Innovative Food Science and Emerging Technologies 39 (2017) 216–222

Contents lists available at ScienceDirect

Innovative Food Science and Emerging Technologies

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

Encapsulation by electrospray coating atomization of probiotic strains

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ARTICLE INFO

Article history:
Received 8 November 2016
Received in revised form 18 December 2016
Accepted 19 December 2016
Available online 22 December 2016

Keywords:
Electro-hydrodynamic processing
Bifidobacteria
Whey protein
Lactobacillus

ABSTRACT

A new encapsulation method termed electrospray coating atomization (ECA) is presented here for the first time as a new encapsulation concept to enhance the viability of dry powders in general and of freeze-dried probiotic strains in particular. A freeze-dried powder of a probiotic strain (~10 log cfu/g) was homogeneously electrospray coated with ultrafine particles of different food hydrocolloids of permitted use in supplemented food and nutraceutical products. The water soluble polymer polyvinylpyrrolidone (PVP) was included in the study as reference pharma permitted encapsulation material. The protected microorganisms were stored at different conditions for periods between 10 and 700 days. Significant differences were found during storage at most of the assayed stressing conditions when compared to the freeze-dried uncoated powder. The results show significantly enhanced comparative survivability of the encapsulated material over time after more than one year at room temperature and 23% RH, especially for whey protein concentrate (WPC), resistant maltodextrin (Fibersol) and PVP. The protective effect of WPC, Fibersol and PVP at 37 °C and 23% RH was also demonstrated.

Industrial relevance: Electrospray coating atomization (ECA) is proposed as a new processing methodology for the protection of dry forms of probiotics and in general for the protection of functional/active/bioactive added value ingredients used in the food industry. Since the technology is currently in the process of being scaled up it seems of great significant relevance to an industrial level in order to stabilize or further stabilize existing powder form formulations.

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1. Introduction

Global probiotics market, still in its infancy stage, was forecasted to cross 44 billion US$ revenues by 2019 (Technavio, 2016). The market is set to witness growth as consumers become more conscious about their health. The beneficial effects of probiotics on human health have been claimed by several studies, citing their use as an aid in stimulation of host immune response, reduction of lactose intolerance, resistance to enteric pathogens, and in the prevention of cancer, cardiovascular disease, and gastrointestinal disorders (Iannitti & Palmieri, 2010; Ranadheera, Baines, & Adams, 2010).

Probiotics are "live micro-organisms which, when administered in adequate amounts, confer a health benefit on the host" (FAO/WHO, 2002). In order to exert their beneficial effects on the host, probiotics must remain viable and reach the intestine in large numbers. The minimum quantity of microorganisms expected to be in food products, such as fermented milks, is 10^6 cfu/g (Codex Stan, 2003). Therefore, the preservation of microorganisms' viability is one of the most important objectives for probiotic foods companies. Most commonly used techniques for microorganism preservation are cryopreservation, different drying applications (i.e. freeze drying, foam drying, spray drying or fluidized bed drying), and encapsulation technologies, which can provide an extra protection to the previously dried form (Morgan, Herman, White, & Vesey, 2006).

Instead of cryopreserved microorganisms, a dry powder is more desirable since it allows extending the shelf-life and broadening the range of food applications. Moreover, encapsulation is a plausible option because it also facilitates handling of cells and allows controlled dosage. Common techniques used for this purpose are spray drying and freeze-drying (Gouin, 2004; Poddar et al., 2014). Freeze-drying (FD) is currently the most extended technique to preserve microorganisms in dried form. This processing technology is suitable for sensitive biological materials since it exerts less thermal stress on the materials. On the other hand, it is relatively expensive and is a batch process (Eratte et al., 2015). When compared to freeze-drying, the operational and capital costs of spray drying are reportedly 1/6 and 1/9, respectively, per unit mass of the product (Chávez & Ledeboer, 2007; Gharsallaoui, Roudaut, Chambin, Voilley, & Saurel, 2007; Gouin, 2004). However, the spray drying process can cause significant viability loss as a result of simultaneous dehydration, thermal, and oxygen stresses imposed to bacteria during the drying process (Eratte et al., 2015; Fang & Bhandari, 2012; Ghandi, Powell, Broome, & Adhikari, 2013; Picot & Lacroix, 2004). Foam drying has been used to stabilize *Lactobacillus acidophilus* and *Lactococcus lactis* subsp. *cremoris*, and also some Gram negative species although it produces bacteria viability reductions of 40% (Morgan et al., 2006). The

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http://dx.doi.org/10.1016/j.ifset.2016.12.013
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main constrain of these two technologies (spray and foam drying) is the utilization of temperature (higher in case of spray-drying) which can damage temperature sensitive microorganisms (Fathi, Martín, & McClements, 2014). This problem can be overcome in many cases by using other encapsulation techniques that do not require heat such as electro-hydrodynamic atomization (EHDA), either electrospinning or electrospraying.

EHDA uses a high voltage to generate particles (electrospray) or fibers (electrospinning) while avoiding the use of heat or aggressive solvents representing an alternative to other thermal techniques that lead to some bacteria damages. While electrospraying may take several forms, in the cone-jet mode of operation (widely known as the Taylor cone), a jet shoots off the tip of the cone and breaks into droplets, for example as a result of the Rayleigh-Plateau instability, driven by surface tension. In electrospinning, the polymer fluid does not break up into droplets but instead forms a slender, continuous liquid filament due to the suppression of the Rayleigh-Plateau instability by the effect of surface charge and the viscoelastic response of the jet. (Ma & Rutledge, 2012). Typically, the EHDA process works at room temperature and allows the use of biopolymers in water solutions, avoiding the use of organic solvents, just by adjusting the process parameters and/or changing the solution properties through the addition of proper additives (Pérez-Masiá, Lagaron, & López-Rubio, 2014). Both electrospray and electrospinning technique have shown a great potential in the biomedical industry (Xie, Ng, Lee, & Wang, 2008; Xie & Wang, 2007; Luu, Kim, Hsiao, Chu, & Hadjiargyrou, 2003; Cerqueira et al., 2016; Rasekh, Young, Roldo, et al., 2015; Rasekh, Ahmad, Day, Wickam, & Edirisinghe, 2011; Mehta et al., 2016). These techniques have recently appeared as an alternative to other drying processes and it has a tremendous potential in the food science area for the development of novel functional ingredients (Paximada, Echegoyen, Koutinas, Mandala, & Lagaron, 2017; Pui David & Chen, 1997; López-Rubio, Sanchez, Sanz, & Lagaron, 2009; López-Rubio, Sanchez, Wilkanowicz, Sanz, & Lagaron, 2012; Torres-Giner, Martinez-Abad, Ocio, & Lagaron, 2010). Nonetheless, formulating encapsulated bioactives can still be challenging in the food industry because of the limiting use of food grade and generally regarded as safe (GRAS) materials as encapsulants. Most used synthetic encapsulating polymers with controlled release properties, such as Eudagrit® or polyethylene oxide among others, are just permitted for pharmaceutical use and not for food applications (Sobel, Versic, & Gaonkar, 2014). Moreover, the selected processing materials affect not only cell viability but also determine the future properties of the encapsulated bioactive ingredient (i.e. mechanical and thermal resistance, solubility, etc.). Proteins, polysaccharides and their formulations have attracted a considerable interest because these natural biopolymers are surface active materials and are allowed to be used in the food science area (Nieuwland et al., 2013; Stijnman, Bodnar, & Hans Tromp, 2011; van den Heuvel et al., 2013). Moreover, they may be considered as amphiphilic macromolecules that play an essential role in stabilizing food formulations (Jones, Decker, & McClements, 2010). Among proteins, milk-derived ones, casein and whey protein, have been the most widely used by different encapsulation techniques. Generally, all of these materials lead to an increase in bacterial survival during storage at different conditions. Biopolymer combination or combination of previously mentioned techniques can lead to achieve even better results on cell viability with time.

Drying technologies have demonstrated a good capacity for cell stabilization during time. However, the absence of water is not always enough against some stressing conditions (i.e. acid pH, high humidity/temperature environments or the presence of light). To overcome these problems, combined treatments such as coatings are applied to improve the properties of powders, leading to an enhanced protective effect. (Eratte et al., 2015).

Despite the fact that EHDA has been reported as an alternative method for encapsulation as a one step process, very often the industry has spent enormous efforts in developing probiotic products in dry form that require further protection. Thus, the aim of this study is to demonstrate the potential application of an innovative process based on electrospray coating of formulated probiotic products in powder form to further enhance the product shelf-life. Up until recently, the EHDA technology remained at a laboratory scale (Bocanegra, Galán, Márquez, Loscertales, & Barrero, 2005; Deng, Klemic, Li, Reed, & Gomez, 2006; Zhang et al., 2015), however new instrumental designs for scaling up production such as the ones used in the present study, which led to high throughput pilot and industrial plants, enable the implementation of this technology to encapsulate added value products in mass quantities (Paximada et al., 2017; Chalco-Sandoval, Fabra, López-Rubio, & Lagaron, 2015; Hoang et al., 2015).

2. Materials and methods

2.1. Materials

Different biopolymers from different origins were evaluated as potentials matrices for the protection of probiotic bacteria. Whey protein concentrate (WPC) with a protein content of 80% was kindly donated by ARLA Food Ingredients (Viby, Denmark) and used without further purification. A commercial resistant maltodextrin, namely Fibersol®, was purchased from Alifarma (Barcelona, Spain). Polysaccharide maltodextrin (Dextrose Equivalent DE = 16.5–19.5), vegetal protein zein and plastic derived polymer polyvinylpyrrolidone (PVP) (M_w = 1,300,000) were provided by Sigma Aldrich Co. (Spain). The surfactant sorbitan monolaureate (Span20®) was also purchased from Sigma Aldrich Co. (Spain).

2.2. Preparation of polymer solution

Fibersol and maltodextrin solutions were prepared at 20 wt.% in distilled water and stirred gently at room temperature for 30 min. WPC solution was prepared according to López-Rubio and Lagaron (2012) at 20 wt.% in skimmed milk and zein at 12 wt.% in ethanol 85% (v/v). PVP was prepared at 10 wt.% in distilled water at 80 °C and cooled down after complete dissolution. Surfactant was added to all solutions at a concentration of 6 wt.% with respect to the polymer weight in order to improve its sprayability according to previous authors (A. López-Rubio et al., 2012; Pérez-Masiá et al., 2014).

2.3. Solution characterization

The apparent viscosity (η_a) of the polymeric solutions at 100 s^{-1} was determined using a rotational viscosity meter Visco BasicPlus L from Fungilab S.A. (San Feliu de Llobregat, Spain) using a Low Viscosity Adapter (LCP). The surface tension of the biopolymer solutions was measured following the Wilhemy plate method using an EasyDyne K20 tensiometer (Krüss GmbH, Hamburg, Germany). The conductivity of the solutions was measured using a conductivity meter XS Con6 (Lab-box, Barcelona, Spain). All measurements were made at 25 °C.

2.4. Microorganisms

The strain *Bifidobacterium longum* subsp. *infantis* CECT 4552 from the Culture Type Spanish Collection was grown in Man Rogosa and Sharpe (MRS) broth (Scharlau, Barcelona, Spain) supplemented with 0.07% (w/v) L-cysteine (Sigma, St. Louis, MO) (MRS-cys), and incubated for 48 h at 37 °C under anaerobic conditions. Finally, cells were harvested at stationary growth phase by centrifuging at 4000 ×*g* for 10 min at 4 °C. The supernatant was discarded and the precipitated cell mass was washed twice with sterile peptone water (0.2%, w/v). The final wet cell mass was re-suspended in a maltodextrin solution (10% w/v) to approximately 10^{10} cell/ml. This solution was lyophilized in a VirTis Genesis 35 EL freeze-drier (SP Scientifics, New York, EE.UU.). The obtained powder was stored at 0% RH and 5 °C until use. Cell viability

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