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Edible films as carrier for lactic acid bacteria

Joana Odila Pereira, José Soares, Sérgio Sousa, Ana Raquel Madureira, Ana Gomes, Manuela Pintado*

CBQF – Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Universidade Católica Portuguesa/Porto, Rua Arquiteto Lobão Vital, Apartado 2511, 4202-401 Porto, Portugal

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ABSTRACT

The use of edible coatings and films formulated with bioactive compounds in food products in order to convey new functionalities or extend shelf-life opens new possibilities as a carrier for functional lactic acid bacteria. In this work the main objective was to study the stability of probiotic microorganisms, viz. *Bifidobacterium animalis* Bb-12® and *Lactobacillus casei*-01, in edible film formulations based on whey protein isolate (WPI).

The results demonstrated a loss of bacterial cell viability of ca. 3 log cycles (reaching 10^6 CFU/g film) until 60 d at both 23 and 4 °C, noting that the most marked decrease was at 23 °C for both strains. *Bifidobacterium animalis* Bb-12® remained viable for a longer period of time and with less decrease in its cell numbers (10^8 CFU/g film).

Physical properties, namely color, water activity, thickness, young's modulus, tensile strength, elongation at break and the molecular structure of WPI films were maintained stable throughout the storage period at both temperatures tested.

Edible films incorporated with probiotics can be good carriers for these to be ingested together with food products.

1. Introduction

Edible coatings and films are natural polymers used to retain the appearance and physicochemical properties of foods during storage. When adsorbed on the surface of the food, they may promote protection against moisture migration or oxidation and control of microbial growth (Han, 2000; Naushad & Stading, 2007).

Several reviews have demonstrated that edible films can be prepared from different structural materials such as lipids (Hambleton, Debeaufort, Bonnotte, & Voilley, 2009), polysaccharides (Jiménez, Fabra, Talens, & Chiralt, 2013; Jridi et al., 2014) and proteins (Ramos, Fernandes, Silva, Pintado, & Malcata, 2011; Ramos et al., 2013; Ramos et al., 2012) or by combining two or several of these compounds. Protein based films have received considerable attention because they have advantages over others, in particular, due to their mechanical properties that are generally better since proteins have a distinctive structure, which confers a wider range of functional properties, (Cuq, Gontard, & Guilbert, 1995; Gennadios & Weller, 1990; Wittaya, 2012).

Edible packaging systems mainly deal with maintaining or increasing the quality and safety of packaged foods, extending their shelf life. On the other hand, bioactive packaging systems (coating/films) are a new technology concept to assist in the production of functional foods, where bioactive principles are designed to be contained within coatings or coating materials (Korhonen, 2002) in order to have a direct impact on consumer health, creating healthy foods packed for a specific bioactive attribute (Han, Lederer, McDaniel, & Zhao, 2005). A bioactive edible coating/film is defined as a protective coating applied to the surface of a food with addition of functional compounds such as antioxidants, color agents, flavors, nutrients, probiotics, prebiotics and antimicrobial agents that increase the functionality of the coating/film (Min, Harris, & Krochta, 2005; Pranoto, Rakshit, & Salokhe, 2005; Salmieri & Lacroix, 2006). These coatings/films have the function of protecting and controlling the release rate at which functional compounds are provided in the desired location (Pothakamury & Barbosa-Cánovas, 1995). To promote these characteristics, the coating must be prepared in accordance with the incorporated compound and the nature of the food. In this sense, the development of protein-based films is considered suitable for use as the carrier of functional ingredients and covers a wide range of foods.

Various studies have pointed out the beneficial effects of probiotics (Daoud & Hani, 2013; Nagpal et al., 2012; Vasiljevic & Shah,

* Corresponding author.
E-mail address: mpintado@porto.ucp.pt (M. Pintado).

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2008). Probiotics have key functionalities including: relief from lactose intolerance, increased resistance to intestinal invasion by pathogenic bacterial species, stimulation of the immune system and possible protection against colon cancer (Zubillaga et al., 2001). Another functionality is the antimicrobial capacity, through bacteriocins, or by competition *in situ* (Messaoudi et al., 2013). For these reasons, the incorporation of probiotics into food products has been increasing, to assure safe and healthy products.

Though a large number of genera and species of microorganisms are considered as potential probiotics (Holzapfel, Haberer, Snel, Schillinger, & Huis in't Veld, 1998; Shah & Ravla, 2004), the most common bacteria commercially available are from the genera *Lactobacillus* and *Bifidobacterium*. Probiotics incorporated into food products made by fermentation of milk, cereals, fruits and vegetables and meat are currently receiving great attention (Gupta & Abu-Ghannam, 2012; Kołożyn-Krajewska & Dolatowski, 2012; Rouhi, Sohrabvandi, & Mortazavian, 2013; Rößle, Auty, Brunton, Gormley, & Butler, 2010). However, the main challenge for their successful incorporation is ensuring their viability (Madureira, Brandão, Gomes, Pintado, & Malcata, 2011).

The survival of probiotics during the production of foods can lead to significant losses of viability due to mechanical and/or heat treatments, presence of oxygen and osmotic stress mechanisms (Bustos & Bórquez, 2013; Fu & Chen, 2011), hence, it is important to protect and incorporate them in coated foods. Preparation of such bioactive coatings is a very innovative field in the food industry and therefore only a small number of studies on this topic are available (Kanmani & Lim, 2013; López de Lacey, López-Caballero, Gómez-Estaca, Gómez-Guillén, & Montero, 2012; Soukoulis et al., 2014; Tapia et al., 2007).

The first study describes the incorporation of *Bifidobacterium lactis* Bb-12, in alginate-gellan coatings and films for coating of fresh-cut apple and papaya. Storage values of probiotic viable cell numbers higher than 10^6 CFU/g were maintained for 10 d, thus maintaining the viability of probiotics in films applied to fresh fruit (Tapia et al., 2007). López de Lacey et al. (2012) studied the incorporation of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* into gelatin edible coatings applied to fish and assessed its effect during storage. Lactic acid bacteria remained viable (above 10^8 CFU/g) and the H_2S producing microorganisms were reduced in 2 log cycles. Additionally, this coated fish was suited to treatment with high pressure (200 MPa/10 min/20 °C) which resulted in a reduction of total viable counts (<2 log cycles) after 13 d of storage (López de Lacey et al., 2012). Kanmani and Lim (2013) reported that starch-pullulan based edible films incorporated with probiotic strains, *Lactobacillus reuteri* ATCC 55730, *Lactobacillus plantarum* GG ATCC 53103 and *L. acidophilus* DSM 20079 maintained the viability of the selected strains after 30 d of storage at 4 °C. More recently, Soukoulis, Behboudi-Jobbehdar, et al. (2014) and Soukoulis, Yonekura, et al. (2014) demonstrated the development of a probiotic pan bread by application of edible coatings based on sodium alginate or sodium alginate/whey protein concentrate incorporated with *Lactobacillus rhamnosus* GG. *Lactobacillus rhamnosus* GG viability was improved significantly by the presence of whey protein concentrate throughout 7 d storage (Soukoulis et al., 2014).

All the above studies represent innovations of great interest and of promising nature when it comes to using edible coatings and films as carriers of probiotics, opening new possibilities for the development of novel functional foods. However, no study has considered until now, the characteristics and bacterial cell viability of stable dried edible whey protein films incorporated with probiotics.

So, considering that there are very few studies on this topic, in particular using protein-based films, the objective of this study was to evaluate the stability of two probiotic microorganisms in edible films based on whey protein formulations opening new possibilities for coating food products.

2. Material and methods

2.1. Bacterial strains, media and growth conditions

Probiotic strains, *Bifidobacterium animalis* Bb-12® and *Lactobacillus casei*-01 obtained from Christian Hansen (Denmark) were stored at −80 °C in de Man–Rogosa–Sharpe (MRS) broth (Biokar Diagnostics, France) supplemented with 30% (v/v) sterile glycerol. The aforementioned microorganisms were reactivated, and precultures were prepared in MRS medium supplemented with filter-sterilized 0.05% (w/v) L-cysteine·HCl (Fluka, St. Gallen, Switzerland), in order to lower the redox potential, and incubated at 37 °C during 24 h under anaerobic conditions, in a plastic anaerobic jar with an AnaeroGen sachet (an atmosphere generation system, Oxoid, Basingstoke, England). Subsequently, grown cells were harvested by centrifugation at 4000 rpm for 30 min, at 4 °C. The supernatant was discarded and the pellet was resuspended in a 0.9% (w/v) NaCl solution.

2.2. Formulation of the films

The film-forming solutions, one for each probiotic strain, were prepared by slowly dissolving 10% (w/w) whey protein isolate (WPI) powder (Armor Proteines, Saint Brice en Coglés, France) in deionized water, according to Pérez-Gago and Krochta (2002). Glycerol was added at 5% (w/w), as plasticizer, and the resulting solutions were magnetically stirred for approximately 2 h. Subsequently, the solutions were heated in a water bath at 80 °C, for 20 min under continuous agitation and cooled to room temperature for 1.5 h.

Afterwards, 5% (w/w) inoculum of each probiotic strain was added to each film solution to attain a final concentration of 10^9 CFU/mL. To prepare the films, the same amount (300 mL) of each solution was poured onto level Teflon plates (38 × 34 cm), so as to control the film thickness. The film solutions were allowed to dry at room conditions under a sterile environment (in a vertical laminar-flow cabinet, ca. 23 °C and 50% relative

Table 1
Viable cell numbers (Log CFU/g) of *B. animalis* Bb-12® and *L. casei*-01 incorporated in whey protein based films stored under vacuum for 60 days at 23 ± 1 °C and 4 ± 1 °C.

	23 °C		4 °C	
	B. animalis Bb-12®	*L. casei*-01	*B. animalis Bb-12®*	*L. casei*-01
Film-forming solutions	9.06 ± 0.03	9.07 ± 0.03	9.06 ± 0.03	9.07 ± 0.03
0 days	8.98 ± 0.01[a]	8.93 ± 0.04[a]	8.94 ± 0.03[a]	8.93 ± 0.04[a]
3 days	8.81 ± 0.04[b]	8.53 ± 0.04[b]	8.64 ± 0.03[b]	8.55 ± 0.03[b]
5 days	7.90 ± 0.01[c]	7.43 ± 0.04[c]	8.56 ± 0.03[c]	8.34 ± 0.02[c]
10 days	7.17 ± 0.01[d]	6.53 ± 0.05[d]	8.41 ± 0.03[d]	8.75 ± 0.02[d]
40 days	6.66 ± 0.01[e]	6.40 ± 0.02[e]	8.07 ± 0.04[e]	7.17 ± 0.03[e]
60 days	6.09 ± 0.02[f]	5.98 ± 0.01[f]	7.90 ± 0.01[f]	6.95 ± 0.01[f]

Note: [a, b, c, d, e, f] Means within the same columns, labeled with the same letter, do not statistically differ from each other ($p > 0.05$).