cases experience structural collapse due to physical state transitions (glassy to rubbery) resulting in reduced cell viability. Hence, a vast number of applications have been investigated for edible film and coating technologies, these include bakery products, fishery products, dried fruits, olives, cereal bars (Altamirano-Fortoul, Moreno-Terrazas, Quezada-Gallo, & Rosell, 2012; De Prisco & Mauriello, 2016; López de Lacey et al., 2012; 2014; Soukoulis et al., 2014a; Tavera-Quiroz et al., 2015).

To understand the potential of edible films as vehicles for probiotics inclusion, parameters such as the biopolymer and plasticiser type and amount, the presence of oxygen scavenging agents and prebiotics have been recently evaluated (Gialamas et al., 2010; Kanmani & Lim, 2013; López de Lacey et al., 2014; Piermaria, Diosma, Aquino, Garrote, & Abraham, 2015; Romano et al., 2014; Soukoulis et al., 2014; Soukoulis, Behboudi-Jobbehdar, Yonekura, Parmenter, & Fisk, 2014b; Soukoulis et al., 2016). In a previous work, we demonstrated that the inclusion of *L. rhamnosus* GG in edible films, comprising whey protein concentrate and sodium alginate, assisted bacterial cells to withstand heat and osmotic stress upon bread production and storage whereas it also enhanced their survival throughout ingestion and gastrointestinal passage (Soukoulis et al., 2014). In the present work, we aim to further investigate the technological feasibility of edible films comprising selected biopolymers with established good film forming properties (namely low esterified amidated pectin (PEC), low (LSA) and high (HSA) viscosity sodium alginate, porcine skin gelatine (GEL) and kappa-carrageenan/locust bean gum (κ-CAR/LBG)), in the presence or absence of whey protein concentrate (WPC) as potential vehicles for *L. rhamnosus* GG. Selection of the biopolymers and compositional design of the edible film forming solutions was based on previous formulations for effective films and are constrained by practical and biopolymer specific requirements. Both protein and polysaccharide based films and binary films containing two polysaccharides are included to expand the range of the study (Galus & Lenart, 2013; Martins et al., 2012; Ramos, Fernandes, Silva, Pintado, & Malcata, 2012; Rivero, García, & Pinotti, 2010). Ultimately the aims was to explain the interplay between the survivability of *L. rhamnosus* GG and the structural and physicochemical properties of the embedding biopolymer substrate.

2. Materials and methods

2.1. Materials

For the purposes of this work a *Lactobacillus rhamnosus* GG strain (E-96666, VTT, Espoo, Finland) of established probiotic functionality was used. Low ester content (<50%) amidated pectin (LM-101 AS, Genu®, CPKelco, UK), low viscosity sodium alginate (LFR5/60, Protanal®, 65–75% guluronic acid units, 25–35% mannuronic acid, units, 35–60 kDa, Drammen, Norway), high viscosity sodium alginate (RF6650, Protanal®, 45–55% guluronic acid units, 45–55% mannuronic acid, units, ~100 kDa, Drammen, Norway), locust bean gum (Sigma Aldrich, UK), kappa-carrageenan (Sigma Aldrich, UK) and bovine skin gelatin B (Sigma Aldrich, UK) were used as film forming agents. Whey protein concentrate (81 ± 2% whey protein, 9% lactose, Lacprodan® DI-8090) was used as a co-structuring component, glycerol (97% purity, Sigma Aldrich, UK) was used as the plasticiser.

2.2. Preparation of the film forming solutions

Ten film forming solutions were prepared by dispersing the biopolymers and WPC (as listed in Table 1) in distilled water at 25 °C under agitation for 1 h. Then, glycerol accounting for the 50% (w/w) of the film forming agent total solids was added and the obtained biopolymer aliquots were heated to 80 °C for 30min. Heat treatment assisted the full desolution and hydration of the biopolymers, induced whey protein denaturation (>95%) and reduced residual microbial load. Eventually, the film forming solutions were cooled to 37 °C to be inoculated with *L. rhamnosus* GG.

2.3. Stock culture preparation and growth conditions of L. rhamnosus GG

Stock culture preparation of *L. rhamnosus GG* was carried out according to the procedure as previously described by Soukoulis et al. (2014a). Six frozen culture beads were placed in MRS broth (Oxoid Ltd., Basingstoke, UK) and incubated at 37 °C (48 h) under anaerobic conditions in plastic jars containing AnaeroGen® (Oxoid Ltd., Basingstoke, UK). The final broth was transferred under aseptic conditions into 50 mL sterile centrifuge tubes (Sarstedt Ltd., Leicester, UK) and centrifuged at 3000 g for 5 min. Pellets were washed twice with phosphate buffer saline (PBS), Oxoid Ltd. Basingstoke, UK.

2.4. Preparation and storage of the probiotic edible films

Film forming solutions (100 mL) were inoculated with three pellets (corresponding to ca. 10 logCFU/g of film forming solution, expressed in a dry basis) and successively degassed using a vacuum pump at 40 °C for 10 min. Then, 30 mL of the aliquots were aseptically transferred using a serological pipette to sterile petri dishes (inner diameter 15.6 cm; polystyrene; 101VR20, Sarstedt Ltd., Leicester, UK). The cast solutions were dried for 24 h in a ventilated incubator at 37 °C and ca. 50% RH (Sanyo Ltd., Japan). After air drying, the probiotic edible films were peeled off intact from the petri dishes and conditioned either at room temperature (25 °C) or chilling conditions (4 °C) for microbiological testing under controlled relative humidity conditions (ca. 54 and 59% RH respectively) using a saturated magnesium nitrate solution (Sigma Aldrich, Basingstoke). Separate systems conditioned for at least three days at 25 °C and 54% RH were used for physicochemical, mechanical and structural characterisation.

2.5. Enumeration of the bacteria

One mL of the probiotic film forming solutions was suspended in 9 mL sterile PBS and vortexed for 60s to ensure adequate mixing. For the recovery of *L. rhamnosus* GG from the probiotic edible films the method described by (Soukoulis, et al., 2014a,b,c) was adopted. Specifically, 1 g of the film containing *L. rhamnosus GG* was mixed with 9 mL of PBS and vortexed for 2 min to ensure sufficient dissolution of the film. Enumeration of the bacteria was performed