Encapsulation of probiotic yeast cells with pectin

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INTRODUCTION AND OBJECTIVES

In recent years, there is a demand for nutraceutical products with probiotics in the food and supplement markets. Yeast of certain strains with a sufficient number of viable cells have been shown to offer probiotic benefits (Berg, 1998). There are yeast probiotic products in gelatin capsules and powder form on the market, which are not always appealing to consumers. In this work a new dosage form of tablets has been developed in order to prolong the product shelf life, improve product efficacy and enhance consumer acceptibility.

However, direct compaction of yeast cells with excipients to product rigid tablets may cause significant damage to the cells. Therefore, yeast cells were encapsulated in calcium pectinate beads in order to create a microenvironment for cells to minimize damage. The beads were then mixed with excipients for compaction into tablets. The objective of this study is to encapsulate yeast cells with calcium pectinate and to compact the resulting beads into tablet dosage form with minimum damage to the cells. Cell viability was determined by two techniques: plate counting and flow cytometry.

MATERIALS AND METHODS

Encapsulation procedure 15 g of wet yeast was mixed with 40 mL of 5%w/v pectin solution using a stirrer for 30 min. This suspension was pumped through a G23 needle into a crosslinking solution (calcium chloride of varying concentration) to form calcium pectinate beads. The beads were dehydrated by freeze drying for 24 h (Edwards high vacuum Int Limited, model EF03, UK operating at 3 mbar pressure).

Tabletting procedure Dried pectin beads were mixed with microcrystalline cellulose (MCC) in different ratios and compacted into a tablet at different compaction pressures using a universal testing machine (Zwick/Roell 2030, UK)

Viability determination The viability of yeast was assessed by plate counting and flow cytometry. A yeast suspension was diluted in TS buffer and plated onto YM agar and incubated at 25°C for 72 h.

Bis-oxonol (BOX) was used to determine if the cytoplasmic membrane of the cells is polarized and propidium iodide (PI) was used to discriminate between cells with intact and porous membranes. Dual staining using these dyes prior to flow cytometry analysis (BD Accuri C6, MI, USA) can discriminate between healthy, injured and dead cells (Hewitt and Nebe-Von-Caron, 2004; Davey and Kell, 1996).

Tensile strength The tensile strength of tablets was determined by diametral compression to their failure. Single tablets were placed upright at the middle of a base plate and compressed vertically. The force required to break the tablet was recorded and tensile strength (σ_T) was calculated by the following formula:

$$\sigma_T = \frac{2F}{\pi DT}$$

Where F is maximum force needed to break the tablet, d and T are diameter and thickness respectively.

Encapsulation efficiency (EE)

$$= \frac{Number\,of\,cell\,in\,beads}{Initial\,number\,of\,cells\,(INPUT)} \times 100\%$$

Cell viability based on CFU

$$= \frac{CFU\,in\,calcium\,pectinate\,beads}{CFU\,in\,wet\,yeast\,(INPUT)} \times 100\%$$

RESULTS AND DISCUSSIONS

Calcium pectinate beads after encapsulation have an average particle size of 3-4 mm. After 24 h freeze drying, the size reduced to 1-1.5 mm in average, Fig. 1 (a) and (b).

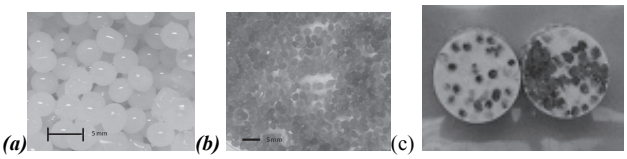

Figure 1 : Images of pectin beads before (a) & after (b) freeze drying and tablets (c) from compaction of calcium pectinate beads mixed with MCC in 50/50 (wt/wt) ratio

In order to produce calcium pectinate beads with different mechanical rigidity, the concentration of the crosslink solution ($CaCl_2$) was varied at 1%, 1.5%, 2% and 2.5% w/v. Viability of cells in the calcium pectinate beads and encapsulation efficiency at each $CaCl_2$ concentration were determined.

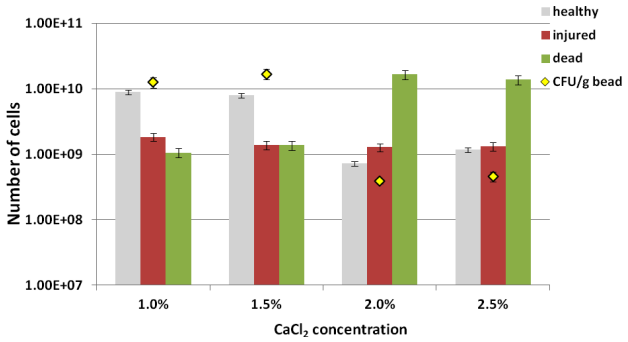

Figure 2 : CFU and the number of healthy, injured and dead cells in 1g of calcium pectinate beads prepared at different $CaCl_2$ concentrations

A decrease in yeast cell viability was observed when $CaCl_2$ concentrations higher than 1.5% w/v were used (Fig. 2). Calcium is one of the important ions in eukaryotic cell function, being-involved in metabolic process and cell replication. However, a high calcium chloride concentration was thought to damage the cell membrane by disturbing the state of cell electrolyte, cell membrane, and subsequently cell function (Cao, 2012).

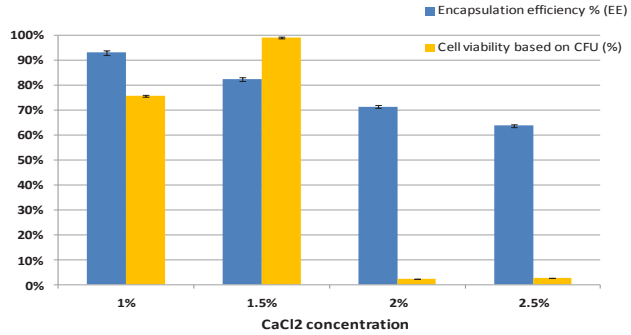

Figure 3 : Encapsulation efficiency (%EE) and cell viability based on CFU (%) of calcium pectinate beads prepared at different $CaCl_2$ concentrations

It has been demonstrated that yeast cells were encapsulated with pectin and entrapped into calcium pectinate beads. The encapsulation of yeast cells with pectin using calcium chloride concentrations of 1% and 1.5% w/v proved to be effective as they showed relatively high encapsulation efficiency and cell viability based on CFU (Fig. 3).

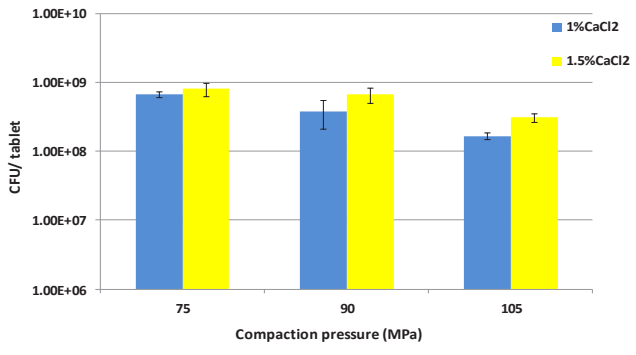

Figure 4 : CFU of cells in tablets produced by mixing calcium pectinate beads and MCC at 50/50 (wt/wt) ratio

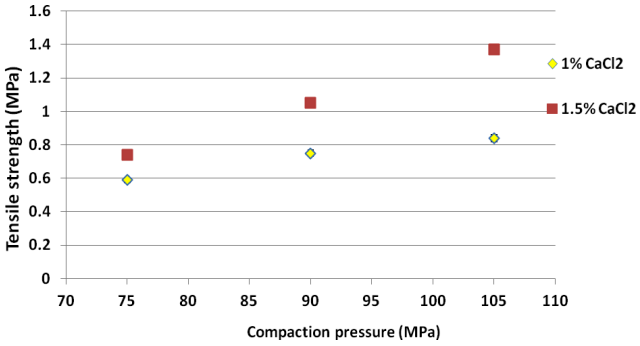

Figure 5 : Tensile strength of calcium pectinate beads tablets produced by mixing calcium pectinate beads and MCC at 50/50 (wt/wt) ratio

The pectinate beads made with a calcium chloride concentration of 1.5% w/v and mixed with MCC compacted at 90 MPa (Fig. 1C) resulted in tablets with $6.7x10^8 \pm 1.68x10^8$ CFU/tablet and a tensile strength of 1.05 ± 0.3 MPa, which may have potential industrial applications (Figs. 4 and 5).

CONCLUSIONS

Yeast cells were successfully encapsulated with pectin in a crosslinking solution of calcium chloride. Using different concentrations of $CaCl_2$ resulted in varying encapsulation efficiency and cell viability based on CFU (%) in the formed calcium pectinate beads. Calcium pectinate beads made with 1.5% w/v $CaCl_2$ mixed with MCC formed rigid tablets which contained a high number of viable cells and may be exploited for industrial applications.

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