Food Research International 106 (2018) 81–89

Contents lists available at ScienceDirect

Food Research International

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

Valorization of okara oil for the encapsulation of *Lactobacillus plantarum*

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ARTICLE INFO

Keywords:
Lactic acid bacteria
Oil-in-water emulsions
Spray-drying
Freeze-drying
Stability

ABSTRACT

Oil-in-water (O/W) emulsions of okara oil-caseinate (1:2; 1:3 and 1:4 O/W ratios) were used to encapsulate *Lactobacillus plantarum* CIDCA 83114. Once encapsulated, microorganisms were freeze-dried or spray-dried, and observed by scanning electronic and confocal microscopies. A physical characterization of the dehydrated capsules was carried out by determining their moisture content, water activity, particle size, polydispersity index and zeta potential. Determining the induction times and peroxide values provided information about their susceptibility to oxidation. In turn, bacterial stability was analyzed by plate counting before and after freeze-drying and spray-drying, and during storage at 4 °C.

Spray-dried emulsions had lower *Z*-sizes and polydispersity indexes, higher induction times and lower peroxide values than the freeze-dried ones, thus resulting better systems to protect *L. plantarum* CIDCA 83114. In addition, the culturability of spray-dried bacteria did not decrease neither after spray-drying nor up to 60 days of storage at 4 °C.

The results showed that the better physical-chemical stability of spray-dried capsules determined the greater stability of microorganisms. This demonstrates the importance of defining adequate emulsions' formulations for an efficient encapsulation of microorganisms, with promising applications in the development of novel functional foods.

1. Introduction

Okara is the by-product remaining from the soy milk production, after filtration of the smashed soybeans seeds (Stanojevic, Barac, Pesic, Jankovic, & Vucelic-Radovic, 2013). It is obtained in large quantities (about 1.1 kg per kilogram of soybean processed for soymilk production), thus posing an important disposal problem (Li et al., 2012). To avoid such problem, great efforts have been employed to use okara for the formulation of different products (Vong & Liu, 2016).

Dehydrated okara contains about 9–10% humidity, 21% proteins, 55% whole fiber, 1.5% ash and 13–14% fats and oils (Quintana, Gerbino, & Gomez-Zavaglia, 2017). Because of the nutritional value of its components (proteins of high nutritional value, dietary fiber, antioxidants, unsaturated lipids), okara appears as a valuable source of different ingredients for the formulation of functional foods (Vong & Liu, 2016). Defatted okara can be used as primary ingredient for the formulation of dry breakfast cereals or as meat extender (Shurtleff & Aoyagi, 2000). Okara oil has potential applications in cosmetic, pharmaceutical and food industry. In this regard, using super-critical CO_2 enables the recovery of nutritionally valuable compounds from okara oil, namely polyunsaturated fatty acids, phytosterols and phenolic compounds, such as soy isoflavones, genistein and daidzein (Borhan, Gani, & Shamsuddin, 2014; Quitain, Oro, Shunsaku, & Moriyoshi, 2006). This represents an added value in preventing chronic diseases (FAO, 2010) and opens opportunities for the development of novel applications aiming at using this by-product.

Lactic acid bacteria have an important role in the food and pharmaceutical industries, as they are extensively used as starters in the development of food and probiotic products. However, the decrease of water activity occurring during preservation and technological processes is often detrimental, leading to damages on the cellular structures or death (Santos, Gerbino, Araujo-Andrade, Tymczyszyn, & Gomez-Zavaglia, 2014; Tymczyszyn et al., 2008). To prevent these problems, bacterial microencapsulation into polymer matrices appears as an adequate strategy to ensure mechanical integrity during production processes (exposure to high/low temperatures, oxidation, shear, etc.), desiccation and storage (packaging and environment conditions, including moisture, oxygen, temperature, etc.) (Chávarri, Marañón, & Villarán, 2012). Encapsulation shell agents include a variety of polymers, carbohydrates, fats and waxes, depending on the material to be

Abbreviations: ζ potential, zeta potential; Đ, polydispersity index; MRS, de Man, Rogosa, Sharpe broth; CFU, colony forming units; PBS, phosphate buffer saline; O/W, oil-in-water; ds, dried sample; a_w, water activity; ANOVA, Analysis of variance

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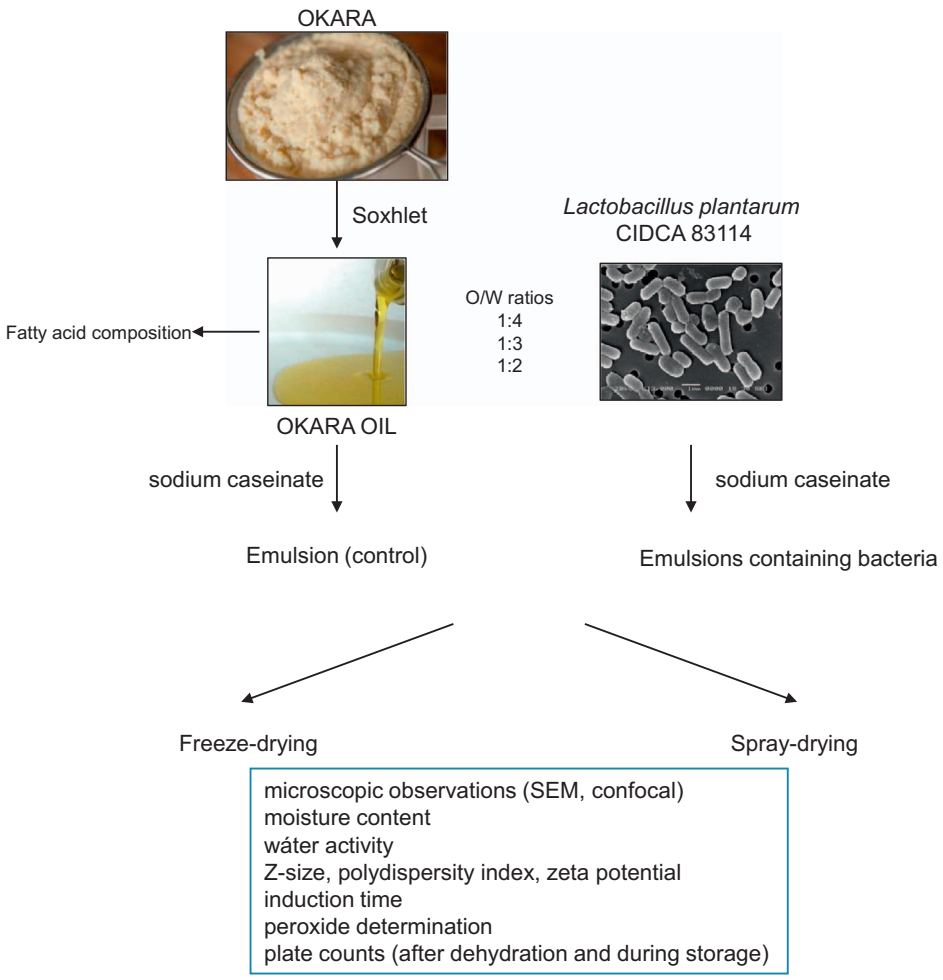
https://doi.org/10.1016/j.foodres.2017.12.053
Received 14 October 2017; Received in revised form 2 December 2017; Accepted 18 December 2017
Available online 19 December 2017
0963-9969/

Scheme 1. Experimental sequence of experiments.



protected. The coating of sealed capsules must be semipermeable and mechanically resistant to the adverse conditions above mentioned (Chávarri et al., 2012).

Microorganisms are usually suspended in a given encapsulating agent, forming emulsions or suspensions. Such emulsions are then dehydrated, generally by freeze-drying or spray-drying (Petrovic, Nedovic, Dimitrijevic-Brankovic, Bugarski, & Lacroix, 2007). Entrapment of probiotic bacteria in emulsion droplets has been suggested to stabilize different species of lactic acid bacteria (Hou, Lin, Wang, & Tzen, 2003; Pimentel-González, Campos-Montiel, Lobato-Calleros, Pedroza-Islas, & Vernon-Carter, 2009; Rodríguez-Huezo et al., 2014; Zhang, Lin, & Zhong, 2015). In this regard, Hou et al. (2003) succeeded at increasing about 10^4 times the intestinal survival rate of *Lactobacillus delbrueckii* ssp. *bulgaricus* by entrapping microorganisms in the droplets of reconstituted sesame oil body emulsions. Pimentel-González et al. (2009) successfully encapsulated *Lactobacillus rhamnosus* using water-in-oil-in-water emulsions prepared with canola oil and sweet whey (Pimentel-González et al., 2009). Milk fat was also used for encapsulation of lactic acid bacteria. In addition, preparing emulsion droplets with multiple lipid-protein-pectin layers provides additional protection for *Lactobacillus salivarius* strains (Zhang et al., 2015). Canola oil was also used to encapsulate *Lactobacillus plantarum* in double emulsions containing *aguamiel* or sweet whey as inner aqueous phases, and the obtained emulsions were successfully incorporated during cheese manufacture (Rodríguez-Huezo et al., 2014). The presence of whey proteins, inulins or fructo-oligosaccharides are reported to play an important role in the protection of the encapsulated microorganisms. Although emulsification technologies have demonstrated a great efficiency for the encapsulation of probiotics, there is still a long way to undergo in the development of more efficient entrapment systems. In this sense, when analyzing the recovery of entrapped bacteria, the focus has been always put on the composition of the aqueous components of emulsions. In fact, for improving bacterial recovery, different aqueous components have been assayed to formulate emulsions (Hou et al., 2003; Pimentel-González et al., 2009; Rodríguez-Huezo et al., 2014; Zhang et al., 2015). However, little attention has been paid to the oil components. This aspect is especially important because the chemical and physical properties of such oils determine their ability to form small droplets, leading to stable emulsions after homogenization (Bai & McClements, 2016). Lipid oxidation is another factor defining the stability of the emulsions, as it usually limits the shelf-life of fat containing products (Jacobsen, 2010). For this reason, an adequate selection of oils is very important to prevent oxidation during storage, thus contributing for the long term stability of encapsulated microorganisms.

The aim of this work was to formulate different okara oil-caseinate emulsions and use them to encapsulate *L. plantarum* CIDCA 83114, a strain with demonstrated inhibitory properties against *E. coli* O157:H7, *Shigella* and *Salmonella* [Hugo, Kakisu, De Antoni, & Pérez, 2008; Golowczyc, Silva, Teixeira, De Antoni, & Abraham, 2011; Kakisu, Abraham, Tironi Farinati, Ibarra, & De Antoni, 2013; Kakisu, Bolla, Abraham, de Urraza, & De Antoni, 2013]. The O/W emulsions were freeze-dried and spray-dried, and the obtained particles were characterized by determining their size, zeta-potential (ζ-potential) and polydispersity index (Đ). The stability of oil to oxidation was assessed by determining the induction time and peroxide values. In turn, the bacterial stability was evaluated by plate counting before and immediately after encapsulation, and during 90 days of storage at 4 °C.