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Short communications

Development of probiotic beads similar to fish eggs

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ABSTRACT

Probiotic foods are mainly restricted to dairy and soy products. This study aimed to develop a new probiotic beads similar to fish eggs, commonly used in oriental cuisine. Beads were produced by the extrusion encapsulation technique with calcium alginate, added to one of the following cultures: *Lactobacillus rhamnosus* GG ATCC 53103 and *Bifidobacterium animalis* DN-173 010 and stored for 30 days at 4 °C. The beads were characterized by the size, weight, morphology and viability of the probiotic strains in different storage temperatures and in simulated gastric juice adjusted to different pH values. The beads were also evaluated by a sensorial affective hedonic scale. The beads present a 2.8 mm diameter and a weight of 0.01 g ($p > 0.05$). Free and encapsulated cells were tolerant to pH 3.0. At pH 2.5 only of the encapsulated cells presented counts above 6 Log colony-forming units per gram (CFU/g). Beads containing *L. rhamnosus* showed higher viability 10^7 CFU/g in storage for 30 days under refrigeration. The beads may be stored at abusive temperature for 5 h without loss of viability cells. The probiotic product developed showed an 82.2% acceptability index of overall characteristics and good market potential as a new probiotic product.

1. Introduction

Diseases transmitted by consuming raw fish are associated with several pathogens present in the aquatic environment (WHO, 2012). The use of lactic acid bacteria in fish as a biopreservation process to prevent the development of pathogens and deteriorating bacteria has been studied (Diop, Destain, Tine, & Thonart, 2010; El Bassi, Hassouna, Shinzato, & Matsui, 2009). However, the application of these bacteria as probiotics in fish products, with the aim of exerting health effects on consumers, has not been well explored.

Probiotic foods contain live microorganisms which, when administered in adequate amounts, confer health benefits to the host (FAO/WHO, 2006). Regular consumption of probiotic foods can prevent diseases caused by intestinal pathogens. However, most of the commercial products are dairy or soy based, which cannot be consumed by some individuals with dietary restrictions.

Fish eggs are used as ingredients and decorative products in oriental cuisine, and their characteristic structure can be an opportunity for the incorporation of physically similar components with probiotic properties in these food items. This study aimed to develop probiotic beads similar to fish eggs. The inclusion of these microorganisms in oriental cuisine could represent a protective factor in food and exercise beneficial effects desirable for the consumer, as well as being a viable consumption alternative of non-dairy probiotics by individuals with restriction on dairy products.

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2. Material and methods

2.1. Microbial strains and cultive conditions

The following microbial strains were used as probiotic: *Lactobacillus rhamnosus* GG *American Type Culture Collection* (ATCC) 53103 and *Bifidobacterium animalis* ssp. *lactis* DN-173 010. The stock was maintained at −20 °C in De Man, Rogosa and Sharpe (MRS) broth (Merck, Darmstadt, Germany) with the addition of 20% glycerol (v/v). The working cultures were stored at 4 °C in slanted MRS agar.

2.2. Production of probiotic beads

The probiotic beads were developed in a way to simulate salmon eggs (Salmo salar). The probiotic strains were immobilized by a modification of the extrusion encapsulation described by Krasaekoopt, Bhandari, and Deeth (2004).

The bacterial strains were actived by two consecutive cultives in MRS broth for 18–24 h at 37 °C. The cells were harvested by centrifugation at 1500*g* for 10 min at 4 °C (SORVALL® SUPERSPEED RC2-B Automatic), washed twice in 20 ml of an 0.85% (w/v) saline solution under the same centrifugation conditions. The washed probiotic cells were mixed with a 1.0% Na-alginate solution (w/v) (LV, Keltone®, ISP Corporation, Wayne, NJ, USA) to form beads. This suspension (10^9 CFU/ml) was mixed with 1.0% (w/v) mackerel tuna-based (Euthynnus affinis) flavoring solution (Hondashi Ajinomoto, Limeira, São Paulo, Brazil). This mixture was dripped into a 0.1 M solution of CaCl2 with a 3 mm syringe injector nozzle under agitation at 82 rpm. A 3.0% dye mixture was added to the $CaCl_2$ solution (Starfest Produtos Alimentícios, Rio de Janeiro, RJ, Brazil). The beads were allowed to stand for 30 min in the $CaCl_2$ solution for gelification under aseptic conditions. They were then removed from the solution and stored in sealed sterile vials at 4 °C for 30 days. The viability of probiotic strains inside the beads was evaluated immediately after production. For the analysis, 0.5 g of beads were liquefied in 4.5 ml sodium citrate (0.1 M) by shaking at room temperature (21 ± 2 °C) for 5 min, in order to completely release the cells from the beads. Liquefied samples were homogenized in saline, decimally diluted in peptone water 0.1% (w/v), plated in MRS agar and incubated at 37 °C for 48 h in a microaerophilic atmosphere.

2.3. Encapsulation efficiency

Encapsulation efficiency was calculated according to the procedure proposed by Donthidi, Tester, and Aidoo (2010), as follows:

$$\text{Encapsulation efficiency}\ (\%) = \frac{\text{Bacteria in the beads(CFU/g)} \times 100}{\text{Bacteria added to feed suspension(CFU/g)}}$$

2.4. Diameter and weight of the probiotic beads

The diameter of the probiotic beads was measured with a caliper. Twenty units were chosen at random and the results were expressed as means ± standard deviation. To evaluate bead weight, sampling was conducted randomly, based on the equation below:

$$\text{w beads}\ (\text{g}) = \frac{(\text{w 10 beads}/10) + (\text{w 10 beads}/10) + (\text{w 10 beads}/10)}{3}$$

where w indicates weight.

2.5. Scanning electron microscopy

Probiotic beads were analyzed by scanning electron microscopy (SEM) as described by Jiménez-Pranteda et al. (2012). Beads were fixed with 2.5% glutaraldehyde, dehydrated by increasing concentrations of ethanol solutions (50%, 70%, 80%, 90% and 100%, v/v ethanol) and dried up by using a critical point dryer BAL-TEC CPD 030. Samples were gold-coated and observed with the scanning electron microscope (model JEOL JSM-5310 Tokyo, Japan).

2.6. Resistance to simulated gastric juice

The tolerance of free and encapsulated probiotics strains to gastric juice was determined according to Monteagudo-Mera et al. (2012). Simulated gastric juice was prepared fresh daily by suspending pepsin (Sigma Chemical Co.) in sterile saline (0.5%, w/v) to a final concentration of 3 g/l and adjusting the pH to 2.0, 2.5 and 3.0 with concentrated HCl.

An aliquot (0.2 ml or 0.2 g) of each washed cell suspension as well as probiotic beads was transferred to a 2 ml capacity Eppendorf tubes, mixed with 0.3 ml of saline solution and 1 ml of simulated gastric juice and was incubated at 37 °C for 180 min. For screening tolerance, samples were taken at time 0 and periodically (45, 90 and 180 min) for the determination of total viable count following the spread plate method as described previously. Assays were carried out in triplicate.

2.7. Entrapped probiotic cells viability

The viability of probiotic bacteria inside the beads was evaluated at different temperatures as previously described. To monitor storage under abusive temperature (25 and 30 °C) the beads were analyzed over 5 h of storage. Beads stored under refrigeration (4 °C) were analyzed for 30 days.

2.8. Sensory analysis

The acceptance of the overall features and color, aroma, texture and flavor attributes were evaluated in an open trial by a team of 60 untrained panelists, through a 9-point sensorial affective hedonic scale, ranging from “Like extremely”, a grade 9, to “Dislike extremely” a grade 1 (Instituto Adolfo Lutz, 2008; Meilgaard, Civille, & Carr, 2006). The samples were offered in randomized blocks (Macfie & Bratchell, 1989).

An “intent to purchase form” was inserted in this test, as well as panelist profiles, which were designed based on sex, age, schooling and consumption frequency.

To calculate the acceptability index (AI), we adopted the mathematical expression:

$$\text{AI} = \frac{\text{X.100}}{\text{N°}}$$

where AI = Acceptability Index, X = Average grade attributed by the tasters, *N*° = Highest grade attributed by the tasters.