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Effect of water activity on the stability of *Lactobacillus paracasei* capsules

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ABSTRACT

Lactobacillus paracasei subsp. paracasei LBC81 microorganisms were encapsulated by fluidized bed drying using water-alginate system incorporating potato starch. The capsules were stored for seven weeks in a_w (s) from 0.103 to 0.846 at 25, 35 and 45 °C. The quantitative analysis of microorganisms indicated that the survival of the strain decreased more rapidly over water activity of 0.536, which was considered as critical for maintaining these alive. This water activity can be obtained using the Rockland analysis. The enthalpy-entropy compensation revealed that encapsulated probiotics were kept alive when the adsorption process was controlled by entropy, which was where the critical water activity occurred.

1. Introduction

In the world food market, a growing sector corresponds to functional foods, especially those containing probiotics (Semyonov, Ramon, & Shimoni, 2011; Senok, Ismaeel, & Botta, 2005). By definition, probiotics are living microorganism, which upon ingestion in adequate amounts confer health benefits on the host (Food and Agriculture Organization of the United Nations [FAO]/World Health Organization [WHO], 2001). The species *Lactobacillus paracasei* is frequently present on human gastro-intestinal (GI) mucosa of healthy individuals (Molin et al. 1993), but is also often dominating the spontaneous, secondary bacterial-flora in semi-dry cheese, especially if the cheese has been manufactured with pasteurized milk (Antonsson, Molin & Ardö, 2003). Research suggests that *L. paracasei* appears helping to reduce inflammation and improves movement in the bowels of those suffering from irritable bowel syndrome, reduces development of dental caries, help fight allergic rhinitis as well as have antimicrobial activity against urogenital infection (Peng & Hsu, 2005; Tanzer et al., 2010; Verdú et al.,2004; Zárate, Santos, & Nader-Macias, 2007). In order to provide health benefits the probiotics must contain at least dose of 10^6–10^8 CFU per gram of foodstuff (FAO/WHO, 2001) or consumed doses of 10^8–10^{10} CFU/day (Champagne, Ross, Saarela, Hansen, & Charalampopoulos, 2011). However, the conditions for the incorporation of new probiotic food matrices keeping them alive need to be studied, because they can be lost during the manufacturing process, transportation and storage (Vesterlund, Salminen, & Salminen, 2012). There are several environmental factors affecting the viability of probiotics such as tritatable acidity, pH, hydrogen peroxide, dissolved oxygen, storage temperature, moisture content, water activity, the presence of other cultures, etc. (Dave & Shah, 1997; Kailasapathy & Supriadi, 1996; Lankaputhra, Shah, & Britz, 1996).

The function of the encapsulation-dehydration is to immobilize the microorganisms in a protective matrix with subsequent drying process such as spray drying, fluidized bed or freeze-drying. The water activity (a_w) of the final product, the oxygen content, the wall

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material and the temperature of storage are key factors in maintaining the viability of probiotics (Dianawati, Mishra, & Shah, 2013; Peri & Pompei, 1976; Vesterlund et al., 2012). It has been determined that there is a critical value of moisture content and water activity (a_w) in which lactic acid bacteria can maintain their structural and biological functioning after a drying treatment (Fonseca, Obert, Béal, & Marin, 2001; Jouppila & Roos, 1994a, 1994b; Kurtmann, Carlsen, Risbo, & Sibsted, 2009; Kurtmann, Carlsen, Sibsted, & Risbo, 2009).

Information on the physicochemical nature of water bound to the solid matrix is required to understand the effect of water content on the storage stability of foods. Stability is greatly influenced by the moisture sorption characteristics of the product. Rockland (1969) applied Henderson's equation to water sorption data in several foods and introduced the concept of "localized isotherms" where was related the change of free energy (ΔG) with the moisture content to provide a closer relationship between sorption isotherms and stability of food. The thermodynamic of water sorption has drawn interest because provides a more thorough interpretation of the sorption phenomenon and helps to understand better the sorption mechanism. The most commonly employed thermodynamic functions in predicting stability conditions are the Gibbs free energy, enthalpy and entropy because they have direct relation to the physico-chemical state of the water molecules in the food (Viganó et al., 2012). Thermodynamic properties studies have shown in a variety of processes parallel changes in enthalpy and entropy, i.e., the stronger the intermolecular interaction, or bonding (enthalpy related), the greater the reduction in the configurational freedom and hence, greater order of the system (entropy related) (Choděra & Mobley, 2013; Rudra, Sarkar, & Shivhare, 2008). This is the so-called enthalpy–entropy compensation effect (Leffler, 1995). The kinetic compensation effect has been widely observed in various areas such as physics, chemistry, biology and thermal analysis. Labuza (1980) describes the problems that can be encountered in applying the concept of a linear relationship between enthalpy-entropy compensation plot in food related reactions such as thermal degradation of microorganisms, protein denaturation and ascorbic acid degradation. In water sorption of food, this compensation has been studied for several systems such as plum skin and pulp, starch materials and pinion seeds, where these compensations were found enthalpy driven (Cladera-Olivera, Ferreira-Marczak, Zapata-Noreña, & Pettermann, 2011; Gabas, Menegalli, & Telis-Romero, 2000; McMinn, Al-Muhtaseb, & Magee; 2005). Beristain, García, & Azuara (1996) suggested that was possible to found entropy-controlled adsorption processes at low moisture contents in foodstuffs which had their microstructure modified by temperature.

Adsorption in micropores is generally known to be dominated by interactions between the diffusing molecules and the pore walls. Thus, in small pores steric and other effects associated with proximate of the pores walls (entropic effects) become important. It has been reported that the adsorption process for yogurt is entropy driven when water molecules are adsorbed in the micropores and suggested that greatest physicochemical stability is achieved within this zone (Azuara & Beristain, 2006; Pascual-Pineda et al., 2013). No reports were found about the stability of probiotic microorganisms using thermodynamic properties of water vapor adsorption as a criterion of stability, which is greatly influenced by the moisture sorption characteristics of the product.

The objective of this work was to investigate the protection to the viability of *L. paracasei* provided by calcium alginate capsules stored at several water activities and temperatures and to analyze the enthalpic and entropic mechanisms occurring during the moisture sorption on capsules and the relationship with its stability.

2. Materials and methods

2.1. Materials

The lyophilized pure bacterial strain was obtained from LY0 *L. paracasei* Danisco LBC81, Mexico. Sodium alginate was obtained from Sigma Aldrich. All the reagents used were analytical grade.

2.2. Cultures and enumeration of free and encapsulated probiotics

Pure freeze-dried probiotic culture of *L. paracasei* was activated by inoculation in the MRS-broth (de Man-Rogosa-Sharpe) at 37 °C for 17 h. The probiotic biomass in late-log phase was harvested by centrifugation at 900 g for 10 min at 4 °C (Centrifuge Universal 320 R, Hettich, USA), then washed twice in sterile 0.9% saline under the same centrifugation conditions, and used in the encapsulation process. Enumeration of probiotic bacteria was achieved as described by Homayouni, Azizi, Ehsani, Yarmand, and Razavi (2008). Samples of 1 g were decimally diluted in 10 mL sterile peptone water (0.1%) and 1 mL aliquot dilutions were poured onto plates of the MRS-agar in triplicate. All enumerating plates of *L. paracasei* were incubated at 37 °C for 72 h under anaerobic conditions. The averages of the results were expressed as colony-forming units per gram of sample (CFU/g). To count the encapsulated bacteria, before and after the drying process, 1 g of sample was re-suspended in 20 mL of phosphate buffer (0.2 M, pH 7.0), followed by shaking until complete rupture. The counts (CFU/g) were determined by plating on MRS-agar as discussed above.

2.3. Preparation and drying of the capsules of L. paracasei

All glassware and solutions used in the protocols were sterilized at 121 °C for 15 min. Alginate capsules were prepared using a modified encapsulation method originally reported by Sultana et al., (2000). A 2% alginate mixture in distilled water was prepared containing 2% potato starch (Sigma–Aldrich) and 0.1% culture (17 h old culture grown in MRS-broth). The mixture was added into 200 mL canola oil (Proteinas y Oleicos S.A. de C.V. Mérida, Yucatán., México). The mixture was stirred vigorously (600 rpm for 10 min, Silverson homogenizer, Chesham, Bucks., England) until was fully emulsified. The capsules were obtained by dripping solution onto a 2% $CaCl_2$ aqueous solution and allowed to stand 30 min. These were collected by filtration, washed with 0.9% saline and immediately were fed to a Retsch fluidized bed model TG204 (Haan, Germany). The drying conditions were inlet air temperature of 30 °C, outlet temperature of 50 °C and air volumetric flow rate of 99 m^3/s.

2.4. Moisture sorption isotherms

Samples of the *L. paracasei* capsules were placed in desiccators containing P_2O_5 at constant vacuum (0.0133 kPa) for 2 weeks at room temperature. The moisture sorption data were obtained using the gravimetric method described by Lang, McCune, & Steinberg, 1981. Two to 3 grams of samples were weighed in triplicate into standard weighing dishes with a circular section on the bottom, where a quantitative filter paper Whatman No. 1 was used to support the sample and at the same time allow transmission of moisture. In order to obtain a true moisture gain by the sample alone, the filter paper was allowed to equilibrate over the salts solution. Samples were placed in separate desiccators containing saturated salt slurries in the range of water activity from 0.103 to 0.846 using the a_w reported by Labuza, Kaanane, and Chen (1985). The samples, were held at 25, 35, and 45 °C until equilibrium was reached. Values of water activity were generated using equations