Meat Science 111 (2016) 198–203

Contents lists available at ScienceDirect

Meat Science

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

Use of alginate beads as carriers for lactic acid bacteria in a structured system and preliminary validation in a meat product

Maria Rosaria Corbo, Antonio Bevilacqua *, Barbara Speranza, Barbara Di Maggio, Mariangela Gallo, Milena Sinigaglia

Department of the Science of Agriculture, Food and Environment, University of Foggia, Via Napoli 25, 71122 Foggia, Italy

ARTICLE INFO

Article history:
Received 2 October 2014
Received in revised form 18 August 2015
Accepted 8 October 2015
Available online 13 October 2015

Keywords:
Lactic acid bacteria
Alginate
Beads
Release
Acidification
Structured system

ABSTRACT

This paper proposes the microencapsulation into alginate beads of 4 isolates of lactic acid bacteria (*Lactobacillus* spp.), previously isolated from pork meat. First, the beads were studied in relation to the encapsulation yield (EY), kinetic of cell release in a structured system, and survival throughout bead storage at 4 °C. EY was 93–96% and the survival of the encapsulated microorganisms was variable, with two isolates showing a bacterial population of 6.1–6.9 log cfu/g after 35 days under refrigerated conditions. Thereafter, the paper addressed a preliminary validation in a meat model system, containing salt, nitrites and nitrates, lactose, pepper, and then in a commercial preparation of pork meat. For the validation in pork meat, free cells were used as controls.
Cell released from beads were able to achieve a significant acidification; in particular, after 7 days they showed the same results of free cells.

1. Introduction

The interest towards microencapsulation increased significantly for its promising application to design suitable carriers for probiotics or as a way to improve food fermentation through the optimization of re-usable bio-catalysts (Corbo, Bevilacqua, Gallo, Speranza, & Sinigaglia, 2013; Gallo, Bevilacqua, Speranza, Sinigaglia, & Corbo, 2014).

Microencapsulation helps to separate a core material from its environment until it is released, thereby improves its stability, extends the core's shelf life and provides a sustained and controlled release, masking flavors, colors or odors (Kailasapathy, 2002).

The most commonly reported microencapsulation procedure is based on calcium-alginate gel capsule formation. Spherical polymer beads with diameters ranging from 0.3 to 3.0 mm and immobilizing active biomass are produced using extrusion with syringe and stirred calcium bath; alginate is used in concentration range of 0.5–4% (Burgain, Gaiani, Linder, & Scher, 2011; Corbo et al., 2013).

Different microorganisms were encapsulated in alginate: lactobacilli (Nualkaekul, Lenton, Cook, Khutoryanskiy, & Charalampopoulos, 2012; Sabikhi, Babu, Thompkinson, & Kapila, 2010), lactococci (Dianawati, Mishra, & Shah, 2013), bifidobacteria (Fritzen-Freire et al., 2012), and *Saccharomyces cerevisiae* (Gallo, Bevilacqua, Speranza, Sinigaglia & Corbo, 2014). Many authors reported that entrapped cells could start a fermentation in yoghurt (Picot & Lacroix, 2004); cheese (Özer, Kirmaci, Şenel, Atamer, & Hayaloğlu, 2009), ice cream (Prevost & Divies, 1992), mayonnaise (Khali & Mansour, 1998), soy milk (Corbo et al., 2013), and dry fermented sausages (Muthukumarasamy & Holley, 2006).

The use of beads was proposed in the past for liquid media; to the best of our knowledge, few data are available on the use of capsules in a structured system. This paper addressed the microencapsulation into alginate beads of 4 isolates of *Lactobacillus* spp., isolated from pork meat and selected for their technological (acidification, growth in wide ranges of temperature, salt concentration, pH values) and probiotic traits (survival in the presence of bile salt and at pH 2.0, bioactivity towards foodborne pathogens, hydrophobicity and auto-aggregation, antibiotic resistance/sensitivity). The isolates were chosen as representative of promising functional starter cultures to design a biocatalyst (alginate beads with microorganisms) intended for sausage fermentation.

The main topic (use of beads in structured systems) was addressed through some intermediate milestones:

a) Assessing encapsulation yield and viability of encapsulated cells throughout storage.
b) Studying cell release from the beads into a structured and solid

* Corresponding author.
E-mail address: antonio.bevilacqua@unifg.it (A. Bevilacqua).

http://dx.doi.org/10.1016/j.meatsci.2015.10.005
0309-1740/

system.

c) Evaluating bead performances (acidification) in a model system and then in a pork-meat preparation, as a function temperature and sausage ingredients (nitrites, nitrates, lactose, pepper).

2. Materials and methods

2.1. Microorganisms

Four isolates of Lactic Acid Bacteria (LAB) isolated from pork meat and characterized for their technological and probiotic traits were used throughout this research. The isolates (162, 178, 204, 239) were identified as *Lactobacillus plantarum* (Bevilacqua, unpublished results) and stored at −20 °C in MRS broth (Oxoid, Milan, Italy) added with 33% of sterile glycerol (J.T. Baker, Milan, Italy). Before each assay the microorganisms were grown in MRS broth incubated at 37 °C for 24 h.

2.2. Microencapsulation

Alginate beads were produced as reported by Corbo et al. (2013). Bacterial cultures were centrifuged at 1000 *g* for 10 min; then, the cells were washed with sterile distilled water (cell suspension). 20 ml of cell suspension (8–9 log cfu/ml) was added with Na-alginate 2% *w*/*v* (Carlo Erba, Milan, Italy) and mixed gently for 1–3 min. After 2–3 h the gel was manually extruded through a sterile 10-ml-syringe and the capsules dipped for ca. 5 min in a $CaCl_2$ solution (0.50% and 8.00% *w*/*v*) (J.T. Baker).

The encapsulation yield (EY) was evaluated as follows: 5 g of beads was diluted with 50 ml of a sterile 0.1 M sodium citrate solution (J. T. Baker) and homogenized through a laboratory blender, to achieve the complete dissolution of capsules, as described by Chávarri et al. (2010). Both bead homogenates and cell suspension (i.e., the original cell suspension used to prepare beads) were serially diluted in a saline solution (0.90% NaCl) and bacterial population was evaluated through pour plating on MRS agar (incubated at 30 °C for 48 h under anaerobic conditions in a jar by using the kit Anaerogen from Oxoid).

EY was evaluated as follows:

$$EY = \left(\frac{N_{bead}}{N_{suspension}}\right) * 100$$

where N_{bead} (expressed as cfu/g) and $N_{suspension}$ (expressed as cfu per gram of solution) are the viable counts in the beads and in cell suspension, respectively.

2.3. Bacterial population within bead storage

Beads were put into an empty sterile plate and stored at 4 °C and analyzed periodically (7, 14, 21, 35 days) for the evaluation of bacterial population through the pour plate method on MRS Agar.

2.4. Kinetic of cell release from capsules in agar medium

0.40 g of beads was put in sterile Petri plates and mixed with 20 ml of a 1.20% sterile liquid medium (Agar technical n. 3, Oxoid, autoclaved and cooled to 45–50 °C); beads were placed into the plates after medium pouring. Then, the medium was gently mixed, let to solidify; after solidification the plates were stored at 25 °C and analyzed periodically (immediately after sample preparation and after 2, 5, 7, 12, 14, 21 days) to evaluate the amount of cells released from beads. The agar was gently removed from the plates, diluted (1:10) with a sterile saline solution (0.90% NaCl), and homogenized through a Stomacher for 30 s. This kind of homogenization did not disrupt or affect the integrity of alginate beads. The viable count was assessed through the pour plate method.

Table 1
Meat broth.

Ingredient	Amount (g/l)
Beef extract (Oxoid)	5.00
Bacteriological peptone (Oxoid)	10.00
Tryptone (Oxoid)	5.00
Agar (Oxoid)	12.00
Nitrites (C. Erba, Milan)	Variable
Nitrates (C. Erba)	Variable
NaCl (C. Erba)	Variable
Lactose (C. Erba)	Variable
Black pepper (C. Erba)	Variable

2.5. Meat model system: validation of microencapsulation

Beads were loaded into a meat broth (Cardenas, Giannuzzi, & Zaritzky, 2007), slightly modified as reported in Table 1. The medium was sterilized at 121 °C for 15 min; then, at 50 °C 0.40 g beads per 20 ml of medium was added.

The amounts of nitrites, nitrates, lactose, sugar, and black pepper, and the storage temperature varied according to a 3^{k-p} Fractional Factorial Design (Table 2). The factors of the design were combined as reported by Box, Hunter, and Hunter (2005).

The samples were stored at 20 and 30 °C. Bacterial population and pH were evaluated after 24 and 48 h through the pour plate method and a Crison pH-meter (Crison Instruments, Barcelona, Spain), respectively.

2.6. Lab-scale validation

This assay was carried out using the isolate 178. Alginate beads were produced as previously described and added to a commercial homogenized infant preparation of pork meat (1.40 g of capsules for 70 g of product). The composition was as follows: pork meat, 40.00%; salt, 0.50%; rice flour, 20.00%; and water. Aliquots of commercial preparation inoculated with free cells (i.e., not encapsulated cells, 8 log cfu/g) were used as control samples. The samples were added with nitrites, nitrates, lactose, and black pepper, and stored at 20 or 30 °C, according to the Fractional Design reported in Table 2. Bacterial population and pH were evaluated after 2, 5, and 7 days of storage.

2.7. Statistical analysis and data modeling

The experiments were performed over two different batches; for each batch duplicate measurements were done. The results were analyzed through one-way ANOVA and Tukey's test as the *post-hoc* comparison test ($P < 0.05$); paired comparisons (i.e. difference between 2 samples) were analyzed through t-test. Moreover, data from the experiments on performances of encapsulated cells were modeled using the theory of DoE (Design of Experiments); bacterial population, acidification (decrease of pH), and pH were set as dependent variables whereas nitrites, nitrates, lactose, temperature, and pepper were used as independent variables. The statistical analyses were performed through the software Statistica for Windows (StatSoft, Tulsa, OK.).

3. Results

3.1. Performances of microencapsulation

Encapsulation yield of beads (EY) was 92.90–96.80% (data not shown), without any significant difference amongst the isolates ($P < 0.05$). Concerning the survival of the isolates, the initial bacterial population was 9.00 ± 0.30 log cfu/g; however, the trend within the storage relied upon the microbial target, as the isolate 204 experienced a fast death kinetic and decreased until the detection limit after 14 days. On