TABLE OF CONTENTS

	Page
Acknowledgements	9 iii
Abstract (in English)	v
Abstract (in Thai)	vii
List of tables	xiii
List of figures	xiv
Abbreviations and symbols	XV
Chapter 1 Introduction	20
1.1 Rationale	1
1.2 Research objectives	4
1.3 Usefulness of the Research	4
Chapter 2 Review of literature	5
2.1 Agricultural development on honey bees in Thailand	5
2.2 Contamination in honey and bee health management	6
2.3 The quality of honey	7
2.4 Antibacterial properties of honey	9
2.5 Main chemicals used in honey bees of Thailand	10
2.6 Residues of antibiotics in honey	12
2.7 Techniques for antibiotic residue test in honey	16
2.7.1 Agar diffusion test	16
2.7.2 The microbial growth inhibition assay	17
2.7.3 The immunological method	18
2.7.4 Other screening test kits	19
	20

ix

31 Raw material		22
3.1 Kaw materia		22
3.2 Equipment		23
3.4 Chemical rea	cants Ny S	25
3.4 Chemical Tea	al modio	3 23
3.6 Pasaarch dasi	an methods	27
3.6.1 Optimal	nethod for reduction of inhibines, in honey	27
3.0.1 Optimal	nethod for reduction of hudronerovides in honey	27
5.0.1.1 L	fact of all and concentration of honory on inhibition zone diameter	27
3.0.1.2 E	nect of per and concentration of honey on inhibition zone diameter	20
3.6.2 Productio	in of test kit	29
5.0.2.1 P	reparation of hesteric sulture	29
5.0.2.2 P	reparation of patibiotic standard colutions	22
5.0.2.3 P	reparation of antibiotic standard solutions	32
5.0.2.4 P	reparation of acaricide standard solutions	32 22
5.0.2.5 P	(2.5.1 Normal text bit	22
3		32
3	.0.2.5.2 freeze-aried test kit	22
3.6.3 Preparati	on to test the test kit	33
3.6.4 Validatio	on of test kit with HPLC technique	34
3.6.5 Effect of	storage time on effectiveness of test kit	34
3.6.6 Applicat	ion of test kit for acaricide and antibiotic residues	35
3.6.6.1 D	etermination of acaricides in honey	35
3.6.6.2 D	etermination of antibiotic in honey	35
3.6.7 Determi	nation of antibiotic residues by HPLC technique	35
3.6.8 Statistica	levaluation	36
Converter 4 Results a	nd discussion lang Mai Uni	37
Chapter 4 Results a		57

Х

4.2 Optimal method for reduction of inhibines in honey	37
4.3 Production and test of test kit	40
4.3.1 Media formulation of test kit	40
4.3.2 Result of negative control	9 41
4.3.3 Result of positive control	46
4.4 Result of Freeze-dried test kit	47
4.5 Validation of normal test kit with HPLC technique	51
4.6 Storage time on effectiveness of normal test kit	54
4.7 Analytical acaricide residues by normal test kit	55
4.8 Physico-chemical properties of commercial honey	56
Chapter 5 Conclusion and recommendation	59
5.1 Conclusion	59
5.2 Recommendation	60
References	61
Appendices	73
Appendix A: Results of study	74
A.1 Data of HPLC technique	74
A.2 Details of commercial honey	76
A.3 Experimental figures	79
Appendix B: The measurement of the assay	81
B.1 Color analysis by a colorimeter	81
B.2 pH values by pH meter	82
B.3 Total soluble solid by a refractometer	83
B.4 Water activity measurement	84
B.5 Viable counts of Geobacillus stearothermophilus	85
B.6 Viable counts of total bacteria, yeasts and mold	86

xi

B.7 QUANTOFIX [®] Peroxide 25	87
Appendix C: Details of commercial test kits for honey	88
C.1: Primi® test	88
C.2: Quicking Tetracycline Rapid test	90
C.3: Tetrasensor honey kits	93
C.4: Charm II test antibiotic	95
Appendix D: Regulation for Chemical residues in Honey	96
Appendix E: Presentations and publications	98

117

xii

Curriculum vitae

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่ Copyright[©] by Chiang Mai University All rights reserved

Table	Page
3.1 Composition of the media	29
4.1 Effect of temperature, time and concentration of honey on H_2O_2	38
4.2 Effect of pH and concentration of honey on inhibition zone diameter	40
4.3 Effect on diluents and concentration of honey on a negative reaction	43
4.4 Effect of diluents and concentration of honey on pH values	44
4.5 Colour of negative reaction at various temperatures and dilutions of honey	45
4.6 Show height (mm) of yellow colour formation on positive reaction at various temperature and dilution of honey	46
4.7 Colour of positive control in freeze-dried test kit	48
4.8 Height (mm) of yellow colour formation on positive control of normal F2 test kit	50
4.9 Detection limits of the screening test kit and HPLC technique	51
4.10 Retention time of tetracycline group by HPLC technique	52
4.11 Values of spiked honey samples with antibiotic by HPLC technique	52
4.12 Validity of normal F2 test kit	53
4.13 Effect of storage time on yellow colour formation in normal F2 test kit	54
4.14 Analytical acaricide residues in honey by normal test kit	56
4.15 Physico-chemical properties of commercial honey	58
A.1 Physico-chemical properties of 120 commercial honey	76
D.1 Comparision of different regulation for antibiotics in honey	96
D.2 Comparision of different regulation for acaricides in honey	97

xiii

LIST OF FIGURES	
Figure	Pag
3.1 0.1 ml of bacteria and mixture 0.1 ml of bacteria and 0.4 ml media	3
4.1 An agar well diffusion assay on nutrient agar	3
4.2 10-50 % concentration of honey in micro vial	3
4.3 0.1 ml of 10-50% concentration of honey in test kits	4
4.4 Complete yellow colour of test kit on negative reaction	4
4.5 Incomplete yellow colour of test kit on negative reaction	- 4
4.6 Coloury formation in test kit with positive and negative control	4
4.7 Coloured results of freeze-dried test kit	4
4.8 Before and after to add 0.5 ml deionized water in freeze-dried test kit	4
A.1 Chromatograms of standard solution of Tetracycline group	7
A.2 Calibration report Tetracycline group	7
A.3 Calibration curve and Linearity of Tetracycline group	7
A.4 G. stearothermophilus on nutrient agar	7
A.5 10, 100 and 1000 μ g/kg antibiotics and negative control of normal test kits	7
A.6 Test pH with negative control by pH paper	8
A.7 Test pH with positive control (high concentration) by pH paper	8
A.8 Test pH with positive control (low concentration) by pH paper	8
C.1Quicking Tetracycline Rapid Test (Honey)	9
C.2 Visual interpretation of Tetrasensor Honey dipsticks	9

xiv

ABBREVIATIONS AND SYMBOLS

Percentage % °C **Degree Celsius** Microgram μg Microgram per kilogram µg/kg μl Microliter CFU Colony forming unit CTC Chlortetracycline **ELISA** Enzyme-Linked Immunosorbent Assay EU European Union Gram g G. stearothermophilus Geobacillus stearothermophilus H_2O_2 Hydrogen peroxide HPLC High Performance Liquid Chromatography kg Kilogram Liquid Chromatography-Mass Spectrometry LC-MS LC-MS/MS Liquid Chromatography-Mass Spectrometry/Mass Spectrometry Μ Molar min Minute Milligram mg Milligram per gram mg/g Milligram per kilogram mg/kg MIA Microbial Inhibition assay Milliliter ml mm Millimeter

XV

Maximal residue limit Nanometer Optical density Oxytetracycline Power of hydrogen ion Tetracycline, Oxytetracycline and Chlortetracycline Tetracycline United States of America

ลิ<mark>ปสิทธิ์มหาวิทยาลัยเชียงใหม่</mark> Copyright[©] by Chiang Mai University All rights reserved

xvi

MRL

OTC

Tetracyclines

pН

TC

USA

nm OD