

## Microencapsulation of *Saccharomyces cerevisiae* and its evaluation to protect in simulated gastric conditions

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### ABSTRACT

**Background and Objectives:** Probiotic yeasts are used in production of functional foods and pharmaceutical products. They play an important role in promoting and maintaining human health. Until now, little work has been published on improving the survival of *Saccharomyces* in stimulated gastrointestinal condition.

**Material and Methods:** In this study the exposure of the yeast in the capsule and free forms to artificial gastrointestinal conditions was assessed and the number of viable *Saccharomyces cerevisiae* cells during 0 to 120 minutes in these conditions was evaluated by a pour plate method using Sabouraud dextrose agar.

**Results:** Results showed the shape of the beads was generally spherical, sometimes elliptical with a mean diameter of about 50–90  $\mu\text{m}$ . Also count of viable probiotic cells obtained for all the microcapsules were above the recommended levels for a probiotic food. Also decrease of approximately 4 logs was noted in the number of free cells after 2 h of incubation at pH 2 and 8, when compared to decreases of about 2 logs in the all microencapsulated *S. cerevisiae* under similar conditions.

**Conclusion:** It is concluded that microencapsulation process was significantly able to increase the survival rate of *Saccharomyces* in a simulated gastrointestinal condition ( $p < 0.05$ ).

**Keywords:** Calcium alginate, survival, *Saccharomyces*, repeated condition

### INTRODUCTION

Microencapsulation is one of the most important

forms of controlled release of substances that is widely used in several fields of the industry, particularly the food industry (1). The encapsulated microbial cells have been used in wide range of fields for optimum activity benefiting the suitable conditions for growth and metabolism and at the same time protection from harsh environmental conditions (2, 3). Over the years, there has grown a great interest in developing suitable eukaryotic probiotics in general and probiotic yeasts in particular for human and animal practice (4). However, nowadays yeasts are

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part of nutritional supplements and healthy food realms due to their established beneficial probiotic effects (5). yeasts especially the genus *Saccharomyces* are widely used probiotics in human and animals throughout the world (6, 7). Providing probiotic living cells with a physical barrier against adverse environmental conditions is therefore an approach currently receiving considerable interest (8, 9). In addition microencapsulation via calcium alginate has been used extensively for immobilization of probiotic because of its simplicity of handling, its non-toxic nature, and its low cost (9-11). The aim of this study was to evaluate the survival rate of free and microencapsulated *S. cerevisiae* by calcium alginate in simulated gastrointestinal conditions.

## MATERIALS AND METHODS

**Yeast strain and growth conditions.** The yeast strain used in this study was *S. cerevisiae* (ATCC code 9763). Yeast cells were activated by transferring cells from refrigerated slant to 50 ml of liquid culture medium sabouraud dextrose (SD) broth (Merck Co., Darmstadt, Germany) and incubated overnight at 28 °C with orbital shaking of 180 rpm. 1 ml of the incubation was inoculated into another 50 ml of the same medium and incubated for 24 h under the same culture conditions. Cells were harvested by centrifugation at 10,000 rpm for 10 min and washed five times with distilled water to remove the culture medium from the cells thoroughly.

**Preparation of simulated gastric and intestinal juices.** Simulated gastric juices (SGJ) were prepared by suspending pepsin (P7000, 1:10,000) in sterile sodium chloride solution (0.5%, w/v) to a final concentration of 3 g/l (1038 U/ml) and adjusting the pH to 2.0 with concentrated HCl or sterile 0.1 mol/l NaOH. Simulated intestinal juices (SIJ) were prepared by suspending pancreatin USP (P-1500) in sterile sodium chloride solution (0.5%, w/v) to a final concentration of 1 g/l, with 4.5% bile salts (Oxoid, Basingstoke, UK) and adjusting the pH to 8.0 with sterile 0.1 mol/l NaOH. Both solutions were filtered for sterilization through a 0.22 µm membrane.

**Microencapsulation procedure.** In this study extrusion technique was performed for microencapsulation process described earlier (11). A 2% Na-algi-

nate mixture in distilled water. Then, the mixture of cell suspension and Na-alginate were injected into a 0.1 M CaCl<sub>2</sub> solution through a sterile insulin syringe. The droplets formed gel spheres immediately. The distance between the syringe and CaCl<sub>2</sub> solution was 25 cm.

**Cell tolerance to gastrointestinal and determination of total viable counts.** The tolerance of free and immobilized cells of *S. cerevisiae* on simulated gastric and intestinal juices was determined using the adapted method described by Charteris et al. (1998). The tests were performed using a series of 10ml sterile falcon tubes, one for each sampling point. Two different conditions were tested: in the first and second tests, 0.4 ml of the suspension of either immobilized or free cells were mixed with 1.8 ml of SGJ or SIJ, gently mixed and incubated for 120 min at 28 °C. The control for these tests was done by incubating 0.4 ml of either free or immobilized cells in 1.8 ml sterile sodium chloride solution (0.5 %, w/v) for 120 min at 28 °C. After the addition of free cells or immobilized to SGJ and SIJ, the pH of these were corrected to 2.0 and 8.0, respectively, with sterile 0.1 mol/l NaOH or concentrated HCl. Aliquots of 1 ml were removed at 0, 30, 60, and 120 min (for all trials) for the determination of total viable counts. Total viable counts of *S. cerevisiae* were determined by a pour plate method using SC agar (Merck Co., Darmstadt, Germany) after serial 10-fold dilutions in peptone water. Plates were incubated at 28 °C for 72 h.

**Statistical analysis.** The chi-square (X<sup>2</sup>) test was used to assess statistical differences between the groups. A P value less than 0.05 was statistically considered significant.

## RESULTS

In this study the size, surface morphology and shape of the 50 randomly selected calcium alginate beads were determined with light microscope at a magnification of 400. The shape of the beads was generally spherical, sometimes elliptical with a mean diameter of about 50–90 µm. On the other hand internal, appearance of coated bead at 400× magnification showed that yeasts were distributed randomly in the alginate matrix (Figs. 1 and 2). The loss during

encapsulation was very low due to the gentle methods used. The results of survival rate of encapsulated cells in simulated gastric pH and intestinal bile salt solutions showed in Table 1. No significant reduction in viable count was observed in free as well encapsulated cells in distilled water (pH 6.5) on incubation

for up to 2 h (control condition). But variations in counts of free and microencapsulated *S. cerevisiae* during incubation period at SGJ and SIJ were significant (Table. 1). However, results show there were significant reductions of free yeast in immediate exposure to pH 2 and 8. After 120 min incubation in



Fig. 1. Optical microscope image of coated yeast breads at 400× magnification.

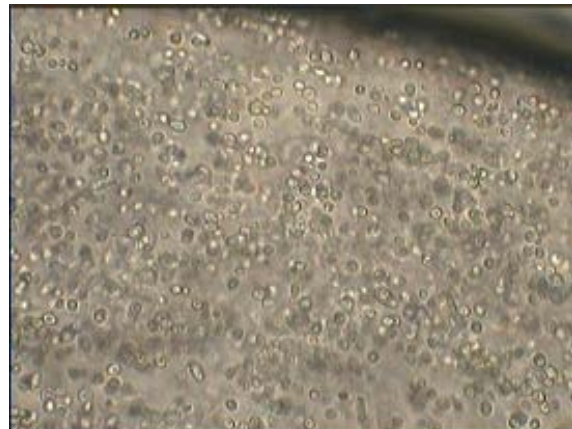


Fig. 2. Vertical cross-section and internal appearance of yeast coated bead at 400× magnification.

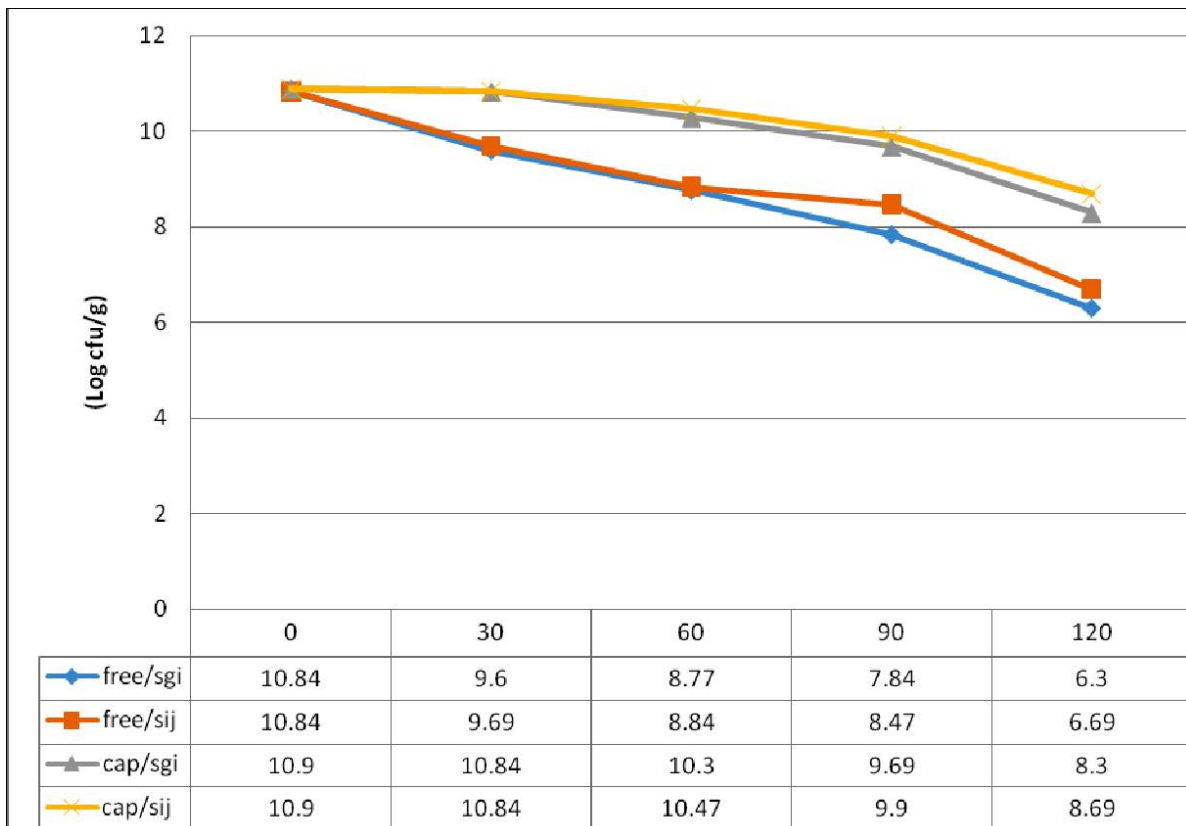


Fig. 3. Survival of microencapsulated and free *Sacchromyces cerevisiae* in simulated gastric conditions

**Table 1.** Number of alive cells after expose to SGI and SIJ for different times (mines) mean  $\pm$  Sea

Condition	Type of cell	Hardening time				
		0	30	60	90	120
distilled water	free	$9 \pm 2 \times 10^{10}$	$9 \pm 4 \times 10^{10}$	$9 \pm 4 \times 10^{10}$	$8 \pm 1 \times 10^{10}$	$8 \pm 3 \times 10^{10}$
	Capsulated	$9 \pm 2 \times 10^{10}$	$9 \pm 3 \times 10^{10}$	$9 \pm 2 \times 10^{10}$	$8 \pm 4 \times 10^{10}$	$7 \pm 4 \times 10^{10}$
SGJ	free	$7 \pm 2 \times 10^{10}$	$4 \pm 1 \times 10^9$	$6 \pm 3 \times 10^8$	$7 \pm 10^7$	$2 \pm 2 \times 10^6$
	Capsulated	$8 \pm 3 \times 10^{10}$	$7 \pm 2 \times 10^{10}$	$2 \pm 3 \times 10^{10}$	$5 \pm 7 \times 10^9$	$2 \pm 6 \times 10^8$
SIJ	free	$7 \pm 3 \times 10^{10}$	$5 \pm 2 \times 10^9$	$7 \pm 4 \times 10^8$	$3 \pm 3 \times 10^8$	$5 \pm 2 \times 10^6$
	Capsulated	$8 \pm 4 \times 10^{10}$	$7 \pm 2 \times 10^{10}$	$3 \pm 2 \times 10^{10}$	$8 \pm 1 \times 10^9$	$5 \pm 1 \times 10^8$

simulated gastric and intestinal condition there was a significant increase ( $p < 0.05$ ) in survival of yeast cells in the capsulated compared with free cells (Fig. 3).

## DISCUSSION

Over the years, probiotics have gained scientific and commercial interest and have now quite commonplace in our daily life starting from health promoting functional foods to therapeutic, prophylactic, and growth supplements (11, 12). On the contrary to prokaryotic cells, the use of eukaryotic as probiotics is limited and only a few probiotics of eukaryotic origin are commercially available for human and animal practices. Survival of the *S. cerevisiae* was found to increase on alginate encapsulation. Also there was no significant difference in the viability of free cells in distilled water, indicating that water had no effect on the survival (13, 14).

Among the eukaryotic probiotics, yeasts especially *Saccharomyces* species are dominant and routinely used in a broad range of hosts that can tolerate a wide range of temperature, salt concentration, and pH depending upon the strain especially *S. cerevisiae* (15).

In addition, the count of viable probiotic cells obtained for all the microcapsules was above the recommended levels for a probiotic food, i.e., equal to or greater than 7 log CFU/g of the product, which is in accordance with earlier reports (15, 16).

On the other hand, the free strain showed a steady loss in viability when exposed to acid conditions. However, the microcapsules containing *S. cerevisiae* survived very well ( $P < 0.05$ ) after exposure to in vitro acid conditions when compared to free cells. Also,

a decrease of approximately 4 log was noted in the number of free cells after 2 h of incubation at pH 2, when compared to decreases of about 2 log in the all microencapsulated *S. cerevisiae* under similar conditions (11, 17).

However, the literature report mentioned the effect of Ox gall bile on the viability of the probiotics, in this study the capsulated yeast had a significant survival rate in SIJ. More recently, *Saccharomyces* probiotics have been found to aid in controlling the pathogenesis of diabetes and other metabolic diseases. It is necessary to mentioned that high tolerance of yeast in different conditions such as intestinal and functional food showed the good potential of this cell as probiotic. Regarding the above mentioned points and agreement of this study results with many other reports about microencapsulation of bacteria, Ca alginate could be good material for protection of probiotic cells (18-20).

## CONCLUSION

*S. cerevisiae* yeast cells, fundamental in the fermentation industry, comprise a natural, safe (food grade), cheap and abundant food material. The present study has shown that Ca alginate microencapsulation significantly improve the yeast survival in simulated gastric environment, and allow viable cells to reach a beneficial level as probiotic. In order to obtain the other aspect of yeast probiotics, this study should be continued by different material and in functional food condition with defined variable in to the future.

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