Microencapsulation and Delivery of *Pediococcus acidilactici* in Chitosan/Polyaniline Composite

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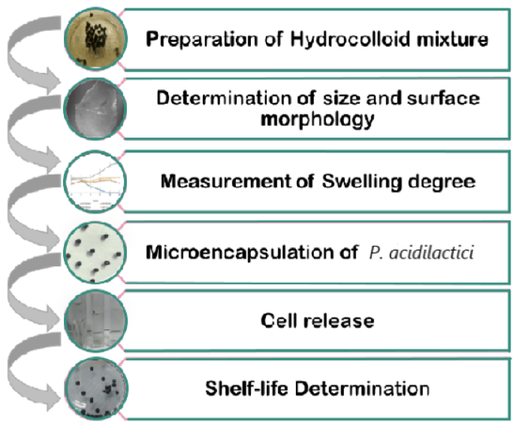
ABSTRACT

Microencapsulation is among several methods and strategies that are being developed to protect probiotic bacteria against adverse environmental conditions in the stomach and increase their recovery rates. This study aims to determine the potential microencapsulation and delivery of *Pediococcus acidilactici* in Chitosan/Polyaniline composite, wherein ionic gelation through extrusion method of microencapsulation is used. It also determined the shelf life and viability of the microencapsulated probiotic. Results show that the optimal CS/PANI ratio that could encapsulate *P.acidilactici* is 3%/0.5% extruded in 1% sodium citrate. The number of probiotic cells that has been entrapped per microbead is 1.61 x 10^6 Cfu/ml ± 0.08. The microencapsulated probiotic was subjected to simulated gastrointestinal (GI) conditions to determine survival through GI transit. The observed cell release of entrapped probiotics in the simulated gastric fluid is considerably higher than expected ranging from 10^5-10^6 cumulative value due to the immediate swelling of the CS/PANI microbeads. However, at the end of exposure to the simulated intestinal fluid, the cumulative release is 10^6-10^7, indicating potential to be released in the gut. While it was found that the cell viability of microencapsulated (46.23%±0.02) probiotics is low as compared to the free cells (69.64%±0.04) after 30 days of storage at 4°C, the results at 30th day of storage at room temperature showed reciprocal findings for free cells (25.92±0.16) and microencapsulated (33.10±0.13). Therefore, microencapsulation of *P. acidilactici* can be a considerable means to achieve higher cell viability both in the course of gastrointestinal delivery and storing at room temperature.

METHODOLOGY:



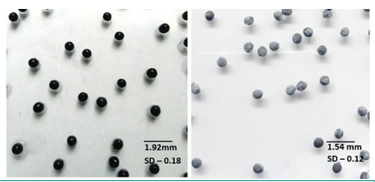


Figure 1. Wet microbeads (left) and freeze-dried microbeads (right)

RESULTS

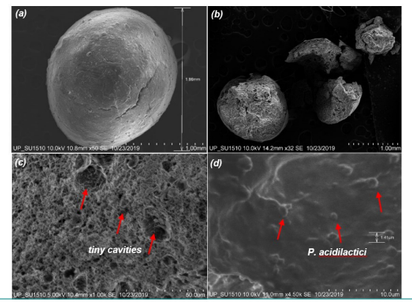


Figure 2. SEM images of (a) whole microbead (b) cracked microbead (c) inner surface showing tiny cavities at 1000x (d) cavity surface at 4,500x magnification

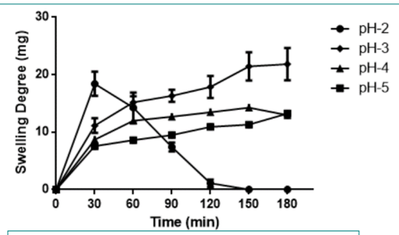


Figure 4. Swelling degree (net weight change) at different pH

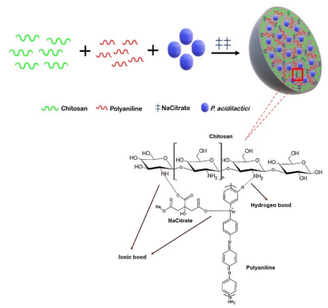


Figure 3. Representation of microencapsulation of *P. acidilactici*

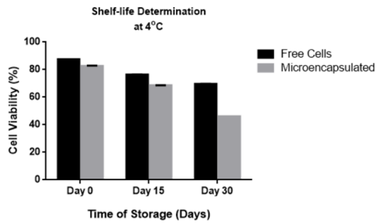


Figure 6. Shelf-life determination: Cell viability at 4^{0}C storage in 30 days

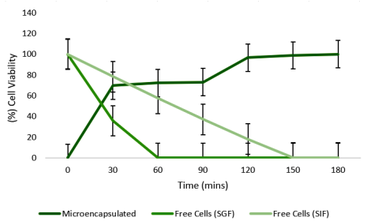


Figure 5. Percent Survival in Simulated gastic (SGF) and intestinal fluid (SIF)

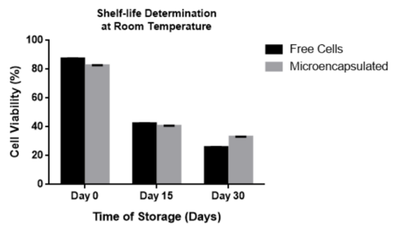


Figure 7. Shelf-life determination: Cell viability at RT storage in 30 days

DISCUSSION

The successful formation of CS/PANI microbeads resulted to encapsulation of *P. acidilactici,* as illustrated in Figure 3 using the drop plate method, each single microbead was found to contain an average of 1.61 x 10^6 Cfu/ml (SD 0.08) of entrapped probiotic cells. Through SEM photomicrograph in Figure 2 (d), it can be seen that the coccus-shaped *P. acidilactici* cells were homogenously embedded and entrapped inside the CS/PANI microbead. Figure 6. shows that there is a significant sudden release of probiotic cells (69.92%) in simulated gastric fluid (SGF) immediately after the microbeads were exposed to it. In the succeeding time of incubation, the observed cell release has significantly increased to 72.21% at 60min. This indicates that the microbeads have already swelled resulting to the release of the entrapped probiotic cells. When the SGF was replaced with simulated intestinal fluid (SIF), the gradual dissolution of the microbeads took place, resulting to 73.06% release at 90mins and immediately increased to 96.7% At 180mins the microbeads have been totally dissolved which released the remaining percentage with 10^5 concentration of entrapped probiotic cells. Although the immediate release of probiotic cells is undesirable at 30mins after subjecting the microbeads in SGF (pH 2), it has been already predicted by the result of the swelling behavior (Figure 4.) of sodium citrate crosslinked CS/PANI composite. The occurrence is due to the very weak ionic bond being formed below pH 4.5 and above pH 6.5 (Rana, Babita, Goyal, and Tiwary, 2005). However, this result is significantly considerable as compared to the free cells (non-encapsulated) subjected in SGF, which have zero survival at 60mins exposure, and free cells that have been dried with trehalose, which have zero survival rate as well after 30 mins exposure to the same medium The viability of free cells is higher (87.52%±0.03) than microencapsulated cells (82.69%±0.18). During storage at 4°C as showed in figure 6. , the mean of 3 independent trials presented in Figure 4.10 showed that on the 15th day, free cells decreased to 76.40%±0.03, while microencapsulated cells significantly decreased by 68.60%±0.04. On the 30th day, furtherly decreased both on free cells to (69.64%±0.04) and microencapsulated (46.23%±0.02). On the other hand, the data in storage at room temperature showed that decrease cell viability both free cells and microencapsulated on 15th day. On contrary, the results at 30th day of storage showed that the viability of microencapsulated (33.10±0.13) is higher than free cells (25.92±0.16).

CONCLUSION

The results obtained have demonstrated that Chitosan/Polyaniline (CS/PANI) composite was successfully formed into microcapsules and capable of probiotic cells entrapment. The optimal CS/PANI ratio that could encapsulate *Pediococcus acidilactici* is 3%/0.5% with 1% sodium citrate as hardening agent. The number of probiotic cells that can be carried by the microbead is 1.61 x 10^6 Cfu/ml ± 0.08. The cell release of entrapped probiotics in the simulated gastric fluid (SGF) is considerably higher than expected ranging from 10^5 to 10^6 cumulative value due to the immediate swelling of the CS/PANI microbeads upon exposure to SGF. At the end of the exposure to simulated intestinal fluid, the cumulative cell release ranges from 10^6 to 10^7. Hence, CS/PANI composite was able to deliver the probiotic cells in the intestine. The cell viability of microencapsulated (46.23%±0.02) probiotics was significantly lower compared to the free cells (69.64%±0.04) after 30 days of storage at 4°C. On the contrary, the results at 30th day of room temperature storage showed reciprocal findings for free cells (25.92%±0.16) and microencapsulated (33.10%±0.13).

ACKNOWLEDGEMENT