



**UNIVERSITI PUTRA MALAYSIA**

***EXTRACTION OF VALUABLE COMPOUNDS FROM WINTER  
MELON (*BENINCASA HISPIDA* (THUNB.) COGN.) SEEDS USING  
ULTRASONICASSISTED SOLVENT, SUPERCRITICAL CARBON  
DIOXIDE AND PRESSURE-SWING METHODS***

**MANDANA BIMAKR**

**FSTM 2012 27**

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PRESSURE-SWING METHODS**

**BY**

**MANDANA BIMAKR**

**Thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia,  
in Fulfillment of the requirement for the Doctor of Philosophy**

**December 2012**

**ESPECIALLY DEDICATED TO MY BELOVED FAMILY**



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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

**EXTRACTION OF VALUABLE COMPOUNDS FROM WINTER MELON  
(*BENINCASA HISPIDA* (THUNB.) COGN.) SEEDS USING ULTRASONIC-  
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**December 2012**

**Chairman: Professor Russly Abdul Rahman, PhD**

**Faculty: Food Science and Technology**

Different extraction techniques have been used to isolate valuable compounds from different plants. Conventional Soxhlet extraction (CSE) is a standard technique which has been used for a long time. The main disadvantages of conventional techniques are long extraction time which is not always accepted by industries. This extraction method is also not suitable for the extraction of thermo-sensitive compounds due to the possibility of thermal decomposition of target compounds as extraction usually occurs at the boiling point of used solvent for a long time. The current study presented the effect of various extraction methods including conventional Soxhlet extraction (CSE), ultrasound-assisted extraction (UAE), supercritical carbon dioxide (CO<sub>2</sub>) extraction (SCE) and SCE with pressure swing technique (SCE-PST) for extraction of valuable compounds from winter melon (*Benincasa hispida* (Thunb.) Cogn.) seeds. Parameters examined were crude extraction yield (CEY), radical scavenging activity (RSA) in terms of 1, 1-diphenyl-

2-picrylhydrazyl (DPPH<sup>·</sup>) radicals scavenging percentage (%DPPH<sub>sc</sub>) and 2,2'-Azinobis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS<sup>++</sup>) radicals scavenging percentage (%ABTS<sub>sc</sub>) and total phenolic content (TPC) of *B. hispida* seeds. The overall optimum conditions for obtaining maximum dependent variables were predicted using response surface methodology (RSM). Under the optimised conditions, the fatty acid composition of extracts was identified and quantified using gas chromatography (GC). In CSE different solvents (*n*-hexane, ethanol and ethyl acetate) were used and the highest CEY ( $294.45 \pm 1.22 \text{ mgg}^{-1}$ ) obtained using ethyl acetate. Ethanol (EtOH) extracts showed the highest %DPPH<sub>sc</sub> and %ABTS<sub>sc</sub> ( $28.7 \pm 0.7$  and  $27.0 \pm 0.9$ , respectively) while *n*-hexane extracts possessed the lowest ( $13.1 \pm 0.9$  and  $12.2 \pm 0.6$ , respectively).

TPC ranging from zero in *n*-hexane extract to  $11.34 \pm 1.3 \text{ mg GAE/g}$  extracts obtained using EtOH which revealed the antioxidant potency of *B. hispida* seeds. In order to improve the quality and quantity of the extracts, UAE was applied and significant ( $p < 0.05$ ) effect of amplitude, temperature and sonication time was detected. The overall optimum conditions predicted to be 65% amplitude, 52 °C and 35.00 min. under the optimum conditions the CEY, %DPPH<sub>sc</sub>, %ABTS<sub>sc</sub> and TPC were  $108.50 \text{ mgg}^{-1}$ , 43.47%, 36.47% and 23.97 mg GAE/g extract. In the following, the SCE was investigated and it was found that studied parameters (pressure, temperature and dynamic extraction time) significantly ( $p < 0.05$ ) affected the dependent variables. The optimum conditions found to be 234.25 bar, 46 °C and 95.05 min and under this optimum condition, the CEY, %DPPH<sub>sc</sub>, %ABTS<sub>sc</sub> and TPC were  $174.91 \text{ mgg}^{-1}$ , 52.96%, 61.65% and 40.45 mg GAE/g extract, respectively. Finally, SCE-PST was investigated at different levels of pressure, holding time and continuous extraction time. The significant ( $p < 0.05$ ) difference in

responses revealed the crucial and effective role of PST for improving the SCE process. The overall optimum conditions predicted to be 179.18 bar, 10.15 min holding time and 52.47 min continuous extraction time. Under the optimum condition the CEY, %DPPH<sub>sc</sub>, %ABTS<sub>sc</sub> and TPC were 235.12 mgg<sup>-1</sup>, 67.36%, 64.42% and 42.77 mg GAE/g extract, respectively. Significant difference ( $p < 0.05$ ) for fatty acid composition was found between CSE and various extraction methods while considerable difference is not found between the unsaturated fatty acids (UFAs) content of extracts using UAE (82.00%), SCE (83.67%) and SCE-PST (84.34%). Generally, the dominant composition of the UFAs was linoleic acid (C18:02) and oleic acid (C18:01). This study, for the first time, confirmed the possibility of using UAE, SCE and SCE-PST to obtain the valuable extracts with high antioxidant potency and containing high amount of UFAs from *B. hispida* seeds.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

**PENGEKSTRAKAN KOMPAUN BERKUALITI DARIPADA BIJI KUNDUR  
(*BENINCASA HISPIDA* (THUNB.) COGN.) MENGGUNAKAN KAEDAH  
PENGEKSTRAKAN BENDALIR GENTING LAMPAU KARBON  
DIOKSIDA DAN KAEDAH-KAEDAH PENGEKSTRAKAN LAIN**

Oleh

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Kaedah pengekstrakan berbeza telah digunakan untuk mengasingkan kompaun berkualiti dari pelbagai jenis tumbuhan berbeza. pengekstrakan konvensional Soxhlet (CSE) merupakan teknik standard yang telah digunakan sekian lama. Walaubagaimanapun, kelemahan utama teknik ini adalah masa pengekstrakan yang panjang yang tidak diterima di industri. Kedah pengekstrakan ini juga tidak sesuai untuk pengekstrakan sebatian atau kompaun yang sensitive terhadap kepanasan. Ini kerana pengekstrakan dilakukan pada suhu tinggi (takat didih pelarut) pada masa yang lama yang akan mengakibatkan kemungkinan berlakunya penguraian terma terhadap kompaun yang ingin diekstrak.

Kajian yang dijalankan membentangkan kesan pelbagai kaedah pengekstrakan seperti pengekstrakan konvensional Soxhlet (CSE), pengekstrakan ultra sound terbantu (UAE), pengekstrakan bendalir genting lampau CO<sub>2</sub> (SCE) dan pengekstrakan SCE teknik tekanan berayun (SCE-PST) bagi mengekstrak kompaun

bernilai daripada biji kundur (*Benincasa hispida* (Thunb.) Cogn.). pemboleh ubah yang diukur adalah hasil pengekstrakan (CEY), aktiviti memerangkap radikal (RSA) berlandaskan 1, 1-diphenyl-2-picrylhydrazyl (DPPH<sup>·</sup>) peratus pemerangkapan radikal (%DPPH<sub>sc</sub>) dan 2,2'-Azinobis (3-ethylbenzothiazoline-6-sulphonic acid) peratus pemerangkapan radikal (%ABTS<sub>sc</sub>) garam ammonium (ABTS<sup>++</sup>) dan total kandungan phenol (TPC) daripada biji kundur (*B. hispida*). Kondisi optimum keseluruhan, untuk mendapatkan pemboleh ubah bersandar maksimum diramalkan dengan menggunakan kaedah rangsangan permukaan (RSM). Pada nilai optimum, komposisi asid lemak yang diekstrak dikenalpasti dan dikira menggunakan kromatografi gas (GC). Bagi CSE, pelarut yang berbeza digunakan (*n*-hexane, ethanol, etil asetat) dimana penghasilan ekstrak tertinggi diperoleh dengan menggunakan etil asetat (294.45 ± 1.22 mgg<sup>-1</sup>). Ekstrak ethanol (EtOH) pula memberikan, nilai paling tinggi pada DPPH<sup>·</sup> dan ABTS<sup>++</sup> (28.70 ± 0.70 dan 27.00 ± 0.90%) kebolehan mengikat radikal bebas, manakala pengekstrakan menggunakan *n*-hexane menghasilkan nilai terendah (1.31 ± 0.90 dan 12.20 ± 0.60% bagi DPPH<sup>·</sup> dan ABTS<sup>++</sup>). Nilai TPC daripada julat kosong dengan menggunakan ekstrak *n*-hexane hingga 11.34 ± 1.33 mg GAE/g dengan menggunakan ekstrak etanol, menunjukkan potensi biji kundur (*B. hispida*) berupaya bertindak sebagai antioksidan. Bagi meningkatkan kualiti dan kuantiti ekstrak, UAE telah digunakan dan kesan ketara (*p*<0.05) bagi amplitud, suhu dan masa sonikasi dikesan. Nilai kondisi purata optimum telah diramalkan pada 65%, 52 °C dan 35 min yang menghasilkan 108.50 mgg<sup>-1</sup> CEY, 43.47 %ABTS<sub>sc</sub>, 36.47 %DPPH<sub>sc</sub> dan 23.97 mg GAE/g ekstrak. Seterusnya adalah kajian terhadap SCE dan didapati parameter yang dikaji (tekanan, suhu dan masa pengekstrakan dinamik) secara umumnya telah memberi kesan ketara (*p*<0.05) terhadap pemboleh ubah bersandar. Pada kondisi optimum (234.5 bar, 46



°C dan 95.05 min) CEY, %ABTS<sub>sc</sub>, %DPPH<sub>sc</sub> dan TPC adalah masing-masing 174.91 mgg<sup>-1</sup>, 52.96%, 61.65% dan 40.45 mg GAE/g ekstrak. Akhir sekali, SCE-PST telah dikaji pada tekanan, masa statik dan masa pengekstrakan berterusan yang berbeza. Rangsangan ketara ( $p < 0.05$ ) menunjukkan kesan efektif PST dalam penambahbaikan proses SCE. Kondisi optimum keseluruhan diramalkan pada 179.18 bar, 10.15 min bagi masa statik dan 52.47 minit bagi proses berterusan. Pada nilai optimum, CEY, %ABTS<sub>sc</sub>, %DPPH<sub>sc</sub> dan TPC adalah masing-masing 235.12 mgg<sup>-1</sup>, 67.36%, 64.42% dan 42.77 mg GAE/g ekstrak. Perbezaan ketara ( $p < 0.05$ ) bagi komposisi asid lemak adalah diperolehi dari pada CSE dibandingkan dengan kaedah pengekstrakan yang lain manakala tiada perbezaan ketara pada kandungan asid lemak tak tepu (UFAs) menggunakan UAE (82.00%), SCE (83.67%) dan SCE-PST (84.34%). Secara umumnya komposisi dominan bagi UFAs adalah asid lenolik (C18:02) dan asid oleik (C18:01). Kajian ini untuk pertama kalinya mengesahkan kemungkinan menggunakan UAE, SCE dan SCE-PST bagi memperolehi bahan berguna, dengan kandungan bahan antioksidan poten serta UFA dengan amaun tinggi hasil pengekstrakan biji kundur (*B. hispida*) ini.

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I certify that an Examination committee met on \_ to conduct the final examination of Mandana Bimakr on her Doctor of Philosophy thesis entitled “Extraction of valuable compounds from winter melon (*Benincasa hispida*) seeds using supercritical carbon dioxide and other extraction methods” in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and University Pertanian Malaysia (Higher Degree) Regulation 1981. The committee recommends that the candidate be awarded the relevant degree. Members of Examination Committee are as follows:

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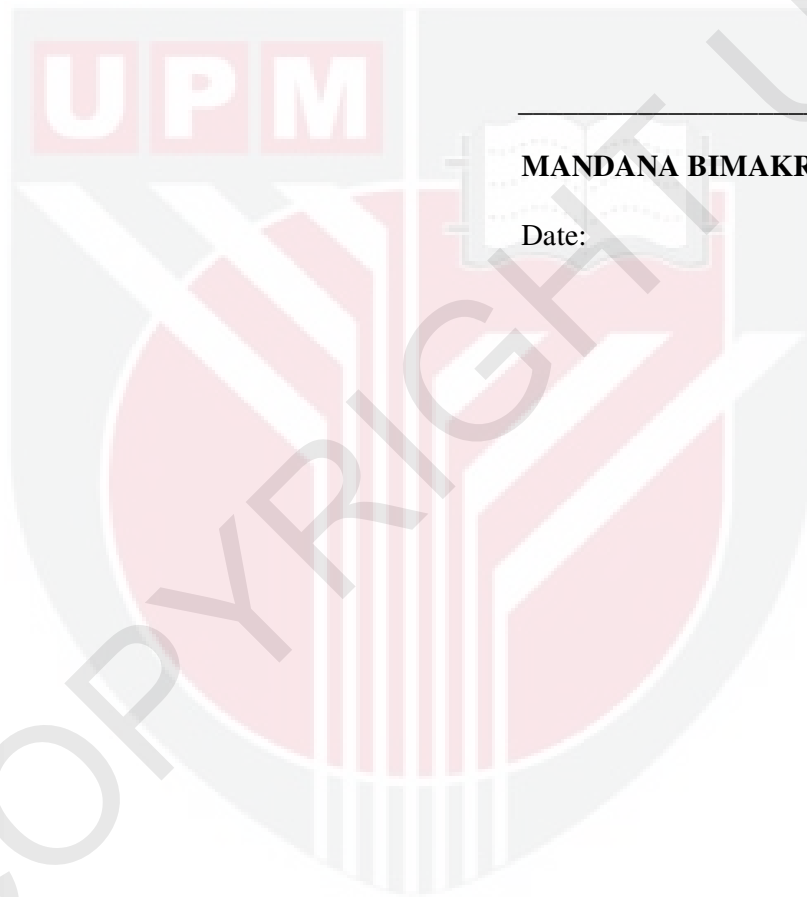
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## DECLARATION

I declare that the thesis is original work except for quotations and citations, which have been duly acknowledged. I also declare that it has not been previously or concurrently, submitted for any other degree at Universiti Putra Malaysia or at other institutions.



MANDANA BIMAKR

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## TABLE OF CONTENTS

<b>ABSTRACT</b>	<b>Page</b> iii
<b>ABSTRAK</b>	vi
<b>ACKNOWLEDGEMENTS</b>	ix
<b>APPROVAL</b>	x
<b>DECLARATION</b>	xii
<b>LIST OF FIGURES</b>	xviii
<b>LIST OF TABLES</b>	xxiii
<b>ABBREVIATIONS</b>	Xxvi

### CHAPTER

<b>1</b>	<b>GENERAL INTRODUCTION</b>	<b>1</b>
<b>2</b>	<b>LITERATURE REVIEW</b>	<b>5</b>
2.1	<i>Cucurbitaceae (Cucurbit) Family</i>	5
2.2	Winter Melon ( <i>Benincasa hispida</i> (Thunb.) Cogn.)	6
2.2.1	Culturing of <i>Benincasa hispida</i>	7
2.2.2	Nutritional Value of <i>Benincasa hispida</i>	8
2.2.3	Medicinal Values of <i>Benincasa hispida</i>	10
2.3	Antioxidants	11
2.3.1	Synthetic Antioxidants	11
2.3.2	Natural Antioxidants	12
2.3.3	Phenolic Compounds	14
2.4	Extraction Methods	16
2.4.1	Conventional Soxhlet Extraction	17
2.4.1.1	Practical Issues in CSE	18
2.4.1.2	Advantages and Disadvantages of CSE	19
2.4.2	Assisted Solvent Extractions	20
2.4.2.1	Ultrasound-Assisted Extraction	20
2.4.2.1.1	Practical Issues in UAE	21
2.4.2.1.2	Effects of Ultrasound Characteristics	22
2.4.2.1.3	Advantages and Disadvantages of UAE	23
2.4.2.1.4	Potential Applications of UAE	23
2.4.2.2	Microwave-Assisted Extraction	25
2.4.2.2.1	Practical Issues in MAE	26
2.4.2.2.2	Operating Conditions for MAE	27
2.4.2.2.3	Advantages and Disadvantages of MAE	28
2.4.2.2.4	Potential Applications of MAE	28
2.4.2.3	Pressurized Liquid Extraction	30
2.4.2.3.1	Advantages and	33

	Disadvantages of PLE	
2.4.3	Supercritical Fluid Extraction	34
2.4.3.1	Practical Issues in SFE	36
2.4.3.2	Raw Materials Preparation	38
2.4.3.3	Operating Conditions for SFE	39
2.4.3.4	Advantages and Disadvantages of SFE	40
2.4.3.5	Potential Applications of SFE	40
<b>3</b>	<b>EXTRACTION OF VALUABLE COMPOUNDS FROM WINTER MELON (<i>Benincasa hispida</i> (Thunb.) Cogn.) SEEDS BY USING CONVENTIONAL SOXHLET EXTRACTION</b>	
3.1	Introduction	45
3.2	Materials and Methods	46
3.2.1	Raw Material and Reagents	46
3.2.2	Conventional Soxhlet Extraction Method	48
3.2.3	Proximate Analysis	49
3.2.4	Radical Scavenging Activity of Extracts	50
3.2.4.1	Determination of DPPH <sup>•</sup> Radical Scavenging Activity	50
3.2.4.2	Determination of ABTS <sup>•+</sup> Radical Scavenging Activity	51
3.2.5	Determination of Total Phenolic Content of Extracts	52
3.2.6	Preparation of Fatty Acid Methyl Esters	52
3.2.7	Analysis of Fatty Acid Composition	53
3.2.8	Statistical Analysis	53
3.3	Results and Discussion	54
3.3.1	Proximate Analysis	54
3.3.2	Effect of Different Solvents on Crude Extraction Yield of Extracts	55
3.3.3	Effect of Different Solvents on Radical Scavenging Activity and Total Phenolic Content of Extracts	56
3.3.4	Analysis of Fatty Acid Composition	59
3.4	Conclusion	62
<b>4</b>	<b>ULTRASOUND-ASSISTED EXTRACTION OF VALUABLE COMPOUNDS FROM WINTER MELON (<i>Benincasa hispida</i> (Thunb.) Cogn.) SEEDS</b>	
4.1	Introduction	63
4.2	Materials and Methods	65
4.2.1	Raw Material and Reagents	65
4.2.2	Ultrasound-Assisted Extraction	65
4.2.3	Radical Scavenging Activity of Extracts	67
4.2.3.1	Determination of DPPH <sup>•</sup> Radical Scavenging Activity	67
4.2.3.2	Determination of ABTS <sup>•+</sup> Radical Scavenging Activity	67

4.2.4	Determination of Total Phenolic Content of Extracts	67
4.2.5	Preparation of Fatty Acid Methyl Esters	67
4.2.6	Analysis of Fatty Acid Composition	68
4.2.7	Experimental Designs and Statistical Analysis	68
4.2.7.1	Response Surface Modeling	69
4.3	Results and Discussion	71
4.3.1	Effects of UAE Variables on the Crude Extraction Yield of Extracts	71
4.3.2	Effect of UAE Variables on the Radical Scavenging Activity of Extracts	76
4.3.3	Effect of UAE Variables on the Total Phenolic Content of Extracts	84
4.3.4	Optimization of UAE Process	89
4.3.4.1	Analysis of UAE Variables on the Crude Extraction Yield	90
4.3.4.2	Analysis of UAE Variables on the Radical Scavenging Activity	94
4.3.4.3	Analysis of UAE Variables on the Total Phenolic Content of Extracts	99
4.3.4.4	Multi Response Optimization	101
4.3.4.5	Verification of the Final Reduced Models	102
4.3.5	Analysis of Fatty Acid Profile	104
4.4	Conclusion	105
<b>5</b>	<b>SUPERCritical CARBON DIOXIDE EXTRACTION OF VALUABLE COMPOUNDS FROM WINTER MELON (<i>Benincasa hispida</i> (Thunb.) Cogn.) SEEDS</b>	
5.1	Introduction	107
5.2	Material and Methods	109
5.2.1	Raw Material and Reagents	109
5.2.2	Supercritical Carbon Dioxide Extraction	109
5.2.3	Radical Scavenging Activity of Extracts	111
5.2.3.1	Determination of DPPH <sup>•</sup> Radical Scavenging Activity	111
5.2.3.2	Determination of ABTS <sup>•+</sup> Radical Scavenging Activity	111
5.2.4	Determination of Total Phenolic Content of Extracts	111
5.2.5	Preparation of Fatty Acid Methyl Esters	111
5.2.6	Analysis of Fatty Acid Composition	112
5.2.7	Experimental Designs and Statistical Analysis	112
5.2.7.1	Response Surface Modeling	113
5.3	Results and Discussion	113
5.3.1	Effects of SCE Variables on the Crude Extraction Yield	115
5.3.2	Effects of SCE Parameters on the Radical Scavenging Activity	121



5.3.3	Effects of SCE Variables on Total Phenolic Content	128
5.3.4	Optimization of SCE Process	134
5.3.4.1	Analysis of SCE variables on Crude Extraction Yield	135
5.3.4.2	Analysis of SCE Variables on the Radical Scavenging Activity	139
5.3.4.3	Analysis of SCE Variables on the Total Phenolic Content	144
5.3.4.4	Multi Response Optimisation	148
5.3.4.5	Verification of the Final Reduced Models	149
5.3.5	Identification and Quantification of Fatty Acid Profile	151
5.4	Conclusion	152
<b>6</b>	<b>SEPARATION OF VALUABLE COMPOUNDS FROM WINTER MELON (<i>Benincasa hispida</i> (Thunb.) Cogn.) SEEDS USING SUPERCRITICAL CARBON DIOXIDE EXTRACTION COMBINED WITH PRESSURE SWING TECHNIQUE</b>	
6.1	Introduction	154
6.2	Materials and Methods	155
6.2.1	Raw Material and Reagents	155
6.2.2	Supercritical Carbon Dioxide Extraction Combined with Pressure Swing	155
6.2.3	Radical Scavenging Activity of Extracts	157
6.2.3.1	Determination of DPPH <sup>•</sup> Radical Scavenging Activity	157
6.2.3.2	Determination of ABTS <sup>•+</sup> Radical Scavenging Activity	157
6.2.4	Determination of Total Phenolic Content of Extracts	157
6.2.5	Preparation of Fatty Acid Methyl Esters	158
6.2.6	Analysis of Fatty Acid Composition	158
6.2.7	Experimental Designs and Statistical Analysis	158
6.2.7.1	Response Surface Modelling	159
6.3	Results and Discussion	160
6.3.1	Effect of SCE-PST Variables on Crude Extraction Yield	160
6.3.2	Effect of SCE-PST Variables on Radical Scavenging Activity	165
6.3.3	Effect of SCE-PST Variables on Total Phenolic Content	173
6.3.4	Optimization of SCE-PST Process	179
6.3.4.1	Analysis of Influence of SCE-PST Variables on Crude Extraction Yield	179
6.3.4.2	Analysis the Influence of SCE-PST Variables on Radical scavenging	183

	Activity	
6.3.4.3	Analysis of SCE-PST Variables on Total Phenolic Content	188
6.3.4.4	Multi Response Optimization	192
6.3.4.5	Verification of the Final Reduced Models	193
6.3.5	Identification and Quantification of Fatty Acid Composition	195
6.4	Conclusion	196
<b>7</b>	<b>GENERAL CONCLUSIONS AND RECOMMENDATION FOR FUTURE RESEARCH</b>	
7.1	Conclusions	198
7.2	Recommendations	202
	<b>REFERENCES</b>	204
	<b>APPENDIX</b>	232
	<b>BIODATA OF STUDENT</b>	238
	<b>LIST OF PUBLICATIONS</b>	239