

TABLE OF CONTENTS

	Page
ABSTRACT (THAI).....	I
ABSTRACT (ENGLISH).....	III
TABLE OF CONTENTS.....	VI
CHAPTER	
1. INTRODUCTION.....	1
1.1 Background and significance of the study.....	1
1.2 Research objectives.....	5
1.3 Research hypothesis.....	5
1.4 Expected result.....	6
1.5 References.....	6
2. LITERATURE REVIEWS.....	9
2.1 <i>Salmonella</i> spp.....	9
2.2 Virulence factors of <i>salmonella</i> spp.....	12
2.3 <i>Salmonella</i> spp. outbreaks.....	15
2.4 <i>Salmonella</i> Typhimurium.....	17
2.5 Antibiotic resistance of <i>salmonella</i> bacteria.....	18
2.6 Bacteriophages.....	19
2.7 Bacteriophages: a solution for bacterial treatment.....	21
2.8 <i>Salmonella</i> bacteriophages.....	23
2.9 Extrusion technique.....	24
2.10 Production of Ca-alginate beads by the extrusion technique.....	26
2.11 Entrapment.....	28
2.12 Entrapment with alginate beads.....	31
2.12.1 Alginate beads modified by soy protein isolate and cassava starch.....	32
2.13 Reference.....	33
3. EFFECT OF SOY PROTEIN ISOLATE ADDED IN CA-ALGINATE BEADS ON STABILITY OF ENTRAPPED <i>vB salP-pYM</i> BACTERIOPHAGE.....	43

TABLE OF CONTENTS (Continued)

	Page
3.1 Abstract.....	43
3.2 Introduction.....	43
3.3 Material and Methods.....	45
3.3.1 Bacteria and bacteriophage.....	45
3.3.2 <i>Salmonella</i> cultivation and enumeration.....	45
3.3.3 Propagation and enumeration of bacteriophage titer.....	46
3.3.4 Entrapments efficiency of bacteriophage.....	47
3.3.5 Scanning electron microscopy (SEM).....	47
3.3.6 Lytic assay of entrapped salmonella phage.....	48
3.3.7 Stability of salmonella bacteriophage in Ca-alginate beads under different temperatures and pH.....	48
3.3.8 Effect of bile salt on the stability of salmonella bacteriophage.....	49
3.3.9 Stability of the phage in Ca-alginate beads under simulated gastric fluid (SGF).....	49
3.3.10 Phage releasing under simulated intestinal fluid (SIF).....	49
3.3.11 The survival of entrapped phages in Ca-alginate beads during Storage.....	50
3.3.12 Statistical analysis.....	50
3.4 Results and discussion.....	50
3.4.1 Entrapment efficiency (EE).....	50
3.4.2 Ca-alginate beads size and morphological characterization of Ca-alginate beads via scanning electron microscopy (SEM).....	51
3.4.3 Lytic assay of entrapped salmonella phage.....	54
3.4.4 Stability of salmonella bacteriophage under different Temperatures.....	55
3.4.5 Stability of salmonella bacteriophage under various pHs.....	56
3.4.6 Effect of bile salt on the stability of entrapped salmonella	

TABLE OF CONTENTS (Continued)

	Page
phage.....	57
3.4.7 Stability of the phage in Ca-alginate beads under simulated gastric fluid (SGF).....	59
3.4.8 Evaluation releasing of phages from Ca-alginate beads in simulated intestinal fluid (SIF).....	60
3.4.9 Stability of phages in the Ca-alginate bead during storage at 4 °C.....	62
3.5 Conclusions.....	63
3.6 References.....	63
4. EFFECT OF CASSAVA STARCH ADDED IN CA-ALGINATE BEADS ON STABILITY ENTRAPPED <i>vB_salP-pYM</i> BACTERIOPHAGE.....	68
4.1 Abstract.....	68
4.2 Introduction.....	68
4.3 Material and Methods.....	69
4.3.1 Bacteria and bacteriophage.....	69
4.3.2 <i>Salmonella</i> cultivation and enumeration.....	70
4.3.3 Propagation and enumeration of bacteriophage titer.....	70
4.3.4 Entrapments efficiency of bacteriophage.....	71
4.3.5 Scanning electron microscopy (SEM).....	71
4.3.6 Lytic assay of entrapped salmonella phage.....	72
4.3.7 Stability of salmonella bacteriophage in Ca-alginate beads under different temperatures and pH.....	72
4.3.8 Effect of bile salt on the stability of salmonella bacteriophage.....	73
4.3.9 Stability of the phage in Ca-alginate beads under simulated gastric fluid (SGF).....	73
4.3.10 Phage releasing under simulated intestinal fluid (SIF).....	74
4.3.11 The survival of entrapped phages in Ca-alginate beads during	

TABLE OF CONTENTS (Continued)

	Page
4.4 Results and discussion.....	74
4.4.1 Entrapment efficiency (EE).....	74
4.4.2 Ca-alginate beads size and morphological characterization of Ca-alginate beads via scanning electron microscopy (SEM).....	75
4.4.3 Lytic assay of entrapped salmonella phage.....	78
4.4.4 Stability of Salmonella bacteriophage under different temperatures.....	79
4.4.5 Stability of Salmonella bacteriophage under various pHs.....	80
4.4.6 Effect of bile salt on the stability of entrapped salmonella phage.....	82
4.4.7 Stability of the phage in Ca-alginate beads under simulated gastric fluid (SGF).....	83
4.4.8 Evaluation releasing of phages from Ca-alginate beads in Simulated intestinal fluid (SIF).....	85
4.4.9 Stability of phages in the Ca-alginate bead during storage at 4°C.....	87
4.5 Conclusions.....	88
4.6 Reference.....	88
5. CONCLUSION AND RECOMMENDATION.....	92
5.1 Result of the entrapment efficacy of Ca-alginate beads with SPI or CS.....	92
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.....	92
5.3 Result of the release of salmonella bacteriophage from modified beads under simulated intestinal fluid.....	94
5.4 Result of the stability of salmonella bacteriophage in modified beads for six months at 4°C.....	95

TABLE OF CONTENTS (Continued)

	Page
5.5 Summary of findings.....	95
BIOGRAPHY.....	96

LIST OF TABLES

Table	Page
3.1 Entrapment efficiency for bacteriophage <i>vB_salP-pYM</i> in Ca-alginate beads.....	56
3.2 Internal morphology and pore size analysis of Ca-alginate beads.....	60
4.1 Entrapment efficiency for bacteriophage <i>vB_salP-pYM</i> in Ca-alginate beads.....	81
4.2 Surface morphology and pore size analysis of Ca-alginate beads.....	83

LIST OF FIGURES

Figure	Page
2.1 The Classification of Salmonella according to the most recent classification by the Centers for Disease Control and Prevention (CDC).....	11
2.2 The Type III Secretion System (T3SS) consists of ring structures spanning both the inner membrane (IM) and outer membrane (OM) of the bacterium.....	15
2.3 classification bacteriophages based on morphology; A: Siphoviride, B: Myoviridae and C: Podoviridae.....	22
2.4 Diagram of the bacteriophage life cycle. Lytic cycle: The bacteriophage infects a bacterial cell, resulting in the synthesis of multiple new viral particles.....	23
2.5 illustrates the encapsulation of an active compound within a hydrogel matrix, demonstrating two extrusion methods.....	28
2.6 The structure of alginate and its gelation mechanism.....	30
2.7 The gelation process of alginate is explained through the “egg-box” model, which illustrates the interaction between calcium ions (Ca^{2+}) and the alginate polymer chains.....	30
2.8 Illustration showing entrapment methods.....	33
3.1 Illustration of Ca-alginate beads. A: 0% SPI, B: 0.15% w/v SPI and C: 0.3% w/v SPI.....	57
3.2 The SEM image surface and internal structure of Ca-alginate beads.....	59
3.3 Lytic of entrapped salmonella phage on number of <i>Salmonella</i> Typhimurium.....	60
3.4 The survival of Salmonella bacteriophage in the modified bead at various temperatures.....	61
3.5 The stability of Salmonella bacteriophage in Ca-alginate bead under various pHs.....	63
3.6 The number of phages remaining in Ca-alginate beads were incubated in bile salt.....	64

LIST OF FIGURES (Continued)

Figure	Page
3.7 The survival of <i>Salmonella</i> phage in the modified beads under SGF.....	59
3.8 Morphology of Ca-alginate beads after in vitro incubation in simulated gastric fluid (SGF) for 120 min.....	59
3.9 The release of phages was entrapped with Ca-alginate beads in simulated intestinal fluid at pH 6.8.....	60
3.10 Morphology of Ca-alginate beads after in vitro incubation in simulated intestinal fluid (SIF) for 120 min.....	60
3.11 Phage survival in the Ca-alginate beads during storage at 4 °C for 6 months.....	62
4.1 Illustration of Ca-alginate beads. A: 0% CS, B: 0.15% w/v CS and C: 0.3% w/v CS.....	76
4.2 The SEM image surface and internal structure of Ca-alginate beads.....	76
4.3 Lytic of entrapped <i>Salmonella</i> phage on number of <i>Salmonella</i> <i>Typhimurium</i>	78
4.4 The survival of <i>Salmonella</i> bacteriophage in the modified bead at various temperatures.....	79
4.5 The stability of salmonella bacteriophage in a Ca-alginate bead under various pHs.....	80
4.6 The number of phages remaining in Ca-alginate beads were incubated in bile salt solution (pH 6.8) at 41°C for 1 hour.....	82
4.7 The survival of salmonella phage in the modified beads under SGF.....	83
4.8 Morphology of Ca-alginate beads after in vitro incubation in simulated gastric fluid (SGF) for 120 min.....	83
4.9 Phage stability released in simulated intestinal fluid at pH 6.8.....	85
4.10 Morphology of Ca-alginate beads after in vitro incubation in simulated intestinal fluid (SIF) for 120 min.....	85
4.11 The survival of phages <i>vB_salP-pYM</i> in the Ca-alginate beads during storage at 4 °C for 6 months.....	87