

TABLE OF CONTENTS

	Page
Acknowledgement	iii
Abstract	iv
Table of Contents	viii
List of Tables	xii
List of Illustrations	xiv
Abbreviations	xvi
Chapter 1 Introduction	1
1.1 Introduction of angkak	1
1.2 The Objectives of this study	2
1.3 Application advantages of this study	3
Chapter 2 Literature reviews	4
2.1 Angkak	4
2.2 The Botanical data	5
2.3 Cultivation conditions of <i>Monascus</i> sp.	6
2.4 Adlay	8
2.5 The Pigment of <i>M. purpureus</i>	10
2.6 Mevinolin	12
2.7 Effects on cholesterol and lipid metabolism	15
2.8 Citrinin	17
2.9 Toxicology	20
2.10 Glucosamine	21
2.11 Principles of HPLC/DAD/MSD on angkak metabolites analysis	24
2.11.1 High Performance Liquid Chromatography (HPLC)	24
2.11.2 The Diode Array Detector (DAD)	25
2.11.3 The Mass Spectrometer Detector (MSD)	26

Chapter 3 Materials and methods	28
3.1 Validation of analysis methods	29
3.1.1 Development and optimization of the HPLC/DAD/MSD	29
3.1.2 Precision and Accuracy determination	30
3.1.3 Linearity	30
3.1.4 Limit of detection and limit of quantitation determination	30
3.2 Selection of <i>Monascus</i> strains for adlay angkak production	30
3.2.1 Microorganisms	30
3.2.2 Preparation of substrate and fermentation	31
3.2.3 Mevinolin analysis	31
3.2.4 Citrinin and glucosamine analysis	32
3.2.5 Pigment measurement	33
3.2.6 Color measurement	33
3.2.7 Moisture and pH determination	33
3.2.8 Statistical analysis	34
3.3 Methods to study kinetic behavior of <i>M. purpureus</i> DMKU cultured on adlay angkak	34
3.4 Optimization of carbon and nitrogen sources on adlay angkak	34
3.4.1 Glucose and peptone were supplemented in adlay	34
3.4.2 Lactose and yeast extract were supplemented in adlay	36
3.5 Studies on effect of carbon and nitrogen sources on adlay angkak	37
3.6 Comparison of adlay angkak with commercial red rice	38
Chapter 4 Results and discussion	40
4.1 Validation of analysis method	40
4.1.1 Development and optimization of the HPLC method	40
4.1.2 Precision and Accuracy determination	41
4.1.3 Linearity	41
4.1.4 Limit of detection and limit of quantitation	41
4.2 Effects of <i>Monascus</i> strains on properties of adlay angkak	44
4.2.1 Metabolites produced from <i>Monascus</i> strains in adlay angkak	44
4.2.2 Pigments in adlay angkak produced from <i>Monascus</i> strains	45
4.2.3 Color in adlay angkak produced from <i>Monascus</i> strains	45

4.2.4 Moisture content and pH in adlay angkak produced from <i>Monascus</i> strains	46
4.3 Kinetic behavior of <i>Monascus purpureus</i> DMKU on adlay angkak	47
4.3.1 Effects of cultivation time on mevinolin production	47
4.3.2 Effects of cultivation time on citrinin production	48
4.3.3 Effects of cultivation time on glucosamine content of adlay angkak	49
4.3.4 Effects of cultivation time on pigment production and Hunter Lab color system	51
4.4 Optimization of carbon and nitrogen source for adlay angkak	54
4.4.1 Effects of glucose and peptone on properties of adlay angkak	54
4.4.2 Effects of lactose and yeast extract on mevinolin production	57
4.4.3 Effects of lactose and yeast extract on properties of adlay angkak	58
4.5 Effects of carbon and nitrogen source on adlay angkak production	60
4.5.1 Effects of glucose-peptone or lactose-yeast extract on <i>M. purpureus</i> DMKU	60
4.5.2 Effects of glucose-peptone or lactose-yeast extract on <i>M. ruber</i> TISTR3006	63
4.6 Comparison of properties of adlay angkak added carbon-nitrogen source with commercial red rice	66
4.6.1 Glucosamine, citrinin and mevinolin content in adlay angkak added carbon-nitrogen source and commercial red rice	66
4.6.2 Physical and chemical properties of adlay angkak added carbon-nitrogen source and commercial red rice	68
Chapter 5 Conclusion	72
5.1 Validation of HPLC/DAD/MSD for analysis <i>Monascus</i> metabolites	72
5.2 Effects of <i>Monascus</i> strains on properties of adlay angkak	73
5.3 Kinetic behavior of adlay angkak fermented by <i>Monascus</i> <i>purpureus</i> DMKU	73
5.4 Optimization of carbon and nitrogen source for adlay angkak	74
5.5 Effects of carbon and nitrogen source on adlay angkak production	74

5.6 Comparison of adlay angkak added carbon-nitrogen source and commercial red yeast rice	75
References	77
Appendix	84
Appendix A Picture	84
1. Picture of Adlay angkak	84
2. Picture of Instrument	88
Appendix B Statistic analysis	77
1. Effects of <i>Monascus</i> strains on adlay angkak metabolites	91
2. Kinetic behavior of <i>Monascus purpureus</i> DMKU fermentation on adlay	102
3. Optimization of carbon and nitrogen source on adlay angkak	106
4. Effects of carbon and nitrogen source on adlay angkak production	108
5. Comparison of properties of adlay angkak added carbon-nitrogen source with commercial red rice	131
Appendix C Chromatogram	154
Appendix D Calculation of the validation method of mevinolin, citrinin and glucosamine	162
1. Limit of Detection (LOD)	162
2. Limit of Quantitation (LOQ)	163
3. Percent of Relative Standard Deviation (%RSD)	164
4. Percent of Recovery	165
Appendix E The range of adlay angkak properties on carbon and nitrogen source supplement	166
Appendix F Preparation of mobile phase solution	168
Curriculum vitae	169

LIST OF TABLES

Table	Page
3.1 Levels in the glucose and peptone on response surface design of secondary-order model	36
3.2 Levels in the lactose and yeast extract on response surface design of secondary-order model	37
4.1 Validation of Glucosamine, Citrinin and Mevinolin	42
4.2 Mevinolin, citrinin and glucosamine content of adlay angkak during fermentation at room temperature for 28 days	44
4.3 Pigments concentration in adlay angkak produced from difference <i>Monascus</i> strains	45
4.4 L, a and b values in adlay angkak produced from difference <i>Monascus</i> strains	46
4.5 Moisture content and pH of adlay angkak produced from different strains of <i>Monascus</i>	47
4.6 Glucosamine, citrinin and mevinolin content of adlay angkak cultivated with <i>M. purpureus</i> DMKU	61
4.7 Pigments of adlay angkak cultivated with <i>M. purpureus</i> DMKU	62
4.8 L, a and b values of adlay angkak cultivated with <i>M. purpureus</i> DMKU	62
4.9 Moisture content and pH of adlay angkak cultivated with <i>M. purpureus</i> DMKU	63
4.10 Glucosamine, citrinin and mevinolin content of adlay angkak cultivated with <i>M. ruber</i> TISTR3006	64
4.11 Pigments of adlay angkak cultivated with <i>M. ruber</i> TISTR3006	65
4.12 L, a and b values of adlay angkak cultivated with <i>M. ruber</i> TISTR3006	65
4.13 Moisture content and pH of adlay angkak cultivated with <i>M. ruber</i> TISTR3006	66
4.14 Glucosamine, citrinin and mevinolin content of adlay angkak (<i>M. purpureus</i> DMKU) and commercial red rice	67
4.15 Glucosamine, citrinin and mevinolin content of adlay angkak (<i>M. ruber</i> TISTR3006) and commercial red rice	68

4.16	Pigments of adlay angkak (<i>M. purpureus</i> DMKU) and commercial red rice	69
4.17	Pigments of adlay angkak (<i>M. ruber</i> TISTR3006) and commercial red rice	69
4.18	L, a and b values of adlay angkak (<i>M. purpureus</i> DMKU) and commercial red rice	70
4.19	L, a and b values of adlay angkak (<i>M. ruber</i> TISTR3006) and commercial red rice	70
4.20	Moisture content and pH of adlay angkak (<i>M. purpureus</i> DMKU) and commercial red rice	71
4.21	Moisture content and pH of adlay angkak (<i>M. ruber</i> TISTR3006) and commercial red rice	71

LIST OF ILLUSTRATIONS

Figure	Page
2.1 Pedicellate ascomata with ascospores of <i>M. purpureus</i> Went.	6
2.2 Pigments produced by <i>Monascus</i> fungi	10
2.3 Chemical structure of mevinolin	13
2.4 The hypothetical pathway of lovastatin biosynthesis, LDKS = lovastatin diketide synthase; LNKS = lovastatin nonaketide synthase	14
2.5 Chemical structure of citrinin	18
2.6 Scheme of the biosynthesis of citrinin by <i>M. ruber</i> .	19
2.7 Biosynthesis of citrinin and red pigment in <i>M. ruber</i> .	20
2.8 Structure formula of glucosamine	23
2.9 Structure formula of <i>N</i> -acetylglucosamine	23
2.10 High Performance Liquid Chromatography system	25
2.11 The UV Photo Diode Array Detector	26
2.12 General conceptual scheme for vapor-phase analysis by mass spectrometry	27
4.1 Standard curve of glucosamine (by MSD detection)	42
4.2 Standard curve of citrinin (by MSD detection)	43
4.3 Standard curve of mevinolin (by DAD detection)	43
4.4 Kinetic behavior of mevinolin production by <i>Monascus purpureus</i> DMKU	48
4.5 Kinetic behavior of microorganism	48
4.6 Kinetic behavior of citrinin production	49
4.7 Kinetic behavior of glucosamine production	50
4.8 Moisture content of adlay angkak (wet weight) varied on cultivation time	51
4.9 pH of adlay angkak varied on cultivation time	51
4.10 <i>Monascus</i> pigments varied on cultivation day	53
4.11 Hunter Lab value of powdered adlay angkak varied on cultivation day	53
4.12 a value of Hunter color system of <i>Monascus ruber</i> TISTR3006 on adlay added with glucose and peptone	55

4.13	Orange pigment of <i>Monascus ruber</i> TISTR3006 on adlay added with glucose and peptone	56
4.14	Red pigment of <i>Monascus ruber</i> TISTR3006 on adlay added with glucose and peptone	56
4.15	Mevinolin content of <i>Monascus ruber</i> TISTR3006 on adlay added with lactose and yeast extract	58
4.16	L value by Hunter color system of <i>Monascus ruber</i> TISTR3006 on adlay added with lactose and yeast extract	59
4.17	a value of Hunter color system of <i>Monascus ruber</i> TISTR3006 on adlay added with lactose and yeast extract	60

ABBREVIATIONS

DAD	Diode Array Detector
Fig	Figure
g	gram
h	hour
HPLC	High Performance Liquid Chromatography
l	liter
LD ₅₀	Lethal Dose
LOD	Limit of Detection
LOQ	Limit of Quantitation
<i>M.</i>	<i>Monascus</i>
min	minute
ml	milliliter
mm	millimeter
MSD	Mass Spectrometer Detector
nm	nanometer
ppm	part per million
R ²	coefficient of determination
rpm	rotation per minute
RSD	Relative Standard Deviation
SD	Standard Deviation
SSF	Solid-State Fermentation
μl	microliter
v	volume