EFFECTS OF FORMULATION VARIABLES ON THE PARTICLE SIZE AND VIABILITY OF *L.CASEI* - LOADED IN WHEY PROTEIN-CA-ALGINATE MICROPARTICLES

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INTRODUCTION

Lactobacillus casei has been found to colonize human GIT and exert many health benefits. The probiotic cells are very susceptible to harsh conditions and various approaches have been used to improve their viability and to reduce the cell damage and loss of viability during processing and storage. A new approach in combination of emulsion method and subsequent coating was used in order to reduce cell damage during processing and increase the final cell count. The aim of this work was to evaluate the influence of the formulation variables of *L. casei*-loaded whey protein-Ca-alginate microparticles on the particle size and the survival of the mentioned probiotic during the processing.

MATERIALS AND METHODS

Emulsion technique was applied to aqueous dispersion of alginate and *L. casei* in olive oil, containing 0,2% Tween 80 to obtain spherical particles, which were then cross-linked in $CaCl_2$ solution. Microparticles were subsequently coated with hydrated native whey protein for 1 h, isolated, washed and freeze-dried. Ca-alginate and whey protein-Ca-alginate microparticles loaded with the probiotic were measured immediately after the preparation, using a Mastersizer Hydro-2000S, Malvern Instruments Ltd., UK.

The survival of the microencapsulated *L. casei* was evaluated prior to- and after lyophilisation. The viability of the cells was assessed in dispersion of beads in 0.05M PBS, pH 6.5 and the number of viable cells was obtained using the plate-count method on MRS agar, after serial dilutions in peptone water.

The influence of the formulation variables was assessed using polynomial regression model at 2nd level, with the experimental matrix of 11 batches. Concentration limits of three variables were alginate (1 and 4%*w/w*), whey protein (1 and 3%*w/w*) and $CaCl_2$ (1 and 5%*w/w*). The cell load in the initial suspension was ca. 10-11 $\log_{10}$cfu/g.

RESULTS AND DISCUSSION

The particle size obtained was in the range from 36.32-63.10μm for the Ca-alginate microparticles (Fig. 1a), and 42.78-77.43μm for the whey protein–Ca-alginate microparticles (Fig. 1b). The results pointed to the dominant influence of the conc. of whey protein on the particle size, followed by the conc. of alginate. Higher conc. of whey protein and alginate in the coating medium resulted in increased particle size. The influence of the conc. of $CaCl_2$ was insignificant. (Fig. 2)

The survival rate of the probiotic in the whey protein-Ca-alginate microparticles was between 9.30 and 10.78 $\log_{10}$cfu/g, in the range from 90.34-99.58% in the particles prior to lyophilisation and between 84.16-98.37% in the particles after lyophilisation.

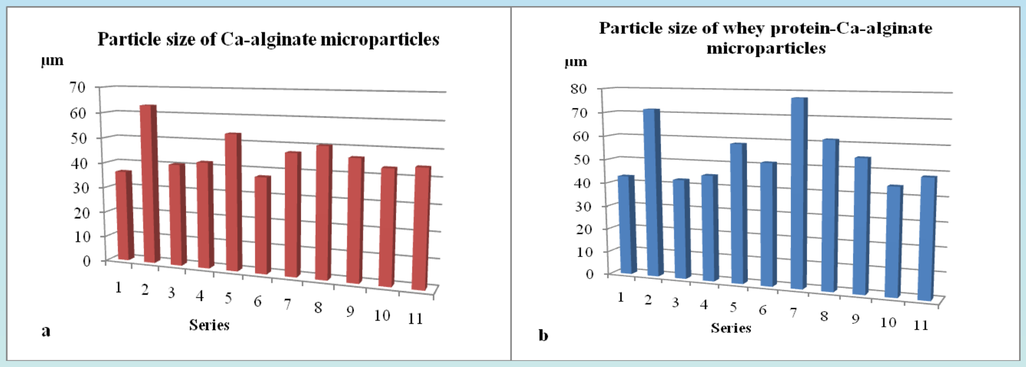

Fig.1: Particle size of *L. casei*-loaded microparticles: a) Ca-alginate, b) whey protein-Ca-alginate microparticles

The data pointed to the dominant influence of the concentration of alginate and $CaCl_2$ on the viability prior to-, and this trend continues after lyophilisation. Significant negative effect of the alginate - whey protein interactions on the viability after lyophilisation was observed (Fig. 3), suggesting competition of the polymers and the probiotic for the same bonding sites.

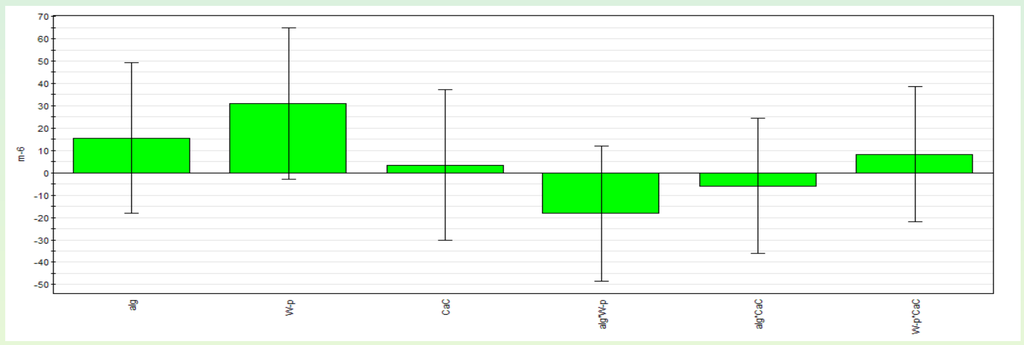

Fig.2: Effect of the experimental variables on microparticle size

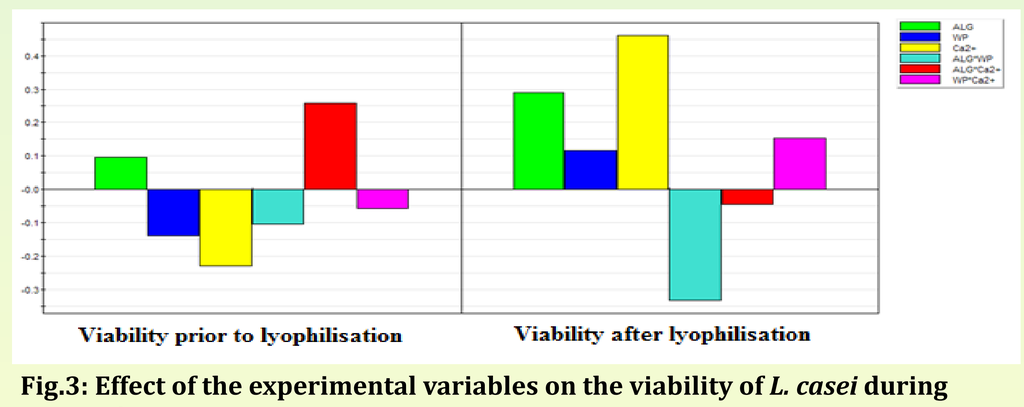

Fig.3: Effect of the experimental variables on the viability of *L. casei* during processing

L. casei loaded whey protein-Ca-alginate microparticles were prepared with survival rate of the probiotic above the minimum therapeutic dose of 10^7–10^9cfu/g per day and particle size distribution suitable for the delivery of the probiotic to the lower intestine.

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