LWT - Food Science and Technology 90 (2018) 412–417

Contents lists available at ScienceDirect

LWT - Food Science and Technology

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

Development and characterization of microcapsules containing *Bifidobacterium* Bb-12 produced by complex coacervation followed by freeze drying

Thaiane Marques da Silva[a], Eduardo Jacob Lopes[a], Cristiane Franco Codevilla[a], Alexandre José Cichoski[a], Érico Marlon de Moraes Flores[b], Mariana Heldt Motta[c], Cristiane de Bona da Silva[c], Carlos Raimundo Ferreira Grosso[d], Cristiano Ragagnin de Menezes[a,*]

[a] Department of Food Science and Technology, Universidade Federal de Santa Maria, Santa Maria, Brazil
[b] Department of Chemistry, Universidade Federal de Santa Maria, Santa Maria, Brazil
[c] Department of Pharmacy, Universidade Federal de Santa Maria, Santa Maria, Brazil
[d] Department of Food Nutrition, Universidade Estadual de Campinas, Campinas, Brazil

ARTICLE INFO

Keywords:
Microcapsules
B. lactis
Complex coacervation
Freeze drying

ABSTRACT

Microcapsules containing *B. lactis* were produced by complex coacervation in gelatin and gum Arabic followed by freeze drying and characterized by optical microscopy and scanning electron microscopy, as well as the resistance of probiotics to the in-vitro release in the simulated gastrointestinal tract and storage under different temperature conditions. Therefore, it formed microcapsules with high encapsulation efficiency (86.04% and 99.52%) and size between 100.12 and 203.32 μm. In addition, microencapsulated probiotics, both in wet and dry forms, maintained viability against the simulated gastrointestinal conditions. Finally, complex coacervation method was also efficient in maintaining the viability of probiotics during storage at temperatures of −18 °C for 120 days, 7 °C for 120 days and 25 °C for 90 days. Thus, our results demonstrated that complex coacervation method is an appropriate alternative to increase the viability of probiotics.

1. Introduction

Fuller (1989) defined probiotics as live microorganisms, which when consumed in sufficient amounts, confer a beneficial health effect on the host, by improving and maintaining the balance of the intestinal microbiota. Currently, probiotics are defined as live microorganisms which when administered in adequate amounts confer a health benefit on the host (World Gastroenterology Organisation, 2011).

Probiotic foods have been widely used for providing beneficial effects to health. Species of *Lactobacillus* and *Bifidobacterium* are most commonly used as probiotics (World Gastroenterology Organisation, 2011). Recent studies have shown that ingestion of foods containing these probiotics may prevent or reduce enteric infections and lower respiratory tract infections. Specifically, *Bifidobacterium lactis* strain Bb12® is reported to have beneficial effects on metabolism including lowered serum LDL (Low Density Lipoproteins)-cholesterol in patients with type 2 diabetes, increased HDL (High Density Lipoproteins) cholesterol in adult women and improved glucose tolerance during pregnancy (Kabeerdoss et al., 2011).

However, these bacteria are sensitive to a number of factors, especially acid pH values and the presence of oxygen, thus, they may not remain viable during shelf-life of the product and passage through the gastrointestinal tract. In this sense, microencapsulation emerges as an alternative of protection and controlled release from these microorganisms, since there is separation of the encapsulated material from the environment. With this, it is possible to improve the survival of probiotics on exposure to oxygen and during the passage through the gastrointestinal system, as well to promote controlled release. (Alvim & Grosso, 2010; Chen, Li, Liu, & Meng, 2017; Shori, 2017). Among different microencapsulation techniques used, there is the complex coacervation, which consists of the electrostatic interaction between oppositely charged polyelectrolytes, forming microcapsules under specific conditions of pH and temperature (Comunian et al., 2016; Oliveira et al., 2007).

Complex coacervation is the combination of two oppositely-charged hydrocolloid solutions, causing interaction and precipitation of complex polymers. The method presents many advantages when compared to the others, such as versatility, high encapsulation efficiency, efficient

* Corresponding author.
E-mail address: cristiano.ufsm@gmail.com (C.R. de Menezes).

https://doi.org/10.1016/j.lwt.2017.12.057
Received 7 August 2017; Received in revised form 20 December 2017; Accepted 24 December 2017
Available online 28 December 2017

control of particle size, possibility of working with biopolymers, absence of organic solvents, low cost and soft conditions of processing (Shoji et al., 2013). In addition, the microcapsules produced by coacervation are versatile and have excellent controlled release characteristics modulated by changes in ionic strength, pH and temperature (Bosnea, Moschakis, Nigam & Biliaderis, 2017; Menezes et al., 2013; Oliveira et al., 2007).

However, complex coacervation is still little used for the encapsulation of probiotics, compared to other techniques, such as spray dryer and ionic gelation, for example. Therefore, there is a need to explore more studies to improve its application in food. The study of the use of coating compounds, variation of concentrations and association with drying techniques, are desirable so that they increase the protection of probiotic cultures and allow a more effective application in foods. This is especially important for food matrices that do not offer the best conditions to ensure probiotic viability. (Bosnea, Moschakis, & Biliaderis, 2014).

Given the above-mentioned, the objective of this study was to microencapsulate *Bifidobacterium lactis* Bb-12 using complex coacervation, followed by freeze drying, evaluate the size and morphology of the capsules, as well as the viability of probiotics against the in-vitro release, the simulated gastrointestinal tract and storage under different temperature conditions.

2. Material and methods

2.1. Materials

For the microcapsules formation were used: Gelatin type A (Gelita, Eberbach, Germany); Gum Arabic (CNI, São Paulo, Brazil); Probiotic culture *Bifidobacterium lactis* (Bb-12) obtained from Chr. Hansen from Brazil (Valinhos, São Paulo, Brazil).

2.2. Zeta-potential measurement of coating materials

The zeta potential of polymer pair, gelatin and gum Arabic, was determined using Zetasizer Nano ZS (Malvern Instruments Ltd., Worcestershire, UK). The polymer pair was analysed in three proportions, 1: 3, 1: 1 and 3: 1 (GE: GA) at pH 3.0, 4.0, 5.0, 6.0 and 7.0, at 25 °C and in triplicate.

2.3. Inoculum preparation

Prior to the time of use, the probiotic culture *Bifidobacterium lactis* (Bb-12) was maintained at −18 °C. For the inoculum preparation, the probiotic culture was actived in 12% reconstituted milk and incubated for 12 h at 37 °C. Then, the activated culture was centrifuged at 2470 × g, at 4 °C for 15 min and washed with saline (0.85%) twice. The cells were then suspended in saline to obtain a solution of approximately 10 log CFU.mL^{-1}.

2.4. Microencapsulation using complex coacervation method

The production of the microcapsules was performed according to methodology described by Alvim and Grosso (2010), with modifications. First, 1g of the culture was added to 100 mL of 2.5% gelatin, maintaining under stirring with hotplate magnetic stirrer (Centauro, Paraná, Brazil) (48-50 °C) for 10 min for homogenization of microorganisms. Afterwards, 100 mL of 2.5% gum Arabic and 400 mL of sterile distilled water were added, while stirring and heating (50 ± 2 °C) in magnetic stirring plate (Centauro), then adjusting the pH to 4.0 (according to item 2.2) with 0.1 mmol/L hydrochloric acid solution (HCl). Thereafter, it was cooled naturally at about 30 °C, and then, the temperature lowering was carried out until the temperature reached 12-10 °C. After, it was settled and the coacervate was removed by sieve filtration with 0.038 mm (400 mesh) porosity.

A portion of the microcapsules produced were frozen (−18 °C for 24 h) on the same day of production. Freeze drying was performed in lyophilizer (L101, Liotop, Brazil), with vacuum: 0.200–0.300 μHg and condenser temperature of −37 °C.

2.5. Encapsulation efficiency

Encapsulation efficiency (EE) was calculated using the following Equation (1) (Annan, Borza, & Hansen, 2008).

$$EE = (N/ N_0) \times 100 \quad (1)$$

Equation (1). Where N is the number of viable cells (log CFU.g-1) released from the microcapsules and N_0 is the number of viable cells (log CFU g^{-1}) in the cell concentrated prior to microencapsulation.

2.6. Morphological characterization of microcapsules by optical and scanning electron microscopy

Optical microscopy of the wet microcapsules was performed using an optical microscope (Scope A.1, Zeiss, Germany) equipped with Axio Cam MRc digital camera (Carl Zeiss). The morphology of the dried microcapsules was evaluated using a Scanning Electron Microscope (Sigma 300 VP, Zeiss, Germany).

2.7. Evaluation of mean diameter and size distribution of microcapsules

The mean size of the wet and dry microcapsules was measured using the Mastersizer 2000 equipment (Malvern, Germany).

2.8. Viable cell count

Appropriate dilutions were transferred in triplicate to sterile Petri dishes, followed by addition of MRS (de Man, Rogosa, and Sharpe) agar (Kasvi, Paraná, Brazil) supplemented with lithium chloride (0.1%) and L-cysteine (0.05%). The plates were incubated at 37 °C for 72 h in anaerobic jars containing anaerobic generators (Anaerobac, Probac, São Paulo, Brazil). The dilution of the microcapsules consisted of weighing 1 g of wet microcapsules and 0.1 g of dried microcapsules, followed by the addition of 9 mL of sodium phosphate buffer solution (pH 7.5), according to the methodology described by Sheu, Marshall, and Heymann (1993). The results were expressed as log CFU.mL^{-1}.

2.9. Bifidobacterium lactis Bb-12 survival under simulated gastrointestinal conditions

The simulated gastrointestinal conditions for the wet and dried microcapsules were simulated according to Madureira, Amorim, Gomes, Pintado, and Malcata (2011), with modifications. In this method, the viability of probiotic microcapsules was evaluated sequentially in media that simulate the different gastrointestinal tract compartments (oesophagus–stomach, duodenum and ileum). For this analysis, 2 g aliquots of wet microcapsules and 1 g of dry microcapsules were used. The oesophagus–stomach step encompassed 25 mg mL^{-1} pepsin (Sigma), prepared in 0.1 mol/L HCl; this solution was added in equal-sized aliquots at each step during the whole gastric phase, at a rate of 0.05 mL mL^{-1}for a total of 90 min; and pH was decreased until 2, using 1 mol/L HCl. The duodenum step used, at a rate of 0.25 ml ml^{-1}, a solution containing 2 g.L^{-1} pancreatin (Sigma) and 12 g.L^{-1} bovine bile salts (Sigma), diluted in 0.1 mol/L $NaHCO_3$. Finally, the ileum step was brought about by an increase of pH to 6.5 using filter-sterilized 0.1 mol/L $NaHCO_3$. All solutions were prepared in the moment of use and sterilized using a 0.22 μm membrane filter (Minisart, Sartorius Stedim Biotech, Germany). The analysis was conducted in a Shaker refrigerated incubator (TE-421, Tecnal, Brazil) maintained at 37 °C to simulate the temperature of the human body;