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Lactobacillus casei loaded alginate-soy protein microparticles: acidification kinetics and survival of the probiotic in simulated gastrointestinal conditions

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1. INTRODUCTION

The probiotics are defined as "live microorganisms which, when administered in adequate amounts, confer a health benefit for the host" (*WHO, 2001).* Numerous health benefits are claimed in favor of the probiotic *Lactobacilli casei* and this probiotic has been investigated for several biological effects as: improvement and promotion of digestion, control of diarrhea, cholesterol lowering effect, anti-inflammatory effect on the gut, reducing lactose intolerance and modulation of the immune system. However, as most lactobacilli it does not survive well during temperature and osmotic extremes and gastric passage *(Yan Li et al., 2009).*

Nowadays, microencapsulation of probiotic bacteria is the method of choice for reducing losses of sensitive bacteria by external factors. In this study the probiotic was encapsulated in a polymeric matrix formed of alginate and soy protein isolate (SPI) to protect the probiotic from these extremes and harsh environment in the GIT.

The aim of this study was to assay the viability of the probiotic and its metabolic activity after loading in alginate-soy protein microparticles.

2.METHODS

Microparticles were obtained by emulsion technique. Bacterial suspension in aqueous solution of 2.5%w/w alginate (10 ml) was dispersed in olive oil (40 ml) containing 0.2% Tween 80 to obtain probiotic loaded alginate microparticles, which were subsequently cross-linked with 3%w/w $CaCl_2$ and coated with soy protein. The concentration of the soy protein in aqueous solution was varied in a range from 1:4 to 4:1 in respect to alginate. Six experimental series were prepared. The beads of each experimental series were then isolated, washed and stored (4 °C, 0.9% saline) until further evaluation.

- The metabolic activity was determent by comparation of the time taken for free and encapsulated cells to acidify MRS broth medium. Free and encapsulated cells were cultivated in MRS broth (37°C) and the pH and optical density (OD) at 600 nm of the mediums were measured every 4h.

- Viability tests of free and encapsulated cells were performed by exchanging simulated GI juices : gastric-3h, pH 1.5, 3g/L pepsin, intestinal-3h, pH 6.8, 0,6%w/v Ox-gall, 0,1%w/v pancreatin, colon-6h, pH7.4) and incubation (37°C, 75rpm). The enumeration of living cells was assayed by plate count method on MRS agar (37°C, 48h).

3.RESULTS

Negatively charged microparticles (-24,46 to -39.06 mV) were obtained with d_{50}16 to 36μm, Ca-content 5.56 to 9.38% and cell load 9.11 to 11.25$\log_{10}$CFU/g.

The free and the entrapped cells changed the OD and pH of the MRS broth medium with time, indicating the viability of bacterial cells in alginate-SPI microparticles. The time taken to decrease the initial pH of MRS broth to 4 was 20h for free cells and for encapsulated cells 32 to 56h. However the encapsulated cells needed more time to reach the same pH value and OD as the free cells. The series with higher concentration of soy protein isolate needed more time (32 to 56h) to reach the pH values and OD in the MRS broth media of the free cells (24h) (Figure 1).

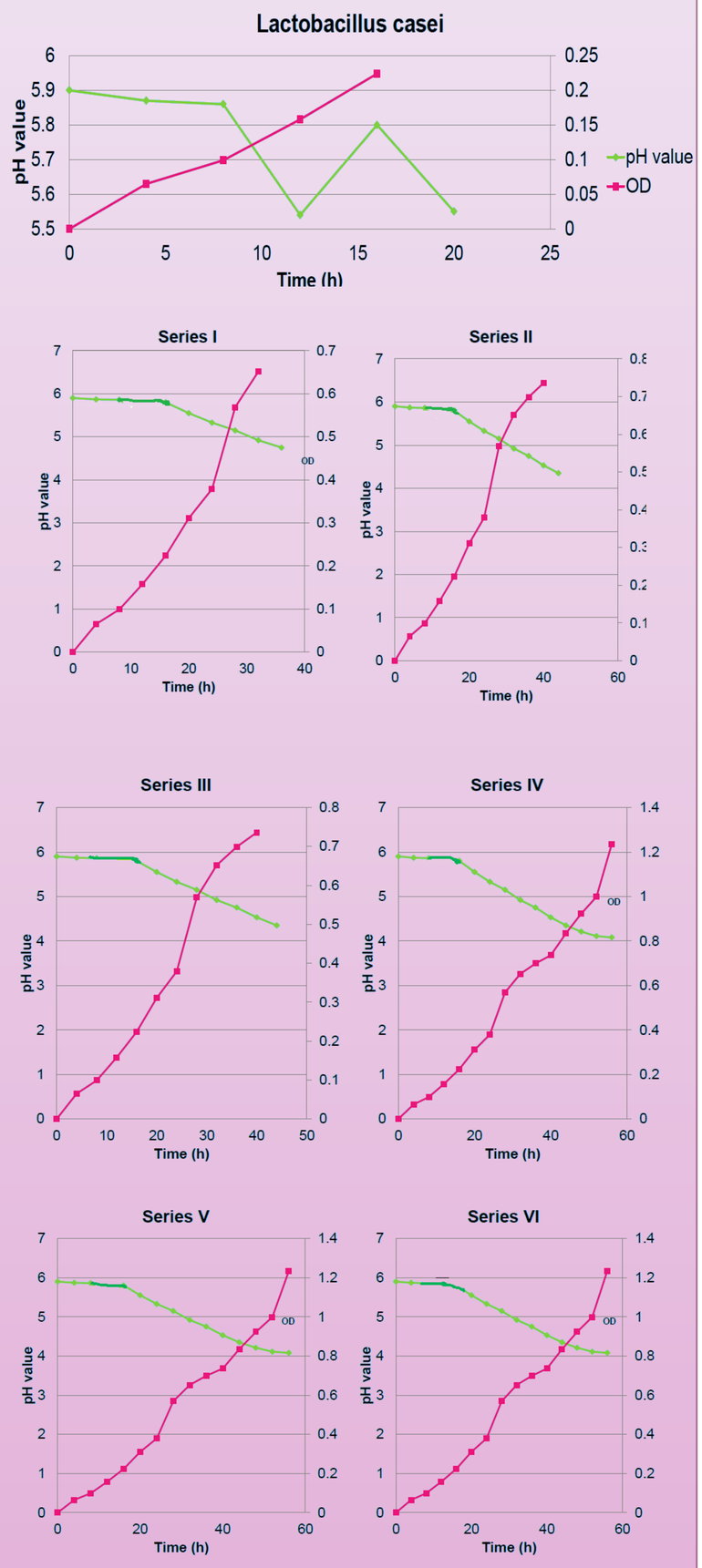


Figure 1. The changes of pH and optical density during 56 h in MRS broth medium for free *L. casei and encapusulated L. casei* in alginate-SPI microparticles

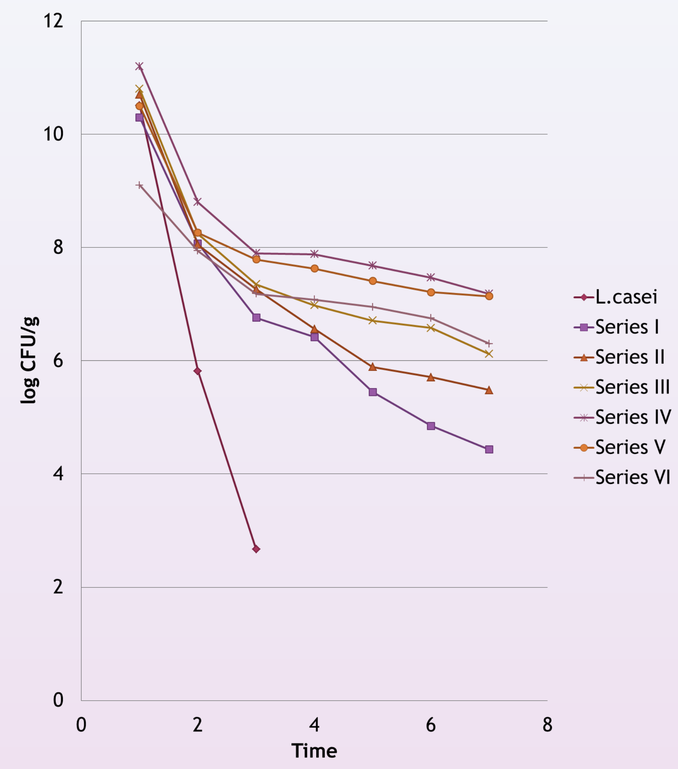
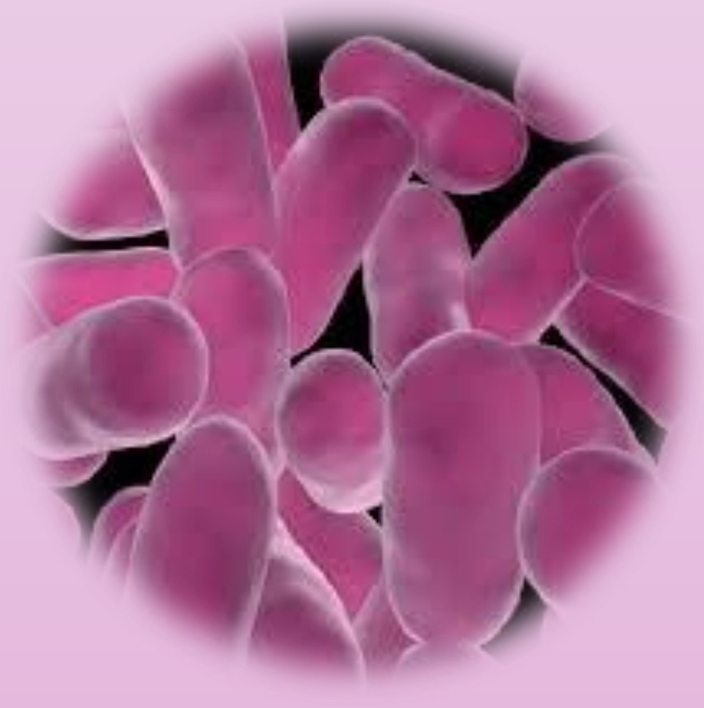


Figure 2. Survival of *L. casei* in simulated gastric juice for free and encapsulated cells

The initial loss of cell viability for free and encapsulated cells was observed after 0.5h in pH 1.5, with 70% for free *L. casei* and 20 to 35% for the encapsulated probiotic. The viability of the probiotic after 4 hours in pH 7.4 ranged from 5.7 to 8.6$\log_{10}$CFU/g (Figure 2) or expressed in percents, from 43.01 to 69.23 %, with increasing of the SPI content.

4.CONCLUSION

L. casei loaded alginate-soy protein microparticles were prepared with high probiotic viability after preparation and in simulated gastrointestinal tract.



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