P-102 **Microencapsulation of probiotic bacteria in alginate- protein mixtures**

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INTRODUCTION AND OBJECTIVES

Due to their health benefits, probiotic bacteria have been increasingly included during the past two decades in food products, especially in yoghurt and in fermented milks. In order to these microorganisms exert positive health effects; they have to reach the large intestine of the host, alive and in sufficient number. However, considerable losses in cell number occur in stomach and in the duodenum due to the low pH and the bile presence. Cell protection by microencapsulation has been used to increase probiotics viability in the upper gastrointestinal tract (Anal and Singh, 2007). For food applications, non-toxic, food grade materials must be used and particle size has to be lower than 100 μm in order to not alter texture properties (Hansen et al., 2002). Proteins are widely used in formulated foods, partly because of their nutritional value, but especially for their functional properties, which include gelling, foaming and emulsification. Among these functional properties, the gel-forming is especially interesting as it allow the development of GRAS biocompatible carriers for oral administration of sensitive nutraceuticals in a wide variety of foods (Chen et al., 2006). Soy protein is used extensively as a functional ingredient in many different food products, such as baked goods and cured meats. It has been shown recently that cold-induced gelation of soy protein can be achieved by adding Ca^{2+} ions to a preheated soy protein suspension (Maltais et al. 2005). Used as a substitute for meat protein or as a nutritious and functional additive, pea protein could also play an important role, similar to what is done with soy protein.

The aim of the study was to investigate the use o soy proteins and pea proteins as encapsulating materials to increase the viability of probiotic strains to adverse conditions such as low pH values.

MATERIALS AND METHODS

Immobilization materials Solbar 950, a commercial soy protein isolate (SPI) was a generous gift of Solbar (Israel). Nutralys® S85F a commercial pea protein isolate (PPI) was a generous gift of Roquette (France). Medium viscosity alginate (Sigma).
Aseptic conditions were used for all of the procedures outlined below.

Bacterial strain and culture conditions The probiotic strain *Lactobacillus casei* was provided by Dr. A. Gomes (Escola Superior de Biotecnologia, UCP, Portugal). Harvested cells were stored as stock solutions in de Man Rogosa Sharpe (MRS) broth (Oxoid) containing 20% (v/v) aqueous glycerol at −80 °C. Bacteria for immobilization experiments were propagated from 1% (v/v) inoculations, incubated overnight at 37 °C under aerobic conditions. The cells were harvested by centrifugation at 4000 rpm for 10 min (Eppendorf 5804R), washed and resuspended in 0.9 % NaCl solution to obtain a concentrated cell suspension. Ultimately, the cell slurry was either employed within the immobilization process, or utilized (as a control) in a free-cell condition.

Microencapsulation of probiotic organism using the emulsion technique A method modified from Sultana et al. (2000) was used. Alginate (1% w/v), or 1% (w/v) alginate plus 4,5 % (w/v) SPI or 1% (w/v) alginate plus 6% (w/v) PPI were used as encapsulating matrix and mixed with 10 mL of suspension containing 9.0–10.0 $\log^{10}$cfu/ml of *L. casei*. Forty millilitres of the probiotic mixture was gently dispensed into a beaker containing 260 mL of commercial vegetable oil containing Tween 80 (0.2%). and stirred at 600 rpm using a an Ika-Eurostar® mixer (Ika) equipped with a marine impeller. After 15 min of emulsification, calcium chloride (0.1 M) was gently added down the side of the beaker until the emulsion was broken. The mixture was allowed to stand for 30 min for the particles to separate and settle at the bottom of the calcium chloride layer. The oil layer was drained and beads were collected by low speed centrifugation (3503g, 15 min) and successively washed with 0.05 M CaCl2 containing 0.5 % Tween 80, 0.9% saline containing 0.5% Tween 80 and 0.9% saline solution.

Survival of free and encapsulated bacteria under gastric conditions For evaluation of the protective effect at low pH-values, a simulated gastric juice (pH 1.5) without pepsin was prepared a buffer mixture composed of 0.2 M HCl solution and 0.2 M KCl solution (USP XXIII). Fresh aqueous microcapsule-slurry (1.0 g) or free cell suspension (1 ml) was added to a test-tube containing 9 mL of prewarmed (37 °C) simulated gastric juice and vortexed for a few seconds. Aliquots of 0.5 mL were removed after 30, 60 and 120 min.

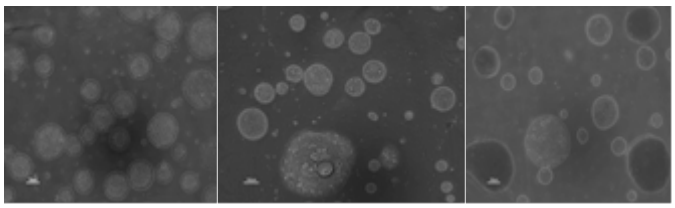
Enumeration of the free and encapsulated bacteria The enumeration of living cells of *L.casei* was assayed by a serial 10-fold dilution. 0.1 mL of the samples were plated in duplicate on MRS agar. Colony forming units (CFU) were determined after 48 h aerobic incubation at 37 °C. The immobilized bacteria were previously released from the capsules by disintegration with a high-speed homogenizer (Ultra-Turrax, Model T18 basic, Ika) in phos-

phate-buffered saline (PBS, 0.01M NaH2PO4, 0.137M NaCl, 2.68mM KCl, pH 70).

Morphology and diameter of the particles The morphology of the beads was observed in an inverted light microscope (Nikon Eclipse, TE2000U equipped with a digital camera DXM1200F (Nikon) Micrographs, at a magnification of 100x, were taken to particles randomly selected and the diameter of each particle measured with ImageJ software (http://rsb.info.nih.gov/ij/).

RESULTS AND DISCUSSION

Figure 1 illustrates shape and size of microparticles obtained with alginate alone or mixtures of alginate and soy protein or pea protein.



(a) (b) (c)

Figure 1: **Morphology of microparticles of (a) alginate 1%, (b) alginate 1% + SPI 4.5% (c) alginate 1% + PPI 6%.**

As expected with this encapsulating method the size and shape of particles is quite heterogeneous. The mean sizes of the particles (Table 1) were higher than desired (less than 100 µm), however, the procedure has not been optimized. The inclusion of soy proteins, decreased the average particle size (P = 0.011). The same occurred when pea proteins were used (P = <0.001).

Table 1: Size of the microparticles obtained by the emulsion technique using different encapsulating matrixes.

Encapsulating matrix	Particle diameter (µm)
Alginate 1%	156.8 ± 65,6
Alginate 1% + SPI 4.5%	120,2 ±68,4
Alginate 1% + PPI 6%	92,1± 67,7

Results are expressed as means ± standard error of three repeated experiments.

The survival of free and encapsulated bacteria in simulated gastric juice (pH 1.5) without enzymes is shown in figure 2. It is possible to verify that there is an initial marked decrease in cell survival, but it remains relatively constant from 30 min. A similar behaviour was already described in the literature (Chandramouli *et al.,* 2004 and Hansen *et al.,* 2002). Encapsulation in any of the matrixes used (i.e, alginate 1%, alginate + SPI 4.5 % and alginate 1% + PPI 6%) improved survival of *L. casei*. However, the use of proteins did not resulted in a better survival compared to alginate alone.

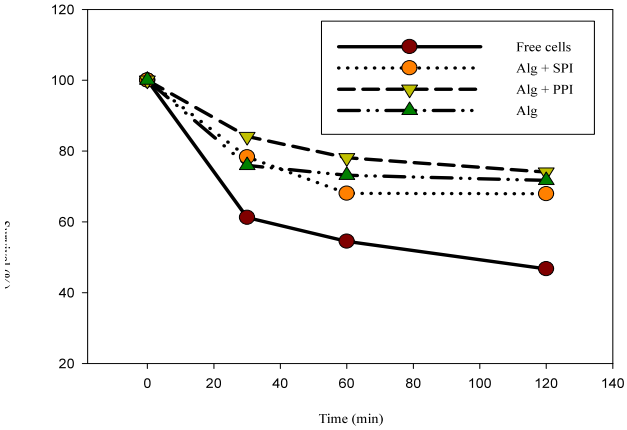


Figure 2: Survival of free and encapsulated *L. casei* in simulated gastric juice (pH = 1.5).

CONCLUSIONS

The results of this study demonstrated that it is possible to make microparticles of alginate and soy or pea protein by the method of emulsification, obtaining spherical particles.

When subjected to simulated gastric juice pH 1.5 encapsulated *L. casei*, showed a significantly higher survival, compared to free cells. However, the use of proteins did not confer a better survival compared to alginate alone.

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