

THE USE OF NEW DUAL LIPASES SYSTEM IN THE  
SYNTHESIS OF FERULATE ESTERS: OPTIMIZATION BY  
RESPONSE SURFACE METHODOLOGY

Nurul Jannah binti Abd Rahman  
(Matric No. 3120323)

Thesis submitted in fulfilment for the degree of  
MASTER OF SCIENCE

Faculty of Science and Technology  
UNIVERSITI SAINS ISLAM MALAYSIA

Nilai

July 2015

**AUTHOR DECLARATION**

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

I hereby declare that the work in this thesis is my own except for quotations and summaries which have been duly acknowledged.

Date:

Signature :

Name : Nurul Jannah binti Abd'Rahman

Matric No : 3120323

Address : No 113, Desa Seri Chepor,  
31200 Chemor, Perak.

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ISLAMIC SCIENCE UNIVERSITY OF MALAYSIA

## APPROVAL

The thesis entitled “**The Use of New Dual Lipases System in the Synthesis of Ferulate Esters: Optimization by Response Surface Methodology**” by Nurul Janiah binti **Abd Rahman** submitted to the Faculty of Science and Technology, Universiti Sains Islam Malaysia and was accepted as fulfilment of the requirements for the degree of Master of Science.

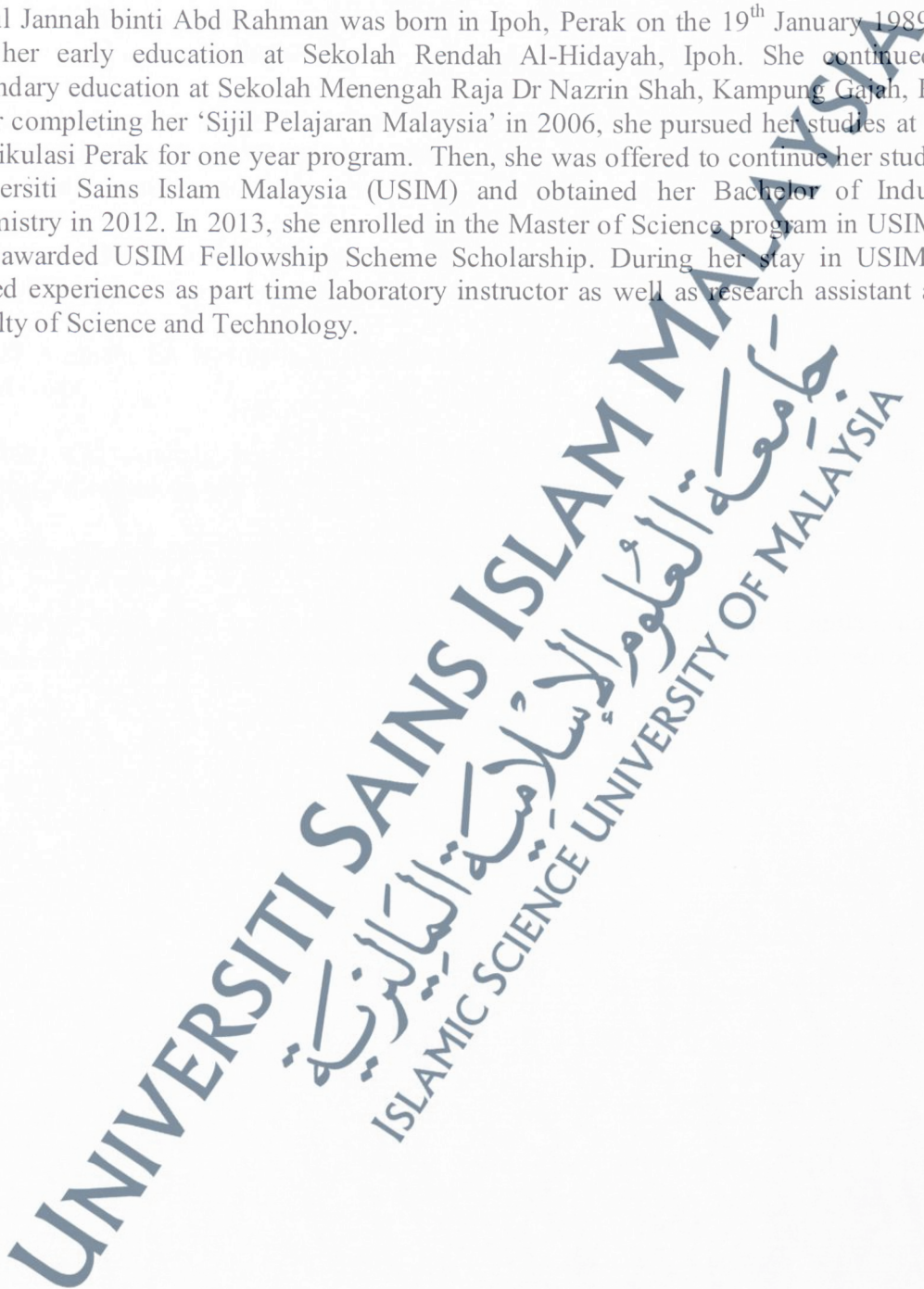
**SALINA BINTI MAT RADZI, PhD**  
Associate Professor,  
Faculty of Science and Technology,  
Universiti Sains Islam Malaysia

Date:

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جامعة العلوم الإسلامية الماليزية  
ISLAMIC SCIENCE UNIVERSITY OF MALAYSIA

## BIODATA OF THE AUTHOR

Nurul Jannah binti Abd Rahman was born in Ipoh, Perak on the 19<sup>th</sup> January 1989. She had her early education at Sekolah Rendah Al-Hidayah, Ipoh. She continued her secondary education at Sekolah Menengah Raja Dr Nazrin Shah, Kampung Gajah, Perak. After completing her 'Sijil Pelajaran Malaysia' in 2006, she pursued her studies at Kolej Matrikulasi Perak for one year program. Then, she was offered to continue her studies at Universiti Sains Islam Malaysia (USIM) and obtained her Bachelor of Industrial Chemistry in 2012. In 2013, she enrolled in the Master of Science program in USIM and was awarded USIM Fellowship Scheme Scholarship. During her stay in USIM, she gained experiences as part time laboratory instructor as well as research assistant at the Faculty of Science and Technology.



## ACKNOWLEDGEMENTS

Alhamdulillah, all praises to Allah S.W.T., for giving me the strength to endure all challenges and complete this study.

I would like to express my sincere gratitude to my adored supervisor, Associate Prof Dr Salina for her kind advice, guidance and ideas. Special thanks to those who have contributed directly or indirectly to the work, especially:

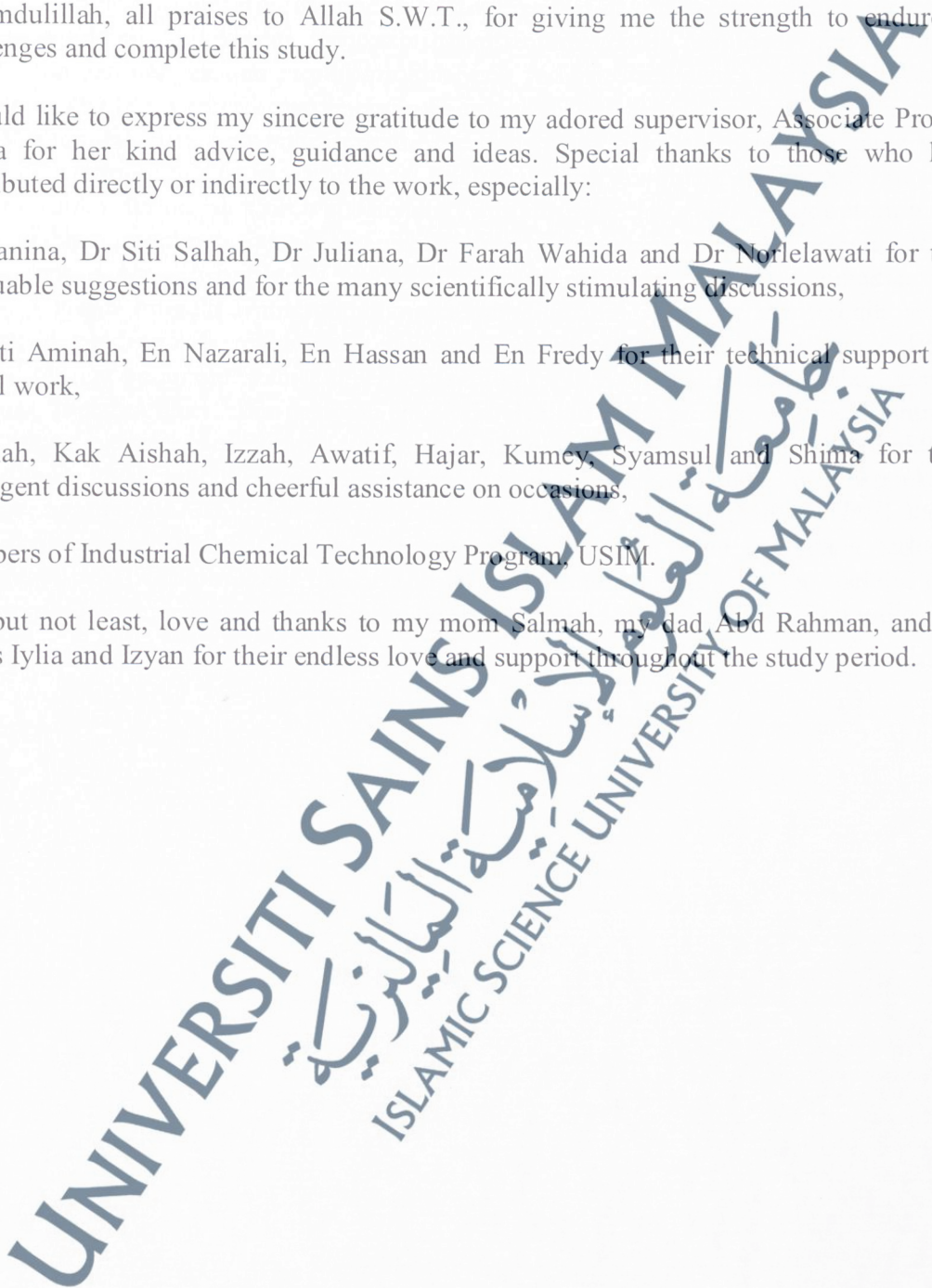
Dr Hanina, Dr Siti Salhah, Dr Juliana, Dr Farah Wahida and Dr Norlelawati for their invaluable suggestions and for the many scientifically stimulating discussions,

Pn Siti Aminah, En Nazarali, En Hassan and En Fredy for their technical support and skilful work,

Aiyshah, Kak Aishah, Izzah, Awatif, Hajar, Kumey, Syamsul and Shima for their intelligent discussions and cheerful assistance on occasions,

Members of Industrial Chemical Technology Program, USIM.

Last but not least, love and thanks to my mom Salmah, my dad Abd Rahman, and my sisters Iylia and Izyan for their endless love and support throughout the study period.



## ABSTRAK

Sistem dwi-lipase yang baru dan mudah telah diperoleh dalam memperoleh ester ferulik yang tinggi hasil tindakbalas transesterifikasi antara ferulik etil dan minyak zaitun. Pengesanan dan pengesanan produk-produk yang terhasil dimudahkan oleh spektroskopi inframerah (FTIR). Pemeriksaan enzim menunjukkan 1: 9 w/w Novozym 435-Lipozyme RM IM merupakan nisbah yang terbaik. Dengan mengubah satu parameter pada suatu masa, suhu setinggi 60 °C, masa inkubasi selama 12 jam, dos lipase sebanyak 80 mg dan nisbah ferulik etil:minyak zaitun sebanyak 1: 2 merupakan parameter yang optimum dan sesuai dalam tindakbalas tersebut. Selanjutnya, kaedah permukaan respon (RSM) berdasarkan 4-faktor-5-peringkat rekabentuk pusat komposit (CCD) digunakan bagi mengkaji kesan interaktif antara parameter yang terlibat. Model yang terbaik adalah polinomial kuadratik di mana masa inkubasi menunjukkan kesan yang sangat ketara berbanding tiga parameter yang lain. Hasil setinggi 94.03 % telah dicapai dalam kondisi optimum, menghampiri nilai ramalan 94.83 %. Kajian biologi ester ferulik ditentukan berdasarkan ciri-ciri antimikrob terhadap Gram-positif dan Gram-negatif bakteria. Penyaringan awal melalui kaedah serapan-agar diaplikasikan diikuti oleh kepekatan rencatan minimum (MIC) dan kepekatan baktisidal minimum (MBC). Hasil kajian membuktikan ester ferulik mempunyai ciri-ciri antibakteria yang sederhana terhadap Gram-positif dan Gram-negatif bakteria. Sifat fizikokimia telah ditentukan melalui nilai faktor perlindungan matahari (SPF), nilai peroksida, nilai saponifikasi dan nilai iodin.

## ABSTRACT

A novel and simple dual lipases system was developed in attaining high performance of ferulate esters by transesterification reaction between ethyl ferulate and olive oil. Detection and identification of reaction products were facilitated by Fourier Transform Infrared (FTIR). Enzyme screening revealed 1: 9 w/w of Novozym 435-Lipozyme RM IM to be the most efficient lipases ratio for the reaction synthesis. By varying one-parameter-at-a-time, 60 °C of reaction temperature, 12 hrs of incubation time, 80 mg of lipase dosage and 1: 2 of ethyl ferulate: olive oil ratio were shown to be the optimal and suitable reaction conditions. Response surface methodology (RSM) based on four-factor-five-level central composite design (CCD) was further used to study the interactive effects among the parameters. The best fitting model was quadratic polynomial where incubation time exhibited most significant effect over the other three parameters. A high percent conversion of 94.03 % was achieved under the optimum conditions, which compared well with the maximum predicted value of 94.83 %. Biological study of ferulate esters was measured based on their antimicrobial properties against both Gram-positive and Gram-negative bacteria. Screening by agar diffusion test was employed as preliminary features followed by minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) assays. The results proved that the ferulate esters possess moderate antibacterial inhibition against Gram-positive and Gram-negative bacteria. Their physicochemical properties were also well examined through sun protection factor (SPF) value, peroxidase value, saponification value and iodine value.

## ملخص

جديد انزيم الليبازيس المزدوج البسيط الذي يوتر بشكل فعال علي تحقيق اداء عالي لسنرات الفيروليت بواسطة الاستره بين الايثيل فيروليت وزيت الزيتون. الكشف والتعرف علي نتائج التفاعل كان بواسطة الاشعه تحت الحمراء. فحص الانزيم بين النسبه الوزنيه الوزنيه 1:9 من نوفوزيم 435-ليبوزيم ارام اي ام لتكون اكثر نسبه فعاله لليبازيس لاجل تخليق التفاعل بواسطة تغيير احد البارميترات عند الزمن ودرجة الحرارةه 60 و الحفظ 12 ساعه , 80 مليجرام من جرعة الليبازيس و ونسبة 1:4 من ايثيل فيروليت الي زيت الزيتون وكانت هذه النسبه ملائمه لظروف التفاعل. منهجية استجابة السطح علي اساس تصميم مركب مركزي كعامل رابع بمستوي خامس استخدم ايضا علي دراسة التأثيرات التفاعليه بين البارميترات . كان افضل نموذج متعدد الحدود من الدرجه الثانيه حيث فترة الحفظ اظهرت اثر كبير علي البارميترات الثلاثه الاخري .نسبه عاليه حوالي 94.3% تما تحويلها تحت ظروف ملائمه والتي قورنت بشكل جيد مع الحد الاقصي للقيمه المتوقعه 94.83% وقد تمت الدراره البيولوجيه لسنرات الفيروليت وكانت مفايه علي اساس خواصها المضاده للميكروبات ضد كلا من الجراثيم الموجبه والجراثيم السالبه للبكتيريا الفحص كان بواسطة اختبار الانتشار كان يتبع بواسطة اقل تركيز للتثبيط واقل تركيز مبيد للجراثيم . النتائج اوضحت هذا استنرات الفيروليت تمتلك تثبيط معتدل للبكتيريا ضد الجراثيم الموجبه بدلا من الجراثيم السالبه . الخواص الفيزيائيه لها كانت ايضا جيده من خلال عامل الحماية من الشمس وقيمة البيروكسيدات , ورقم التصبن وقيمة اليود

## TABLE OF CONTENTS

Contents	Page
AUTHOR DECLARATION	i
APPROVAL	ii
BIODATA OF AUTHOR	iii
ACKNOWLEDGEMENTS	iv
ABSTRAK	v
ABSTRACT	vi
ملخص	vii
TABLE OF CONTENTS	viii
LIST OF TABLES	xiii
LIST OF FIGURES	xiv
LIST OF APPENDICES	xvi
ABRREVIATIONS	xvii
<b>CHAPTER I: INTRODUCTION</b>	<b>1</b>
1.1 Background of study	1
1.2 Objectives	4
<b>CHAPTER II: LITERATURE REVIEW</b>	<b>5</b>
2.1 Sunscreens	5
2.2 Ferulic Acid	6
2.2.1 Applications of Ferulic Acid	8
2.3 Synthesis of Ferulate Esters	11
2.4 Lipases as Biocatalyst	15
2.4.1 Reaction of Lipases	16

2.4.2	Application of Lipases	20
2.4.3	Immobilized Lipases	21
2.4.4	Novozym 435	23
2.4.5	Lipozyme RM IM	23
2.4.6	Dual Lipases System	24
2.5	Olive Oil as Reaction Substrate	26
2.5.1	Chemical Compositions of Olive Oil	27
2.5.2	Dermatologic Effects of Olive Oil	29
2.6	Response Surface Methodology (RSM)	30
2.6.1	Five-Stages of RSM Procedure	30
2.6.2	Advantages and Limitations of RSM	34
<b>CHAPTER III:</b>	<b>SCREENING ON THE PERFORMANCE OF DUAL LIPASES SYSTEM IN FERULATE ESTERS SYNTHESIS</b>	<b>36</b>
3.1	Introduction	36
3.2	Materials and Methods	38
3.2.1	Materials	38
3.2.2	Enzymatic Synthesis of Ferulate Esters: Screening of Dual Lipases System	38
3.2.3	Percentage Conversion of Ferulate Esters	39
3.2.4	Verification of Reaction Components Using FTIR Analysis	39
3.3	Results and Discussion	40
3.3.1	Enzymatic Synthesis of Ferulate Esters	40
3.3.2	Screening of Dual Lipases System in the Synthesis of Ferulate Esters	44
3.3.3	Identification of Reaction Components Using FTIR Analysis	48
3.4	Conclusion	54

<b>CHAPTER IV:</b>	<b>OPTIMIZATION OF REACTION SYNTHESIS VIA CONVENTIONAL AND STATISTICAL APPROACHES USING DUAL LIPASES SYSTEM IN FERULATE ESTERS SYNTHESIS</b>	<b>55</b>
4.1	Introduction	55
4.2	Materials and Methods	57
4.2.1	Materials	57
4.2.2	Conventional Analysis Using One Factor At-A-Time Approach	57
4.2.3	Statistical Analysis by Response Surface Methodology (RSM)	59
4.2.4	Percentage Conversion of Ferulate Esters	61
4.3	Results and Discussion	63
4.3.1	Study on Individual Effects of Reaction Parameters	63
4.3.1.1	Effect of Reaction Time	63
4.3.1.2	Effect of Lipase Dosage	66
4.3.1.3	Effect of Mass Ratio Substrates	68
4.3.1.4	Effect of Reaction Temperature	70
4.3.2	Study on Interactive Effects of Reaction Parameters and Their Optimization Using RSM	72
4.3.2.1	ANOVA and Regression Analysis	72
4.3.2.2	Response Surface Analysis	78
4.3.2.3	Optimum Conditions	90
4.4	Conclusion	91
<b>CHAPTER V:</b>	<b>BIOASSAY STUDY ON SYNTHESIZED FERULATE ESTERS</b>	<b>92</b>
5.1	Introduction	92
5.2	Materials and Methods	93
5.2.1	Materials	93
5.2.2	Media	93

5.2.3	Microorganisms	94
5.2.4	Preparation of Test Microorganisms	94
5.2.5	Preparation of Test Samples	94
5.2.6	Screening Test via Agar Diffusion Method	96
5.2.7	Determination of Minimum Inhibitory Concentration (MIC) by MTT Assay	96
5.2.8	Determination of Minimum Inhibitory Concentration (MIC) by Spectrophotometric Assay	97
5.2.9	Determination of Minimum Bactericidal Concentration (MBC) Value	98
5.3	Results and Discussion	99
5.3.1	Agar Well Diffusion Assay	99
5.3.2	Minimum Inhibitory Concentration (MIC)	102
5.3.3	Spectrophotometric Assay	103
5.3.4	Minimum Bactericidal Concentration (MBC)	106
5.4	Conclusion	107
<b>CHAPTER VI: PHYSICOCHEMICAL PROPERTIES OF SYNTHESIZED FERULATE ESTERS</b>		<b>108</b>
6.1	Introduction	108
6.2	Materials and Methods	108
6.2.1	Materials	109
6.2.2	SPF Value	109
6.2.3	Peroxide Value	110
6.2.4	Saponification Value	111
6.2.5	Iodine Value	112
6.3	Results and Discussion	113
6.3.1	SPF Value	113
6.3.2	Peroxide Value	114

6.3.3	Saponification Value	114
6.3.4	Iodine Value	115
6.4	Conclusion	116
<b>CHAPTER VII: SUMMARY AND RECOMMENDATIONS</b>		<b>117</b>
7.1	Summary	117
7.2	Recommendations for Further Studies	119
<b>BIBLIOGRAPHY</b>		<b>120</b>
<b>APPENDICES</b>		<b>136</b>

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## LIST OF TABLES

	Page
Table 2.1:	A Literature Survey on Ferulate Esters Synthesis 12
Table 2.2:	Industrial Applications of Microbial Lipases 21
Table 2.3:	A Literature Survey on Dual Enzymes System 25
Table 2.4:	Positional Distribution of Fatty Acids in Triglycerides of Olive Oil 27
Table 3.1:	Summary of IR Absorption Peak of Ethyl Ferulate 50
Table 3.2:	Summary of IR Absorption Peak of Olive Oil 51
Table 3.3:	Summary of IR Absorption Peak of Substrates Mixture before Incubation 52
Table 3.4:	Summary of IR Absorption Peak of Ferulate Esters after Incubation 53
Table 4.1:	Summary of Experimental Design of RSM 60
Table 4.2:	Design Matrix of the Actual Level for Four-Factor-Five-Level Central Composite Rotatable Design (CORD) 60
Table 4.3:	Experimental Data, Actual and Predicted Values for Four-Factor-Five-Level Response Surface Analysis 73
Table 4.4:	ANOVA for Joint Test 76
Table 4.5:	R-Squared ( $R^2$ ) Analysis of Quadratic Model 77
Table 4.6:	Optimal Conditions Derived by RSM (Quadratic Model) 91
Table 5.1:	Preparation of Serial Dilution of Ferulate Esters 95
Table 5.2:	Antimicrobial Activity of Synthesized Ferulates Esters 101
Table 5.3:	The Minimum Inhibitory Concentration (MIC) Values of Ferulate Esters 103
Table 6.1:	Properties of Ferulate Esters 115

## LIST OF FIGURES

	Page
Figure 2.1:	Chemical Structure of Ferulic Acid 7
Figure 2.2	Graphical Representation of BCL in (a) Water (b) Near-Critical Propane 19
Figure 2.3	Reaction Mechanism of Lipase 20
Figure 2.4	Enzyme Immobilization Techniques 22
Figure 2.5	Electron Microphotograph of Novozym 435 23
Figure 2.6	Light Microscopic Evaluation of Lipozyme RM IM Particles 24
Figure 3.1:	Possible Products of Transesterification of Ethyl Ferulate with Olive Oil 42
Figure 3.2:	Screening of Dual Lipases System in Transesterification of Ethyl Ferulate with Olive Oil. The Reaction Was Carried Out at 60 °C, 1: 4 g Ethyl Ferulate/g Olive Oil, 5 mL of Toluene and 100 mg of Immobilized Lipases 47
Figure 3.3:	IR Spectrum of Ethyl Ferulate 50
Figure 3.4:	IR Spectrum of Olive Oil 51
Figure 3.5:	IR Spectrum of Ferulate Esters before Incubation 52
Figure 3.6:	IR Spectrum of Ferulate Esters after 12 hrs Incubation Period 53
Figure 4.1:	Effect of Reaction Time on the Conversion of Ferulate Esters. The Reaction Was Carried Out at 60 °C, Ethyl Ferulate and Olive Oil (1: 4, g/g Ratio), 5 mL of Toluene and 100 mg of Immobilized Lipases (Novozym 435-Lipozyme RM IM) 65
Figure 4.2:	Effect of Lipase Dosage on the Conversion of Ferulate Esters. The Reaction Mixture Composed of Ethyl Ferulate and Olive Oil (1: 4, g/g Ratio), 5 mL of Toluene and Immobilized Lipases (1: 9 w/w Novozym 435-Lipozyme RM IM). The Experiment Was Conducted at 60 °C for 12 hrs 67

- Figure 4.3: Effect of Mass Ratio of Ethyl Ferulate to Olive Oil on the Transesterification of Ferulate Esters. The Reaction Was Carried Out at 60 °C for 12 hrs, 80 mg of Immobilized Lipases (1: 9 w/w Novozym 435-Lipozyme RM IM) in 5 mL of Toluene 69
- Figure 4.4: Effect of Reaction Temperature on the Transesterification of Ferulate Esters. The Reaction Mixture Composed of Ethyl Ferulate and Olive Oil (1: 4, g/g Ratio), 5 mL of Toluene and 80 mg of Immobilized Lipase (1: 9 w/w Novozym 435-Lipozyme RM IM). The Experiment Was Conducted for 12 hrs 71
- Figure 4.5: Response Surface Plot of Reaction Time versus Lipase Dosage in Quadratic Model 79
- Figure 4.6: Response Surface Plot of Reaction Time versus Ratio Substrates in Quadratic Model 81
- Figure 4.7: Response Surface Plot of Reaction Time versus Reaction Temperature in Quadratic Model 83
- Figure 4.8: Response Surface Plot of Lipase Dosage versus Mass Ratio Substrates in Quadratic Model 85
- Figure 4.9: Response Surface Plot of Lipase Dosage versus Reaction Temperature in Quadratic Model 87
- Figure 4.10: Response Surface Plot of Mass Ratio Substrates versus Reaction Temperature in Quadratic Model 89
- Figure 5.1: Typical Agar Plates Showing The Inhibition Zone of Ferulate Esters Synthesized (Left-Side of Plate) and Positive Control (Right-Side of Plate) Against Bacterial Strains 101
- Figure 5.2: The Degree of Growth Inhibition *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Salmonella typhimurium* and *Escherichia coli* by Different Concentration of Ferulate Esters after 24 hrs of Incubation 105

## LIST OF APPENDICES

	Page
Appendix A: Experimental Design	137
Appendix B: Formula for Statistical Analysis	138
Appendix C: IR Absorption Peaks for Several Functional Groups	142
Appendix D: McFarland Standard	143
Appendix E: Normalized Product Function in Calculation of SPF	144
Appendix F: List of Publications	145

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## ABBREVIATIONS

%	Percentage
Abs	Absorbance
ABTS	2, 2-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid)
ANOVA	Analysis of variance
B	Blank
°C	Degree Celsius
CCD	Central composite design
CF	Correction factor
CV	Coefficient of variation
DNA	Deoxyribonucleic acid
DPPH	2, 2-diphenyl-1-picrylhydrazyl
EC	Enzyme Commission
EE	Erythral effect spectrum
EF	Ethyl ferulate
FA	Ferulic acid
FRAP	Ferric reducing antioxidant power
FT-IR	Fourier Transform Infrared
g	Gram
hrs	Hours
HATR	Horizontal attenuated total reflectance
HPLC	High-performance liquid chromatography
I	Solar intensity spectrum
IR	Infrared
MBC	Minimum bactericidal concentration
mEq	Milliequivalents
mg	Miligram
MHB	Mueller-Hinton broth
MIC	Minimum inhibitory concentration

mL	Mililiter
mm	Milimeter
MTT	3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide
N	Normality
nm	Nanometer
OD	Optimal density
ORAC	Oxygen radical absorption capacity
PRESS	Prediction error sum of squares
P-value	Probability value
R <sup>2</sup>	Determination of coefficient
RM IM	Immobilized <i>Rhizomucor miehei</i>
ROS	Reactive oxygen species
rpm	Round per minute
RSM	Response Surface Methodology
SPF	Sun protection value
TAG	Triacylglycerol
UV	Ultraviolet
w	Weight