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Edible methylcellulose-based films containing fructo-oligosaccharides as vehicles for lactic acid bacteria

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ABSTRACT

The goal of this work was to investigate the physicochemical properties of methylcellulose (MC) based films as stabilizers of two strains of lactobacilli: *Lactobacillus delbrueckii* subsp. *bulgaricus* CIDCA 333 and *Lactobacillus plantarum* CIDCA 83114. The incorporation of 3% w/v fructo-oligosaccharides (FOS) into the MC film formulation improved the viability of *L. delbrueckii* subsp. *bulgaricus* CIDCA 333 after film preparation. *L. plantarum* CIDCA 83114 was intrinsically more resistant as no viability loss was observed upon preparation of the films in the absence of FOS.

Scanning electronic microscopy images also showed a good incorporation of microorganisms without affecting the homogeneity of the films. FTIR spectroscopy provided structural information about the bacteria-loaded films. Water sorption isotherms showed an impervious behavior at low a_w but on exceeding 0.7 of a_w the film started to dissolve and form syrup, causing a drastic drop of bacterial viability ($\log N/N_0 \leq -5$). Dynamic mechanical analysis (DMA) demonstrated that the incorporation of microorganisms into the MC films had no effect on vitreous transition temperatures. FOS incorporated into the MC films had a plasticizing effect.

Microorganism-loaded films were stored at relative humidities (RH) ranging from 11 to 75%. Both strains could be stored at 11% RH for 90 days. At 33 and 44% RH *L. delbrueckii* subsp. *bulgaricus* CIDCA 333 could be stored up to 15 days and *L. plantarum* CIDCA 83114 up to 45 days. At 75% RH only *L. plantarum* CIDCA 83114 could be equilibrated ($\log N/N_0$: -2.05 ± 0.25), but CFU/g films were undetectable after 15 days of storage.

The results obtained in this work support the use of MC films containing FOS as a good strategy to immobilize lactic acid bacteria, with potential applications in the development of functional foods.

1. Introduction

The importance of lactic acid bacteria (LAB) and probiotics in the food and pharmaceutical industries highlights the need of preservation strategies ensuring an adequate viability in the final product. Hence, the incorporation of these microorganisms into edible films appears as a suitable approach to increase their survival upon dehydration and storage. Besides the small volumes occupied by the films, this strategy represents an effective way to increase cell density and protect them during storage and processing. All these advantages are especially relevant in the development of industrial applications.

Probiotic-loaded films have been frequently used as food coatings to take advantage of the microbial beneficial effects and extend the shelf-life of different products (Gialamas, Zinoviadou, Biliaderis, & Koutsoumanis, 2010; López de Lacey, López-Caballero, Gómez-Estaca, Gómez-Guillén, & Montero, 2012; Moayednia et al., 2009; Tapia et al., 2007). In this regard, Sánchez-González, Quintero Saavedra, & Chiralt (2013) developed bioactive polymeric films as vehicles of bacteriocins produced in situ by a *Lactobacillus plantarum* strain. The antimicrobial activity of *Lactobacillus sakei* immobilized in sodium–caseinate films was also addressed (Gialamas et al., 2010). In addition, alginate films have proved to be adequate carriers for *L. plantarum* release, demonstrating good perspectives for pharmaceutical applications (Brachkova, Duarte, & Pinto, 2009, 2012, Brachkova et al., 2011).

Methylcellulyose (MC) is a low cost, edible and clear viscous polymer in aqueous environments (Li et al., 2002). Its unique properties as film forming agent led to several pharmaceutical and food applications (Bodvik et al., 2010; Lin, Wang, Wei, & Li, 2007; Pérez et al., 2008). However, to our knowledge, no reports on LAB-loaded MC films have been published hereto.

Abbreviations: LAB, lactic acid bacteria; MC, methylcellulose; FOS, fructo-oligosaccharides; FTIR, Fourier transform infrared; DMA, dynamic mechanical analysis; MRS, de Man, Rogosa, Sharpe broth; CFU, cell forming units; RH, relative humidity; ds, dried sample; a_w, water activity; T_g, glass transition temperature; T_m, gel–liquid crystal transition temperature.

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Considering that MC film formulation involves heating and drying processes, sensitive lactic acid bacteria, like *Lactobacillus delbrueckii* subsp. *bulgaricus*, may be injured or dead. For this reason, the incorporation of protective compounds in the MC film formulation can be an adequate strategy to overcome this problem. The role of fructo-oligosaccharides (FOS) as bacterial protectants in processes involving dehydration has been reported in the last years (Chaluvadi et al., 2012; Golowczyc, Gerez et al., 2011; Schwab, Vogel, & Gänzle, 2007). Taking into account the prebiotic properties of FOS, their incorporation into MC films may have a double advantage: protection and prebiotic effect (Gibson, Probert, Van Loo, Rastall, & Roberfroid, 2004).

The structural and mechanical properties of MC films have been recently addressed (Tavera-Quiroz, Lecot, Bertola, & Pinotti, 2013) and FOS-based edible films have also been developed (Ramesh & Siddalingaiya, 2006). However, the incorporation of FOS into MC films results in novel films whose physical–chemical properties deserve a careful analysis. Moreover, when attempting to include microorganisms in MC films, certain parameters like temperature and time of dehydration must be thoroughly controlled in order to avoid losses of viability. This requires a careful investigation of the bacteria-loaded MC films from both the physical–chemical and the microbiological points of view.

In this work, MC films were loaded with two strains of lactobacilli: *L. delbrueckii* subsp. *bulgaricus* CIDCA 333 and *L. plantarum* CIDCA 83114. *L. delbrueckii* subsp. *bulgaricus* CIDCA 333 is a highly sensitive strain upon any preservation process (Tymczyszyn et al., 2012) and *L. plantarum* CIDCA 83114 inhibits the growth and/or the activity of *Escherichia coli* O157:H7, *Shigella* and *Salmonella* (Golowczyc, Silva, Teixeira, De Antoni, & Abraham, 2011; Hugo, Kakisu, De Antoni, & Pérez, 2008; Kakisu, Abraham, Tironi Farinati, Ibarra, & De Antoni, 2013; Kakisu, Bolla, Abraham, de Urraza, & De Antoni, 2013). The effect of FOS included in the MC film on both bacterial viability and physicochemical properties of the films was investigated.

2. Materials and methods

2.1. Materials

MC (A4M, Methocel) (Dow, MI, USA), sorbitol (Merck, USA), FOS (Orafti Beneo p95, Germany), LiCl, $MgCl_2$, K_2CO_3, NaCl, KCl and K_2SO_4 (Anedra, Argentina) were used.

2.2. Methods

2.2.1. Film forming solution

To prepare the hydrocolloid solution, 1.5 g MC were slowly dispersed in 50 mL of distilled water at 80 °C, under constant stirring for 1 h. Once a homogeneous system was obtained, a total volume of 100 mL was made up with cold distilled water and the solution was kept under stirring until it attained room temperature. Afterwards, sorbitol was added as a plasticizer to a final concentration of 0.25% w/v, as determined in a previous work (Tavera-Quiroz et al., 2013). FOS, composed of oligosaccharides of different degree of polymerization (from 2 to 8), were also added to the hydrocolloid solution, to attain concentrations of 0 (control), 1, 2, 3 and 5% w/v. The obtained solution was sterilized using 0.2 μm sterile filters. These experiments allowed selecting the most suitable concentration of FOS for subsequent experiments (Sections 2.2.7 to 2.2.10).

2.2.2. Bacterial strains and growth conditions

L. delbrueckii subsp. *bulgaricus* CIDCA 333 and *L. plantarum* CIDCA 83114 were isolated from fermented milks (Garrote, Abraham & De Antoni, 2001; Gómez-Zavaglia, Abraham, Giorgieri, & De Antoni, 1999). The strains were maintained frozen at −80 °C in 120 g/L non-fat milk solids. Cultures were grown in MRS broth (de Man, Rogosa, & Sharpe, 1960) at 37 °C (*L. delbrueckii* subsp. *bulgaricus* CIDCA 333) and 30 °C (*L. plantarum* CIDCA 83114) in aerobic conditions.

100 mL of cultures in the stationary phase [grown overnight in MRS to attain approximately 5×10^8 CFU/mL for *L. delbrueckii* subsp. *bulgaricus* CIDCA 333 (Tymczyszyn et al., 2012) and 1×10^{10} CFU/mL for *L. plantarum* CIDCA 83114 (Golowczyc, Silva, et al., 2011)] was harvested by centrifugation at 7000 ×*g* for 10 min and washed twice with 0.85% w/v NaCl.

2.2.3. Film preparation

Bacterial pellets were diluted into 17–25 mL of the film forming solution to obtain 1.1×10^{10} CFU/mL of *L. delbrueckii* subsp. *bulgaricus* CIDCA 333 and 2.7×10^{12} CFU/mL of *L. plantarum* CIDCA 83114. An aliquot of 1.5 mL of the resulting suspension was spread onto Petri dishes and dried in a forced air oven at 40 °C for 2.5 h. The obtained films (surface: 28.3 cm^2 and weight: 50 mg) were removed from the dishes and thickness was determined using a coating thickness gauge (Check Line DCN-900, New York, USA) for non-conductive materials on non-ferrous substrates.

2.2.4. Bacterial plate counts

50 mg of the bacteria-loaded films, corresponding to 1.5 mL of film forming solution, were re-hydrated in 5 mL of 0.85% w/v NaCl for 10 min to allow a complete hydration. The obtained suspensions were serially diluted and plated on MRS agar. Plates containing *L. plantarum* CIDCA 83114 were incubated at 30 °C for 24 h in aerobic conditions (Golowczyc, Silva, et al., 2011), and plates containing *L. delbrueckii* subsp. *bulgaricus* CIDCA 333, at 37 °C for 48 h in aerobic conditions (Tymczyszyn et al., 2012). This process was carried out any time that bacterial viability was determined [immediately after drying and during storage (see Section 2.2.10)].

2.2.5. Scanning electron microscopy

The bacteria-loaded films were frozen in liquid nitrogen and fractured using a cold scalpel blade. The samples were examined with a FEI model Quanta 200 electron microscope (The Netherlands). Samples were mounted onto bronze stubs by using a double-sided tape and examined without any metal or carbon coating at low pressure and an acceleration voltage of 12.5 kV.

2.2.6. FTIR spectra of the films with bacteria

FTIR spectra of the MC films were recorded in a transmission mode, on a Thermo Nicolet iS10 spectrometer (Thermo Scientific, MA, USA). The FTIR spectra were obtained in the 4000–400 cm^{-1} range, by co-adding 64 scans with 4 cm^{-1} spectral resolution (Tavera-Quiroz et al., 2013).

2.2.7. Water sorption isotherm

50 mg of the bacteria-loaded films (28.3 cm^2) were equilibrated at 4 °C in atmospheres of the following saturated salts: LiCl, $MgCl_2$, K_2CO_3, NaCl, KCl and K_2SO_4 giving relative humidities (RH) of 11, 33, 44, 75, 85 and 97%, respectively.

Moisture contents of the bacteria-loaded films were determined by measuring their weight loss, upon drying in a vacuum oven at 105 °C until constant weight (AOAC 1980). Moisture results were expressed as grams of water per 100 g of dried sample (ds).

GAB (Guggenheim-Anderson-de Boer) and Iglesias–Chirife models were used to fit sorption isotherm data. GAB isotherm model can be expressed as follows:

$$M_w = \frac{m_0 C K_{aw} (1 - K_{aw} + C K_{aw})}{(1 - K_{aw})} \quad (1)$$

where M_w is the equilibrium moisture content at a given water activity (a_w), m_0 is the monolayer value (g water/g solids) and C and K_{aw} are the GAB constants (Galdeano, Mali, Grossmann, Yamashita, & García, 2009).