CHAPTER 5
CONCLUSION AND RECOMMENDATION

5.1 Result of the entrapment efficacy of Ca-alginate beads with SPI or CS.

Salmonella phages were entrapped in Ca-alginate beads. The entrapment efficiency of each treatment was not significantly different, ranging from 97.67% to 98.34%. This result showed that the addition of SPI or CS to Ca-alginate beads did not affect the entrapment efficiency (EE) of Ca-alginate beads for salmonella phages.

Ca-alginate beads were placed in SM buffer (pH 7.4), followed by the addition of *Salmonella* Typhimurium at a concentration of 10^7 PFU/mL. The samples were then incubated at 41°C for 6 hours to evaluate phage efficacy. After 2 hours of incubation, a significant reduction in *Salmonella* counts was observed in the free phage treatment group, whereas the groups entrapped with Ca-alginate beads, either with or without additional biopolymers, exhibited higher bacterial counts, likely due to delayed phage release from the entrapment beads. At 4 hours, the free phage group continued to show markedly lower *Salmonella* levels compared to all bead treatments. After 6 hours, calcium-alginate beads containing 0.3% w/v CS were found to have the least release amount compared to other treatments.

5.2 Result of the stability of salmonella bacteriophage in Ca-alginate beads under different temperatures, pH levels, under simulated gastric fluid and bile salt solution.

The Ca-alginate beads, modified with or without soybean protein isolate (SPI) or chitosan (CS), were evaluated for their ability to protect entrapped salmonella phages under various temperatures for 24 hours. At 4°C and 37°C, the phage titers in all entrapped treatments showed no significant differences. However, at 25°C, the survival of phages in Ca-alginate beads containing 0.15% or 0.3% CS was higher than in beads without CS and in free phages. Similarly, at elevated temperatures (42 °C and 50 °C), the phage titers varied significantly among treatments.

Both SPI and CS enhanced the thermal protection of salmonella phages when incorporated into Ca-alginate beads, with improved survivability compared to Ca-alginate alone. In contrast, free phages exhibited a marked decline in survivability at high temperatures, with reductions of approximately 65.50% at 42 ℃ and 38.81% at 50 ℃, indicating substantial thermal damage. These findings suggest that the addition of either SPI or CS to Ca-alginate beads enhances the thermal stability of entrapped salmonella phages, making them more resilient to heat-induced inactivation.

The stability of salmonella bacteriophages entrapped in Ca-alginate beads, with or without modifications using SPI or CS, was evaluated under various pH conditions (2.5, 5.0, 5.5, 6.5, and 8.0) at 37 ℃ for 2 hours. At highly acidic conditions (pH 2.5 and 5.0), free phages were undetectable after incubation, indicating complete inactivation. In contrast, phages entrapped in Ca-alginate beads showed significantly higher survival across all tested pH levels. Notably, the incorporation of 0.3% (w/v) SPI or CS into the Ca-alginate beads improved phage viability compared to unmodified beads, with SPI and CS enhanced demonstrating the highest phage survivability. These findings suggest that modifying Ca-alginate beads with either SPI or CS enhances the pH tolerance of entrapped salmonella phages, providing better protection in both acidic and neutral environments.

The stability of salmonella phages entrapped in Ca-alginate beads, with or without supplementation of SPI or CS, was evaluated in a 0.01 M bile salt solution (pH 6.8) at 41 ℃ for 1 hour. The results showed that Ca-alginate beads without any additive retained approximately 75% of phages, while beads containing 0.15% and 0.3% (w/v) of either SPI or CS improved phage survivability to 77.92% and 90.91%, respectively. These findings suggest that the incorporation of SPI or CS into Ca-alginate beads significantly enhances the resistance of entrapped phages against bile salt exposure, thereby improving their overall stability under gastrointestinal-like conditions.

The stability of salmonella phages entrapped in Ca-alginate beads, with or without supplementation of SPI or CS, was evaluated under simulated gastric fluid (SGF; 3 mg/mL pepsin in 0.85% NaCl, pH 2.5) at 41 ℃ for 2 hours. Free phages were rapidly inactivated and became undetectable after 30 minutes of exposure. Similarly, phages entrapped in unmodified Ca-alginate beads lost viability over time, with no

detectable phages after 60 minutes. The incorporation of 0.15% (w/v) SPI or CS provided a modest improvement in survivability, with phage survival at 30 minutes ranging from 44 ± 0.4% (SPI) to higher levels observed with CS. Notably, beads containing 0.3% (w/v) SPI or CS offered significantly better protection. Phages entrapped with 0.3% SPI exhibited 76 ± 0.22% survival at 30 minutes and maintained approximately 66 ± 0.3% at 60 minutes before becoming undetectable. In comparison, 0.3% CS-entrapped phages showed the highest overall resistance, retaining viable phage populations up to 120 minutes. These results indicate that modifying Ca-alginate beads with 0.3% SPI or CS substantially improves phage stability under acidic gastric-like conditions, offering potential for oral delivery applications.

5.3 Result of the release of salmonella bacteriophage from modified beads under simulated intestinal fluid.

Ca-alginate beads, with and without SPI or CS, were incubated for 120 minutes in simulated intestinal fluid (SIF; 1 mg/mL pancreatin in 0.85% NaCl, pH 6.8) at 41°C. For beads containing SPI, phage release was not detected in samples without SPI until after 90 minutes. In contrast, beads containing 0.15% and 0.3% (w/v) SPI released phages as early as 30 minutes, with increasing release levels at 60 and 90 minutes. After 120 minutes, phage titers from beads without SPI reached 3.7 ± 0.1 log PFU/g, while those from beads with 0.15% and 0.3% SPI reached 3.8 ± 0.08 and 4.7 ± 0.7 log PFU/g, respectively, indicating that SPI enhanced phage release compared to alginate alone. Similarly, Ca-alginate beads with CS showed accelerated phage release compared to those without. At 30 minutes, only beads containing 0.15% CS exhibited phage release (3.2 log PFU/g), while no release was observed from CS-free beads. At 60 minutes, beads with 0.15% and 0.3% CS released 4.1 and 5.7 log PFU/g, respectively. This trend continued, with phage titers reaching approximately 5.7 log PFU/g at 90 minutes and at 120 minutes, 3.7, 5.7, and 6.1 log PFU/g for beads without CS and those with 0.15% and 0.3% CS, respectively. These results suggest that both SPI and CS can enhance the release of entrapped phages in SIF, with CS demonstrating a faster onset of release. Additionally, higher concentrations (0.3% w/v) of either additive led to greater phage release than lower concentrations (0.15% w/v).

5.4 Result of the stability of salmonella bacteriophage in modified beads for six months at 4°C.

The long-term survival of phages entrapped in Ca-alginate beads supplemented with SPI or CS was evaluated over a six-month storage period at 4°C. After six months, the concentration of free phages declined by 2.25 log PFU/mL from an initial level of 8 log PFU/mL. Phages entrapped in Ca-alginate beads without any additive showed a reduction of 1.67 log PFU/g. In the SPI-containing beads, phage titers decreased by 1.23 and 1.7 log PFU/g for 0.15% and 0.3% (w/v) SPI, respectively, with no significant differences observed among treatments during the first month of storage. These results suggest that the inclusion of SPI can enhance phage viability over long-term refrigeration, with 0.15% SPI showing the highest stability at the end of six months. Similarly, phages entrapped in Ca-alginate beads with CS also exhibited improved stability compared to beads without additives. The reductions in phage titers were 1.74 and 0.86 log PFU/g for beads containing 0.15% and 0.3% (w/v) CS, respectively. Overall, these findings demonstrate that both SPI and CS contribute to improved long-term stability of entrapped phages during storage at 4°C, with optimal performance observed at specific additive concentrations of 0.15% for SPI and 0.3% for CS.

5.5 Summary of findings

This study demonstrated that Ca-alginate beads effectively entrapped the bacteriophage vB_salP-pYM with consistently high entrapment efficiency across all treatments. The incorporation of soy protein isolate or cassava starch into the Ca-alginate matrix significantly enhanced phage viability under various stress conditions, including elevated temperature, acidic pH, and exposure to 0.01 M bile salts, compared to Ca-alginate beads alone. Furthermore, these modified beads promoted greater phage survival during simulated gastrointestinal transit and facilitated faster phage release in intestinal conditions. The addition of soy protein isolate or cassava starch also contributed to improved phage stability during prolonged cold storage. Overall, Ca-alginate beads supplemented with these biopolymers show promise as a delivery system for enhancing the oral viability of bacteriophages, supporting their potential use in phage therapy.