TABLE OF CONTENTS

ACKNOWLEDGEMENTS **iii**
ABSTRACT (THAI) **iv**
ABSTRACT (ENGLISH) **vi**
TABLE OF CONTENTS **viii**
LIST OF TABLES **xiii**
LIST OF FIGURES **xv**
LIST OF ABBREVIATIONS **xix**

CHAPTER 1 INTRODUCTION **1**

CHAPTER 2 LITERATURE REVIEW **4**
2.1 Biodegradable films **4**
2.1.1 Chitosan **4**
2.1.2 Methylcellulose **7**
2.1.3 Composite films **9**
2.2 Antimicrobial biodegradable films **12**
2.3 Vanillin **14**
2.4 Release of antimicrobial agents from films **19**
2.5 Antimicrobial film applications for food **21**
2.5.1 Inhibitory effect of antimicrobial films **21**
2.5.2 Film properties after incorporation of antimicrobial agents **26**

CHAPTER 3 METHODOLOGY **27**
3.1 Formulations and properties of chitosan/cellulose derivative based film **27**
3.1.1 Film formulations **27**
3.1.2 Film properties **28**
3.1.2.1 Determination of film thickness 28
3.1.2.2 Determination of mechanical properties 28

3.2 Effect of vanillin and plasticizer on chitosan/methylcellulose films 28
3.2.1 Film formulations 28
3.2.2 Film properties 30
3.2.2.1 Determination of film thickness 30
3.2.2.2 Determination of mechanical properties 30
3.2.2.3 Determination of water vapor permeability 30
3.2.2.4 Determination of oxygen permeability 30
3.2.2.5 Determination of opacity and color 31
3.2.2.6 Determination of film thermal properties 31
3.2.2.7 Determination of film solubility 32
3.2.2.8 Sorption behavior 32
3.2.2.9 Morphology observation 32
3.3 Release of vanillin and migration test 32
3.3.1 Factors affecting release of vanillin from the films 32
3.3.1.1 Effect of temperature 32
3.3.1.2 Effect of initial vanillin concentration in film 33
3.3.1.3 Effect of pH 33
3.3.1.4 Swelling behaviour 33
3.3.1.5 Solvent preparation 34
3.3.1.6 Vanillin determination 34
3.3.2 Migration of vanillin from film 34
3.3.3 Release of vanillin on fruit pieces 35
3.4 Inhibition effect of chitosan/methylcellulose film and chitosan/methylcellulose film incorporating vanillin as an antimicrobial agent 36
3.4.1 Standard disk diffusion technique 36
3.4.2 *Escherichia coli* and *Saccharomyces cerevisiae* inoculation and determination number of *E. coli* and *S. cerevisiae* 36

3.5. Application of chitosan/methylcellulose film and chitosan/methylcellulose film incorporating vanillin on fresh-cut cantaloupe and pineapple **37**

3.5.1 Fruit preparation **37**

3.5.2 Quality evaluation of fruit wrapped with antimicrobial films **37**

3.5.2.1 Flesh color 40

3.5.2.2 Firmness 40

3.5.2.3 L-ascorbic acid 40

3.5.2.4 Respiration rate 41

3.5.2.5 Ethanol content 41

3.5.2.6 Total soluble solids (TSS), titratable acidity (TA) and pH 41

3.5.2.7 Weight loss 41

3.5.2.8 Sensory evaluation 42

3.5.2.9 Statistical analysis 42

CHAPTER 4 RESULTS AND DISCUSSION **43**

4.1 Formulations and properties of chitosan/cellulose derivative based films **43**

4.1.1 Film formulations **43**

4.1.2 Thickness and mechanical properties of the films **44**

4.1.3 Effect of plasticizer on properties of chitosan/methylcellulose films **47**

4.2 Effects of vanillin and plasticizer on chitosan/methylcellulose films **47**

4.2.1 Thickness **47**

4.2.2 Mechanical properties **49**

4.2.3 Water vapour permeability **51**

4.2.4 Oxygen permeability **51**

4.2.5 Opacity and color **52**

4.2.6 Film thermal properties **56**

4.2.7 Film solubility **56**

4.2.8 Sorption behavior **58**

4.2.9 Morphology observation **61**

4.3 Release of vanillin and migration test 65
4.3.1 Factors affecting release of vanillin from the films 65
4.3.1.1 Kinetics of vanillin released from the chitosan/methylcellulose film 65
4.3.1.2 Comparison of experimental data with theoretically calculated values 67
4.3.1.3 Effect of temperature 67
4.3.1.4 Effect of initial vanillin concentration in film 71
4.3.1.5 Effect of pH 72
4.3.1.6 Swelling behaviour 74
4.3.2 Migration of vanillin from film 76
4.3.3 Release of vanillin on fruit pieces 76
4.4 Inhibition effect of chitosan/methylcellulose film and chitosan/methylcellulose film incorporating vanillin as an antimicrobial agent 79
4.4.1 Standard disk diffusion technique 79
4.4.2 *Escherichia coli* inoculation and determination of number of *Escherichia coli* 84
4.4.3 *Saccharomyces cerevisiae* inoculation and determination of number of *Saccharomyces cerevisiae* 87
4.5 Quality evaluation of fruit wrapped with antimicrobial films 90
4.5.1 Flesh color 91
4.5.2 Firmness 91
4.5.3 L-ascorbic acid 94
4.5.4 Respiration rate 97
4.5.5 Ethanol content 98
4.5.6 Total soluble solids (TSS), titratable acidity (TA) and pH 98
4.5.7 Weight loss 102
4.5.8 Sensory evaluation 105

CHAPTER 5 CONCLUSIONS **108**

5.1 Overall conclusions **108**

5.2 Future works **110**

BIBLIOGRAPHY **111**

APPENDIX 1 **122**

APPENDIX 2 **124**

PUBLICATIONS **126**

CURRICULUM VITAE **141**

LIST OF TABLES

Table		Page
2.1	Inhibition effect of vanillin on a variety of microorganisms	17
2.2	Application of cellulose derivatives and chitosan in antimicrobial food packaging	22
2.3	Application of antimicrobial packaging in different food systems	23
3.1	The ratio of chitosan, methylcellulose (MC) and hydroxypropyl cellulose (HPC) in film-forming solution	27
3.2	Film formulations with varying PEG and vanillin concentration	29
4.1	Thickness of composite films from different ratios of chitosan and cellulose derivatives	44
4.2	Thickness of chitosan/methylcellulose films containing different combination levels of vanillin and plasticizer (PEG)	47
4.3	L, a*, b*, chroma, hue angle and opacity of films containing different combination levels of vanillin and plasticizer (PEG)	54
4.4	Solubility of chitosan/methylcellulose films containing different combination levels of vanillin and plasticizer (PEG)	58
4.5	K, C, Vm and SSE of chitosan/methylcellulose films containing different plasticizer concentrations	61
4.6	K, C, Vm and SSE of chitosan/methylcellulose film containing different vanillin concentrations	61
4.7	Diffusion exponent (*n*) and constant (*k*) of chitosan/methylcellulose films containing vanillin in different systems and temperatures	66
4.8	Diffusion coefficient of film containing high vanillin concentration in water, cantaloupe juice and pineapple juice at 10, 25 and 35^{o}C	71

4.9 **Inhibition effect of chitosan/methylcellulose films containing varying amount of plasticizer and vanillin against *Escherichia coli* at 10°C** **80**

4.10 **Inhibition effect of chitosan/methylcellulose films containing varying amount of plasticizer and vanillin against *Escherichia coli* at 37°C** **80**

4.11 **Inhibition effect of chitosan/methylcellulose films containing varying amount of plasticizer and vanillin against *Saccharomyces cerevisiae* at 10°C** **83**

4.12 **Inhibition effect of chitosan/methylcellulose films containing varying amount of plasticizer and vanillin against *Saccharomyces cerevisiae* at 25°C** **84**

4.13 **Sensory scores (9=like extremely, 8=like very much, 7=like moderately, 6=like slightly, 5=neither like nor dislike, 4=dislike slightly, 3=dislike moderately, 2=dislike very much, and 1=dislike extremely) of cantaloupe during storage at 10oC for 2 and 8 days** **106**

4.14 **Sensory scores (9=like extremely, 8=like very much, 7=like moderately, 6=like slightly, 5=neither like nor dislike, 4=dislike slightly, 3=dislike moderately, 2=dislike very much, and 1=dislike extremely) of pineapple during storage at 10°C for 2 and 6 days** **107**

LIST OF FIGURES

Figure		Page
2.1	Structure of chitosan	4
2.2	Structure of vanillin	15
3.1	Dried films of (A) chitosan film, (B) and (C) low and high plasticized chitosan/methylcellulose films, (D), (E) and (F) low, medium and high vanillin containing films	29
3.2	Film specimen fix on aluminum cup containing distilled water	31
3.3	Film disks threaded onto a stainless steel wire	35
3.4	Fresh-cut cantaloupe without wrapping (A), wrapped with stretch film (B), chitosan/methylcellulose film (C) and vanillin film (D)	38
3.5	Fresh-cut pineapple without wrapping (A), wrapped with stretch film (B), chitosan/methylcellulose film (C) and vanillin film (D)	39
4.1	Films prepared from chitosan (CH) and methylcellulose (MC) or hydroxypropyl cellulose (HPC)	43
4.2	Tensile strength of (A) chitosan/methylcellulose mixture (CH:MC) and (B) chitosan/hydroxypropyl cellulose mixture (CH:HPC)	45
4.3	Percent elongation of (A) chitosan/methylcellulose mixture (CH:MC) and (B) chitosan/hydroxypropyl cellulose mixture (CH:HPC)	46
4.4	Tensile strength (A) and percent elongation (B) of chitosan/methylcellulose films with varying plasticizer (PEG) concentrations	48

4.5	**Tensile strength (A) and percent elongation (B) of chitosan/methylcellulose films containing different combination levels of vanillin and plasticizer (PEG)**	**50**
4.6	**Water vapor permeability (A) and oxygen permeability (B) of chitosan/methylcellulose films containing different combination levels of vanillin and plasticizer (PEG)**	**53**
4.7	**Opacity (A) and b* value (B) of chitosan/methylcellulose films containing different combination levels of vanillin and plasticizer (PEG)**	**55**
4.8	**DSC thermograms of high plasticized chitosan/ methylcellulose films containing different levels of vanillin**	**57**
4.9	**Heat flow of chitosan/methylcellulose films containing different combination levels of vanillin and plasticizer (PEG)**	**57**
4.10	**Sorption isotherms of chitosan/methylcellulose films containing different levels of plasticizer (A) and vanillin (B)**	**60**
4.11	**Morphologies of low plasticized chitosan/methylcellulose film**	**62**
4.12	**Morphologies of high plasticized chitosan/methylcellulose film**	**63**
4.13	**Morphologies of high plasticized chitosan/methylcellulose film incorporating vanillin as an antimicrobial agent**	**64**
4.14	**Release profiles for vanillin in chitosan/methylcellulose films immersed in water (A), cantaloupe juice (B) and pineapple juice (C) at 10, 25 and 35^{o}C**	**69**
4.15	**Release profiles for vanillin in chitosan/methylcellulose films containing 3 vanillin concentrations at 10^{o}C**	**70**
4.16	**Release profiles for vanillin in chitosan/methylcellulose films immersed in citrate buffer pH 3.5, 5.0 and 6.5 at 10^{o}C**	**70**
4.17	**Arrhenius plot of chitosan/methylcellulose films containing vanillin in water, cantaloupe juice and pineapple juice at 10, 25 and 35^{o}C**	**72**

4.18	**Percentage of vanillin released out of vanillin films in different concentrations of citrate buffer at pH 3.5 and 6.5**	**73**
4.19	**Swelling behavior of chitosan/methylcellulose film containing high vanillin immersed in pineapple juice at 10, 25 and 35^{o}C**	**74**
4.20	**Swelling behavior of chitosan/methylcellulose film containing high vanillin immersed in citrate buffer pH 3.5, 5.0 and 6.5 at 10^{o}C**	**75**
4.21	**Migration of vanillin from chitosan/methylcellulose films into food simulants**	**77**
4.22	**Percentage of vanillin released out of chitosan/methylcellulose films containing high vanillin to fresh-cut cantaloupe and pineapple pieces stored at 10^{o}C**	**78**
4.23	**Percentage of vanillin released out of chitosan/ methyl-cellulose films containing high vanillin to fruit pieces (solid line) compared with fruit juices (dash line) stored at 10^{o}C**	**78**
4.24	***Escherichia coli* growth underneath different films during storage at 10^{o}C for 7 days**	**81**
4.25	***Escherichia coli* growth underneath chitosan/methylcellulose films containing medium vanillin with different plasticizer concentrations during storage at 37^{o}C for 2 days**	**82**
4.26	***Saccharomyces cerevisiae* growth underneath different films during storage at 25^{o}C for 2 days**	**85**
4.27	***Saccharomyces cerevisiae* growth underneath chitosan/methylcellulose films containing medium vanillin with different plasticizer concentrations during storage at 10^{o}C for 6 days**	**86**
4.28	**Numbers of *Escherichia coli* on inoculated cantaloupe (A) and pineapple (B) during storage at 10^{o}C for 8 days**	**88**
4.29	**Numbers of *Saccharomyces cerevisiae* on inoculated cantaloupe (A) and pineapple (B) during storage at 10^{o}C for 20 and 12 days, respectively**	**89**

4.30	**Color of vanillin films after removal from fresh-cut pineapple**	**90**
4.31	**Fresh-cut cantaloupe wrapped with different film types on day 0**	**92**
4.32	**Mold incidence on fresh-cut cantaloupe after storage at 10°C for 12 days**	**93**
4.33	**Hue angle of fresh-cut pineapple during storage at 10°C for 12 days**	**94**
4.34	**Firmness of fresh-cut cantaloupe during storage at 10°C for 12 days**	**94**
4.35	**Standard curve of L-ascorbic acid**	**95**
4.36	**L-ascorbic acid content of fresh-cut cantaloupe (A) and pineapple (B) during storage at 10°C for 10 and 12 days, respectively**	**96**
4.37	**L-ascorbic acid content of pineapple juice supplemented with 1,000 and 2,000 mg/L vanillin during storage at 10°C for 12 days**	**97**
4.38	**Respiration rate of fresh-cut cantaloupe (A) and pineapple (B) during storage at 10°C for 12 days**	**99**
4.39	**Ethanol content of fresh-cut cantaloupe (A) and pineapple (B) during storage at 10°C for 12 days**	**100**
4.40	**Total soluble solids of fresh-cut cantaloupe (A) and pineapple (B) during storage at 10°C for 12 days**	**101**
4.41	**Total acidity of fresh-cut cantaloupe (A) and pineapple (B) during storage at 10°C for 12 days**	**103**
4.42	**Weight loss of fresh-cut cantaloupe (A) and pineapple (B) during storage at 10°C for 12 days**	**104**

LIST OF ABBREVIATIONS

AA	L-ascorbic acid
ASTM	American Society of Testing and Material
BET	Brunauer-Emmett-Teller
CFU	Colony Forming Unit
CH	Chitosan
D	Diffusion Coefficient
FTIR	Fourier Transform Infrared Spectroscopy
GAB	Guggenheim-Anderson-deBoer
GC	Gas Chromatography
GRAS	Generally Recognized as Safe
HDPE	High Density Polyethylene
HPC	Hydroxypropyl Cellulose
HPLC	High Performance Liquid Chromatography
HPMC	Hydroxypropyl Methylcellulose
LDPE	Low Density Polyethylene
MC	Methylcellulose
MIC	Minimum Inhibitory Concentration
MUG	Methylumbelliferyl Glucuronide
OP	Oxygen Permeability
OPP	Oriented Polypropylene
P	Permeability
PA	Polyacetate
PC	Polycarbonate
PCTFE	Polychlorotrifluoroethylene
PEG	Polyethylene Glycol
PETP	Polyethylene Terephthalate
PP	Polypropylene
PS	Polystyrene
RH	Relative Humidity

S	Solubility
TA	Titratable Acidity
TSS	Total Soluble Solid
WVP	Water Vapor Permeability