การเปลี่ยนแปลงองค์ประกอบของสารให้กลิ่นรสและไขมัน ระหว่างกระบวนการแปรรูปถั่วแมคคาเดเมีย

นางสาว สุพรทิพย์ พัฒนยินดี

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาเทคโนโลยีทางอาหาร ภาควิชาเทคโนโลยีทางอาหาร
คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2550

CHANGES IN THE COMPOSITION OF VOLATILE COMPOUNDS AND LIPIDS DURING PROCESSING OF MACADAMIA NUTS

Miss Suporntip Phattanayindee

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science Program in Food Technology
Department of Food Technology
Faculty of Science
Chulalongkorn University
Academic Year 2007

Thesis Title	CHANGES IN THE COMPOSITION OF VOLATILE COMPOUNDS AND LIPIDS DURING PROCESSING OF MACADAMIA NUTS
By	Miss Suporntip Phattanayindee
Field of Study	Food Technology
Thesis Advisor	Chaleeda Borompichaichartkul, Ph.D.
Thesis Co-advisor	George Srzednicki, Ph.D.

Accepted by the Faculty of Science, Chulalongkorn University in Partial Fulfillment of the Requirements for the Master 's Degree

S. Hannongbua ..... Dean of the Faculty of Science

(Professor Supot Hannongbua, Ph.D.)

THESIS COMMITTEE

S. Subhimaros ..... Chairman

(Associate Professor Suwanna Subhimaros, Dr. Ing.)

Chaleeda Borompichaichartkul ..... Thesis Advisor

(Chaleeda Borompichaichartkul, Ph.D.)

George Srzednicki ..... Thesis Co-advisor

(George Srzednicki, Ph.D.)

Thanachan Mahawanich ..... Member

(Thanachan Mahawanich, Ph.D.)

N. Laohaprasit ..... External Member

(Nirutchara Laohapasit, Ph.D.)

สุพรทิพย์ พัฒนยินดี : การเปลี่ยนแปลงองค์ประกอบของสารให้กลิ่นรสและไขมันระหว่างกระบวนการแปรรูปถั่วแมคคาเดเมีย (CHANGES IN THE COMPOSITION OF VOLATILE COMPOUNDS AND LIPIDS DURING PROCESSING OF MACADAMIA NUTS)

อ. ที่ปรึกษา : อ. ดร.ชาลีดา บรมพิชัยชาติกุล,

อ.ที่ปรึกษาร่วม : George Srzednicki, Ph.D. 153 หน้า.

แมคคาเดเมียเป็นพืชยืนต้นไม่ผลัดใบ มีถิ่นกำเนิดที่ประเทศออสเตรเลีย แมคคาเดเมียมีกรดไขมันไม่อิ่มตัวเชิงเดี่ยวสูง เช่น กรดไขมันโอเลอิค ซึ่งสามารถช่วยลดระดับคอเลสเตอรอลในเส้นเลือด อัตราส่วนของกรดไขมันไม่อิ่มตัวต่อกรดไขมันอิ่มตัวในแมคคาเดเมียโดยเฉลี่ยเท่ากับ 5.5 : 1 อัตราส่วนของกรดไขมันที่ไม่อิ่มตัวสูงส่งผลให้เกิดปฏิกิริยาออกซิเดชันได้ง่าย และเป็นสาเหตุให้เกิดกลิ่นหืน ปฏิกิริยาเหล่านี้สามารถเกิดขึ้นในระหว่างกระบวนการแปรรูปและการเก็บรักษา ซึ่งส่งผลต่อการเปลี่ยนแปลงคุณภาพของแมคคาเดเมีย งานวิจัยนี้มีวัตถุประสงค์ เพื่อติดตามการเปลี่ยนแปลงองค์ประกอบของไขมันและกลิ่นรสของแมคคาเดเมียในระหว่าง การอบแห้ง การคั่ว และการเก็บรักษา โดยเปรียบเทียบวิธีการอบแห้ง 2 วิธี คือ การอบแห้งแบบลมร้อนที่ 30°C 7 วัน จากนั้นเพิ่มอุณหภูมิเป็น 40°C 7 วัน และ 60°C 3 วัน และ การอบแห้งโดยใช้ปั๊มความร้อนที่อุณหภูมิ 38 °C นาน เป็นเวลา 1 วัน จนความชื้นลดลงเหลือ 8 % wb จากนั้นอบแห้งต่อด้วยเครื่องอบแห้งแบบอุโมงค์ที่ 55°C 2 วัน อบแห้งแมคคาเดเมียทั้ง 2 วิธี จนความชื้นลดลงเหลือ 1.5% wb แล้วนำไปคั่วที่ 125 °C เป็นเวลา 20 นาที จนความชื้นเหลือ 1 % wb หลังจากนั้นเก็บแมคคาเดเมียที่อุณหภูมิ 4°C เป็นเวลา 3 เดือน ด้วยถุงพลาสติกชนิดเอทิลีนไวนิลแอลกอฮอล์โคโพลีเมอร์ เพื่อติดตามการเปลี่ยนแปลงคุณภาพ โดยวิเคราะห์ปริมาณกรดไขมัน กลิ่นรส สี ค่าเปอร์ออกไซด์ ค่ากรดไขมันอิสระ และวัดความชื้นของแมคคาเดเมียในแต่ละช่วงของกระบวนการแปรรูป ผลการศึกษาพบว่าหลังจากการอบแห้งด้วยลมร้อน กรดไขมันไม่อิ่มตัว เช่น C16:1 และ C18:1 และกรดไขมันอิ่มตัวเช่น C14:0 C18:0 C20:0 และ C22:0 ลดลงอย่างมีนัยสำคัญ ($p \leq 0.05$) หลังจากการอบแห้งโดยใช้ปั๊มความร้อนร่วมกับการอบแห้งแบบอุโมงค์ กรดไขมันไม่อิ่มตัว C16:1 และกรดไขมันอิ่มตัวเช่น C12:0 และ C22:0 ลดลงอย่างมีนัยสำคัญ ($p \leq 0.05$) การสลายตัวของกรดไขมันส่งผลต่อการเพิ่มขึ้นของกลิ่นรส เช่น กลุ่มสารแอลดีไฮด์ แอลกอฮอล์ ไฮโดรคาร์บอน นอกจากนี้หลังการอบแห้ง ปริมาณกรดไขมันอิสระ และค่าเปอร์ออกไซด์เพิ่มขึ้นอย่างมีนัยสำคัญ ($p \leq 0.05$) การอบแห้งโดยใช้ปั๊มความร้อนร่วมกับอบแห้งแบบอุโมงค์สามารถลดการสลายตัวของกรดไขมันในระหว่างการอบแห้งมากกว่าการอบแห้งแบบลมร้อน อีกทั้งปริมาณสุดท้ายของกรดไขมันโอเลอิคลดลงจาก 59.3 เหลือ 45.5 และจาก 62.3 เหลือ 57.3 mg/100 g db เมื่อผ่านการอบแห้งแบบลมร้อนและการอบแห้งแบบใช้ปั๊มความร้อนร่วมกับการอบแห้งแบบอุโมงค์ตามลำดับ จากการอบแห้งด้วยลมร้อนพบว่า เฮกซานอล สารกลุ่มแอลดีไฮด์ แอลกอฮอล์ และไฮโดร์คารบอนเพิ่มขึ้นมากกว่าการอบแห้งแบบใช้ปั๊มความร้อนร่วมกับการอบแห้งแบบอุโมงค์ ดังนั้นการอบแห้งแบบใช้ปั๊มความร้อนร่วมกับการอบแห้งแบบอุโมงค์ช่วยลดระยะเวลาในการอบแห้ง และรักษาคุณภาพของแมคคาเดเมียได้ดีกว่าการอบแห้งแบบลมร้อน แม้ว่าหลังจากการอบแห้ง และการคั่ว การเปลี่ยนแปลงของกรดไขมัน กลิ่นรส สี รวมทั้งเกิดกลิ่นหืน ยังคงเกิดขึ้นอย่างต่อเนื่องในระหว่างการเก็บ แต่ค่ากรดไขมันอิสระ ค่าเปอร์ออกไซด์ และค่าสี หลังจากเก็บแมคคาเดเมียนาน 3 เดือน ยังอยู่ในช่วงที่ยอมรับได้

ภาควิชา.....เทคโนโลยีทางอาหาร..... ลายมือชื่อนิสิต.....สุพรทิพย์ พัฒนยินดี.....

สาขาวิชา.....เทคโนโลยีทางอาหาร..... ลายมือชื่ออาจารย์ที่ปรึกษา.....C. Boonpichaidharkul.....

ปีการศึกษา.....2550..... ลายมือชื่ออาจารย์ที่ปรึกษาร่วม.....G. Srzednicki.....

4972541023 : MAJOR FOOD TECHNOLOGY

KEY WORDS : FATTY ACIDS / VOLATILE COMPOUNDS / FREE FATTY ACIDS / PEROXIDE VALUE /COLOUR

SUPORNTIP PHATTANAYINDEE : CHANGES IN THE COMPOSITION OF VOLATILE COMPOUNDS AND LIPIDS DURING PROCESSING OF MACADAMIA NUTS.

THESIS ADVISOR : CHALEEDA BOROMPICHAICHARTKUL, Ph.D.,

THESIS COADVISOR : GEORGE SRZEDNICKI, Ph.D., 153 pp.

Macadamia is a subtropical tree originating from Australia. The kernels are rich in monounsaturated fatty acids and may reduce serum cholesterol when included in the diet. It can be assumed that this is due to a particularly high oleic acid content of the nut oil. In general, an average ratio of unsaturated to saturated fatty acids in macadamia nuts is 5.5:1. High content of unsaturated fatty acid leads to oxidative reactions and results in rancidity which decreases quality of macadamia nuts. Moreover, processing methods and storage can have a major impact on changes in quality of macadamia nuts. Therefore, the proposed research aims at investigating the changes of flavour and lipid composition during macadamia nut processing. The latter includes drying, roasting and storage of macadamia nuts. Two methods of drying were employed in this study. Hot air drying (HA) (30°C for 7 days, 40°C for 7 days, 60°C for 3 days) and heat pump drying (HP) (38 °C,1 day, 8% wb) followed by tunnel dryer (TD) (55°C, 2 days) were used to dry macadamia nuts until their moisture content came down to 1.5% wb. This was followed by roasting at 125 °C for 20 minutes until the kernel moisture content reached 1% wb. The final phase of the experiment was storage at 4°C for 3 months. After each processing stage the quality of macadamia nuts was assessed in terms of fatty acid content, volatile compounds, colour, peroxide value, free fatty acids and moisture content. The result showed a decrease of fatty acid content ($p \leq 0.05$) among unsaturated fatty acids such as C16:1, C18:1 and saturated fatty acids such as C14:0, C18:0, C20:0 and C22:0 after drying with HA drying as well as the decreasing of fatty acid content ($p \leq 0.05$) among unsaturated fatty acids such as C18:1 and saturated fatty acids such as C12:0 and C22:0 after drying with HP+TD drying. The decomposition of fatty acids contributed to an increase in the level of total aldehydes, total alcohols and total hydrocarbons. Moreover, free fatty acids and peroxide value increased significantly ($p \leq 0.05$) after drying. HP + TD drying can reduce decomposition of fatty acids during drying more than HA drying. Final content of oleic acid decreased from 59.3 to 45.5 and 62.3 to 57.3 mg/100 g db after HA and HP + TD drying, respectively. The increasing values of hexanal, total alcohols, aldehydes and hydrocarbons after HA drying were more pronounced than after HP + TD drying. The degradation of fatty acids continued during roasting and storage. In addition to that, a small degradation was found during roasting and storage of macadamia nuts at low moisture content after roasting (1% wb). It can be concluded that HP + TD drying showed benefit of time saving and natural quality preservation of the macadamia nuts vs. than HA drying. Although, the changes of fatty acids, volatile compounds, colour and rancidity after drying and roasting continued during storage of macadamia nuts, they occurred at a slow rate. The final values of peroxide value, free fatty acid and colour were within acceptable limits after three months of storage.

Department.....Food Technology..... Student's signature.....Suporntip Phatanayindee

Field of study.....Food Technology..... Advisor's signature..C. Borompichaichartkul.

Academic year.....2007..... Co-advisor's signature.....G. Srzednicki

ACKNOWLEDGEMENTS

I would like to extend my sincere gratitude and appreciation to the following people, without whom this thesis would not be possible. To my supervisors, Dr. Chaleeda Borompichaichartkul and Dr. George Srzednicki for continuous guidance and constructive criticism. Dr. John Craske and Prof. Dr. Michael Wootton for the valuable comments and information on the volatile compound and fatty acids analysis. Without their expertise, the aims of this study would not have been achieved. I would also like to thank them for their constant encouragement and trust in my ability to carry out this study.

Food Science and Technology, School of Chemical Sciences and Engineering, Faculty of Engineering, University of New South Wales, Australia for the facilities and laboratory and Denham Plantation, Knockrow, Northern New South Wales, for providing macadamia nut samples and explanations on the initial processing of macadamia nuts.

To Camillo and Peter for their technical assistance. To P'ton, P'tum, Nadiene, Pim, Pom and Food Science students of University of New South Wales, who have helped me several times. I wish to thank them for their constant encouragement and for helping me in many areas.

Thanks to my family for financial and emotional support, and for their love, care and concern of me. Finally, The skills and experience that I gained by studying in Australia will be invaluable for my development in the future.

CONTENTS

Page number

THAI ABSTRACT……..**iv**

ENGLISH ABSTRACT……...**v**

ACKNOWLEDGEMENTS ……**vi**

TABLE OF CONTENTS……**vii**

LISTS OF TABLES ……**xii**

LIST OF FIGURES ……**xiv**

NOMENCLATURE ……**xx**

Chapter 1 INTRODUCTION……**1**

Chapter 2 LITERATURE REVIEW……**5**

2.1 History……...5

2.2 Classification ……6

2.3 Compositions ……8

2.3.1 Proximate composition and nutritional value …….8

2.3.2 Oil composition ……9

2.4 Market and production ……..13

2.5 Processing ……14

2.5.1 Nuts fall ……...15

2.5.2 Harvesting……..15

2.5.3 Dehusking……..16

2.5.4 Pre-drying……..19

2.5.5 Drying ……...20

2.5.5.1 Drying in factory……...20

2.5.5.2 Techniques of drying macadamia nuts …….21

2.5.6 Cracking and grading ……....24

2.5.7 Roasting and packaging……25

2.6 Quality……...26

2.6.1 Moisture Content…….. 26

2.6.2 Water activity……27

2.6.3 Temperature……..27

2.6.4 Grading ……...28

2.6.5 Sugar and Colour……..28

2.6.6 Rancidity……...29

2.6.6.1 Lipolysis or Hydrolytic Rancidity……29

2.6.6.2 Free Fatty Acid ……30

2.6.6.3 Oxidative Rancidity……..31

2.6.6.4 Peroxide Value ……33

2.6.7 Volatile Compounds……..34

2.6.7.1 Volatiles compounds in macadamia nuts…….34

2.6.7.2 Lipid Volatile Formation ……36

2.6.7.3 Isolation of volatile compounds……38

2.6.7.4 The GC-MS technique ……40

(Gas chromatography and mass spectrometry)

2.6.8 Fatty acids……..40

2.6.8.1 Derivative preparation of fatty acids……41

2.6.9 Gas chromatography (GC)……42

2.6.9.1 Carrier gas source……...42

2.6.9.2 GC injection/inlet……42

2.6.9.3 Column……45

2.6.9.4 Detector ……45

Chapter 3 MATERIALS AND METHODS……47

3.1 Materials and Equipment……47

3.1.1 Raw materials……47

3.1.2 List of chemicals……47

3.1.3 List of equipment……...49

3.2 Experimental Design……50

3.2.1 Hot air drying ……...51

3.2.2 Heat pump drying……51

3.2.3 Tunnel drying……53

3.2.4 Macadamia nut roasting……....54

3.2.5 Storage conditions……54

3.2.6 Quality checking 1……54

3.2.7 Quality checking 2 – 4……..54

3.2.8 Quality checking 5……55

3.2.9 Statistical Analysis……55

3.3 Isolation of volatiles compounds from macadamia nut……55

3.3.1 Sample preparation……55

3.3.2 Likens-Nickerson simultaneous distillation and extraction (LNSDE)..55

3.3.3 Internal standard……57

3.3.4 Solvent purification……...57

3.3.5 Anhydrous sodium sulphate purification……..58

3.3.6 Analysis of macadamia volatiles by gas chromatography and mass spectrometry (GC-MS)……..58

3.3.6.1 Apparatus ……58

3.3.6.2 GC-FID Conditions……..59

3.3.6.3 Qualitative analysis……..60

3.2.6.4 Quantitative analysis……60

3.4 Isolation of macadamia fatty acid ……..61

3.4.1 Methylation ……..61

3.4.2 Apparatus……..62

3.4.3 GC-FID Conditions……..62

3.4.4 Qualitative analysis……..63

3.4.5 Quantitative analysis……63

3.4.5.1 Theoretical relative response factor (TRF)

for fatty acid methyl esters…..……..63

3.4.5.2 Standard curve of palmitoleic acid preparation……63

Chapter 4 RESULTS AND DISCUSSION……**64**

4.1 Raw materials analysis……..64

4.1.1 Proximate composition……64

4.1.2 Fatty acid compositions ……...66

4.1.3 Volatile compounds in raw macadamia nut……..68

4.2 Effect of processing and storage on quality of macadamia nut…….. 69

4.2.1 Oil content and composition ……69

4.2.2 Peroxide value, free fatty acid, moisture content

and water activity……..……75

4.2.2.1 Peroxide value……....76

4.2.2.2 Free fatty acid ……78

4.2.2.3 Moisture content and water activity……...79

4.2.3 Colour evaluation……..80

4.3 Effect of processing and storage on volatile compounds of macadamia nuts……..……..82

Chapter 5 CONCLUSIONS……..**91**

Chapter 6 REFERENCES…….**95**

APPENDICES……**105**

APPENDIX A……..**106**

APPENDIX B……..**114**

APPENDIX C……..**122**

APPENDIX D……..**136**

APPENDIX E……..**144**

APPENDIX F……..**151**

VITAE……..**153**

LIST OF TABLES

Page number

Table 2.1 Proximate composition of macadamia nut ……8

Table 2.2 Nutritional information of macadamia nut…….8

Table 2.3 The fatty acid composition of Macadamia *spp*…… 10

Table 2.4 The fatty acid composition of *Macadamia* spp. from Amper Doi Saked, Chiang Mai, Thailand…….11

Table 2.5 Compounds identified from the headspace analysis of roasted macadamia and other nuts…… 35

Table 2.6 Some volatile aldehydes obtained from oxidation of unsaturated fatty Acids……37

Table 3.1 Chemical reagents used in the project…….. 47

Table 3.2 List of equipments used in this study……49

Table 4.1 Proximate analysis of raw macadamia nut……64

Table 4.2 Comparison of chemical properties of raw macadamia oil from nuts grown in Australia and Thailand……..66

Table 4.3 The fatty acid composition of raw macadamia nut……...66

Table 4.4 Volatile compounds in raw macadamia nut……..68

Table 4.5 Fatty acid composition (mg/100 g dry basis) in macadamia nut oil from extracted samples subjected to different drying methods and storage duration……70

Table 4.6 The quality of macadamia nuts during processing and storage……75

Table 4.7 Colour changes of macadamia nut during processing and storage……...81

Table 4.8 Concentration (µg/kg, ppb) of volatile compounds after hot air drying of macadamia nut ……83

Table 4.9 Concentration (µg/kg, ppb) of volatile compounds after heat pump drying of macadamia nut ……..84

Table 4.10 List of volatile compounds found in macadamia nut in this study and in the literature……..89

Table D.1 Corrected area of palmitoleic acid at the different concentration ……141

Table D.2 Theoretical relative response factor (TRF) for fatty acid methyl esters...142

Table D.3 Fatty acids composition of dried macadamia nut with hot air dryer…….143

LIST OF FIGURES

Page number

Figure 2.1 Fresh macadamia nuts ……7

Figure 2.2 Monounsaturated fat content in different nuts…… 9

Figure 2.3 Australian macadamia nut production ……13

Figure 2.4 World Macadamia Production 2004……13

Figure 2.5 Production stages of macadamia from farm to factory ..…….14

Figure 2.6 Macadamia nut plantation……15

Figure 2.7 Macadamia nut sweeper and blower machine…….16

Figure 2.8 Macadamia nuts harvesting machine ……..16

Figure 2.9 Dehusking machine of macadamia nuts ……17

Figure 2.10 The size grading of macadamia nuts in shell …….18

Figure 2.11 The nut in shell grading by floating……19

Figure 2.12 In store dryer in macadamia nut orchard.....……...20

Figure 2.13 Adapted domestic microwave oven……22

Figure 2.14 Schematic representation of general heat pump drying system ……23

Figure 2.15 Cracking the nuts from shell……..25

Figure 2.16 Oxidation of linoleic acid and formation of 2-octenal and hexanal from 2,4-decadienal …….36

Figure 2.17 Likens-Nickerson apparatus ……..39

Figure 2.18 The reaction of methylation of fatty acids……..41

Figure 2.19 Steps of splitless injection …….43

Figure 2.20 Basic design of the split injector ……...44

Figure 2.21 Schematic diagram of a flame ionisation detector ……46

Figure 3.1 The overview of the experiments……...50

Figure 3.2 Heat pump dryer …….52

Figure 3.3 Schematic diagram of the heat pump dryer…….52

Figure 3.4 Laboratory-scale cabinet dryer ……...53

Figure 3.5 Modified Likens-Nickerson simultaneous distillation and extraction apparatus…….57

Figure 3.6 Solvent purification apparatus…….58

Figure 3.7 Gas chromatography – Mass spectrometry…….60

Figure 3.8 Gas chromatography – Flame ionisation detector ……..61

Figure 4.1 Content of palmitoleic acid (C16:1) during different stages of processing and drying …….74

Figure 4.2 Content of oleic acid (C18:1) during different stages of processing and drying ……..74

Figure 4.3 Formation of oxidative rancidity ……77

Figure 4.4 A summary of Maillard reaction ……87

Figure A.1 Colour meter equipment ……107

Figure A.2 Soxhlet extraction apparatus……109

Figure A.3 Rotary evaporation apparatus……..110

Figure A.4 Vice for cracking macadamia nuts……..110

Figure A.5 Standard curve of concentration oleic acid and volume titrate of NaOH for determination of free fatty acid …….....112

Figure B.1 The chromatogram of purified tetradecane from GC-MS……115

Figure B.2 The chromatogram of volatile compounds in fresh macadamia nut subjected to hot air drying treatment ……..116

Figure B.3 The chromatogram of volatile compounds in dried macadamia nut subjected to hot air drying treatment ……..116

Figure B.4 The chromatogram of volatile compounds in roasted macadamia nut subjected to hot air drying treatment ……..117

Figure B.5 The chromatogram of volatile compounds in macadamia nut after 1 month storage subjected to hot air drying treatment ……...117

Figure B.6 The chromatogram of volatile compounds in macadamia nut after 2 months storage subjected to hot air drying treatment …….118

Figure B.7 The chromatogram of volatile compounds in macadamia nut after 3 months storage subjected to hot air drying treatment …….118

Figure B.8 The chromatogram of volatile compounds in dried macadamia nut subjected to heat pump dryer combined with tunnel drying treatment ……...…119

Figure B.9 The chromatogram of volatile compounds in roasted macadamia nut subjected to heat pump dryer combined with tunnel drying treatment ……...…119

Figure B.10 The chromatogram of volatile compounds in macadamia nut after 1 month storage subjected to heat pump dryer combined with tunnel drying treatment ……..…….120

Figure B.11 The chromatogram of volatile compounds in macadamia nut after 2 months storage subjected to heat pump dryer combined with tunnel drying treatment ……..…….120

Figure B.12 The chromatogram of volatile compounds in macadamia nut after 3 months storage subjected to heat pump dryer combined with tunnel drying treatment ……..……. 121

Figure C.1 Mass spectrum of Tetradecane…….123

Figure C.2 Mass spectrum of Isopentanol ……123

Figure C.3 Mass spectrum of 1-Ethoxy-2-propanol ……124

Figure C.4 Mass spectrum of Dimethyl disulfide ……124

Figure C.5 Mass spectrum of Pyridine……..125

Figure C.6 Mass spectrum of 1-Pentanol……..125

Figure C.7 Mass spectrum of Toluene……..126

Figure C.8 Mass spectrum of Octane……126

Figure C.9 Mass spectrum of Trans-2-tridecenal ……127

Figure C.10 Mass spectrum of Hexanal……127

Figure C.11 Mass spectrum of 3-Furaldehyde……..128

Figure C.12 Mass spectrum of 1-Hexanol……128

Figure C.13 Mass spectrum of Heptanal……129

Figure C.14 Mass spectrum of 2-Ethyl-1-hexanol ……129

Figure C.15 Mass spectrum of 2-Octenal, trans-2-Octenal ……130

Figure C.16 Mass spectrum of 1-Nonanol ……130

Figure C.17 Mass spectrum of 2-Heptenal ……131

Figure C.18 Mass spectrum of 1-Heptanol ……131

Figure C.19 Mass spectrum of Benzaldehyde ……132

Figure C.20 Mass spectrum of Octanal ……..132

Figure C.21 Mass spectrum of Benzeneacetaldehyde ……133

Figure C.22 Mass spectrum of N-Octanol ……133

Figure C.23 Mass spectrum of Nonanal ……134

Figure C.24 Mass spectrum of 2-Decenal ……134

Figure C.25 Mass spectrum of Trans-2-undecenal ……135

Figure C.26 Mass spectrum of Trans-2-tridecenal ……135

Figure D.1 The chromatogram of purified isooctane from GC-FID …….137

Figure D.2 The chromatogram of palmitoleic acid methyl ester ……..137

Figure D.3 The chromatogram of standard mixture methyl ester of octanoic acid C8:0, nonanoic acid, C9:0, decanoic acid C10:0 undecanoic acid C11:0 and lauric acid C12:0 with GC-FID ……138

Figure D.4 The chromatogram of standard mixture methyl ester of tridecanoic acid C 13:0, myristic C14:0, pentadecanoic acidC15:0, palmitic acid C16:0 and heptadecanoic acid C17:0 with GC-FID ……138

Figure D.5 The chromatogram of standard mixture methyl ester of palmitic acid C16:0, stearic acid, C18:0, oleic acid C18:1, linoleic acid C18:2 and linolenic acid C18:3 with GC-FID ……..139

Figure D.6 The chromatogram of standard mixture methyl ester of stearic acid C18:0, nonadecanoic acid, C 19:0, arachidic acid C20:0, heneicosanoic acid C21:0 and behenic acid C22:0 with GC-FID ……..139

Figure D.7 The log graph between corrected retention time of saturated fatty acid (C8:0 – C22:0) and carbon number ……140

Figure D.8 The log graph between corrected retention time of monounsaturated fatty acid (C16:1, C18:1 and C20:1) and carbon number ……141

Figure D.9 Standard curve of corrected area of palmitoleic acid and concentration (mg) ……142

Figure E.1 The chromatogram of fatty acid methyl ester in fresh macadamia nut subjected to hot air drying treatment.....145

Figure E.2 The chromatogram of fatty acid methyl ester in dried macadamia nut subjected to hot air drying treatment ……...145

Figure E.3 The chromatogram of fatty acid methyl ester in roasted macadamia nut subjected to hot air drying treatment ……146

Figure E.4 The chromatogram of fatty acid methyl ester in macadamia nut after 1 month storage subjected to hot air drying treatment……...146

Figure E.5 The chromatogram of fatty acid methyl ester in macadamia nut after 2 months storage subjected to hot air drying treatment……...147

Figure E.6 The chromatogram of fatty acid methyl ester in macadamia nut after 3 months storage subjected to hot air drying treatment…….....147

Figure E.7 The chromatogram of fatty acid methyl ester in dried macadamia nut subjected to heat pump dryer combined with tunnel drying treatment ……..……...148

Figure E.8 The chromatogram of fatty acid methyl ester in roasted macadamia nut subjected to heat pump dryer combined with tunnel drying treatment ……..…….148

Figure E.9 The chromatogram of fatty acid methyl ester in macadamia nut after 1 month storage subjected to heat pump dryer combined with tunnel drying treatment ……..…….149

Figure E.10 The chromatogram of fatty acid methyl ester in macadamia nut after 2 months storage subjected to heat pump dryer combined with tunnel drying treatment ……..…….149

Figure E.11 The chromatogram of fatty acid methyl ester in macadamia nut after 3 months storage subjected to heat pump dryer combined with tunnel drying treatment ……..…….150

NOMENCLATURE

a_w	Water activity
A (C)	The peak area of compound C
A (S)	The peak area of sugar component
A (IS)	The peak area of internal standard
Conc (C)	The concentration of compound C
Conc (IS)	The concentration of internal standard
d.b.	Dry basis
w.b	Wet basis
GC	Gas chromatography
GC/MS	Gas chromatography and mass spectrometry
GC-FID	Gas chromatography and flame ionization detector
LNSDE	Likens-Nickerson Simultaneous Distillation and Extraction
TRF	Theoretical relative response factor
m/z	Mass per charge
MC	The moisture content (%)
N	Normal
n.d.	Not detected
ppb	Parts per billions
ppm	Parts per millions
SG	Specific gravity
W (IS)	The weight of the internal standard added
W (S)	The weight of the samples

CHAPTER 1

INTRODUCTION

The macadamia tree *(Macadamia* sp*)*, native to the rainforests of eastern Australia, belongs to the family Proteaceae. It is commonly known as the “Queensland nut”, “Popple nut” and “Bush nut” (Winterton, 1968).

There are ten species in the genus macadamia within this family. Only two species produce edible nuts, namely *Macadamia integrifolia* and *Macadamia tetraphylla.* When ripe, the nuts fall to the ground encased in a fibrous, green husk, or pericarp. Generally, fresh macadamia nuts have high moisture content and are prone to deterioration. Thus, the moisture needs to be removed as quickly as possible. Dried and roasted macadamia nuts have moisture content around 1.5 -1.0 % wb. At this moisture content, the kernel shrinks away from the inside of the shell making cracking process become efficient to maintain whole nut and the kernels at 1% wb moisture, thus preventing them from hydrolytic rancidity during storage (Dela *et al*., 1996).

The kernels are rich in monounsaturated fatty acids and may reduce serum cholesterol when included in a healthy diet (Cavaletto, 1980 cited by Wall and Gentry, 2006). This is believed to result from the particularly high oleic acid content of the nut oil. Therefore, oleic acid content can be used as the key criterion to indicate the quality of macadamia nuts. The popularity of macadamia nuts is increased substantially by processing into snack nuts, candies, confectionery, nut butters and oils.

As macadamia nut contains high amount of unsaturated fatty acid, it is prone to hydrolytic and oxidative rancidity when it contains high level of free moisture.

Drying is a critical step in macadamia processing to maximise shelf life and quality of the end product. The available water needed for microbial growth, enzyme

activity, and chemical reactions are decreased. However, internal browning may occur after roasting if drying conditions are not well controlled (Prichavudhi and Yamamoto, 1987). Moreover, lower storage temperature can prolong the shelf life of the macadamia nuts. At a storage temperature of 1.5°C in vacuum package, raw macadamia kernel (2.3% moisture content) had a shelf life of up to 16 months (Dela *et al*.,1966).

Crain and Tang (1975), Chitundu (1994) and Himstedt (2002) are the only workers to have investigated volatile compounds derived from macadamia kernels. However, the study by Crain and Tang (1975) did not identify oxidative volatiles and was performed on roasted kernels. Chitundu (1994) and Himstedt (2002) performed preliminary work identifying oxidative volatiles from raw and dried kernels during storage but did not relate these volatiles to their fatty acid precursors.

From a study by Dela *et al*. (1966) it appears that moisture content of stable macadamia should be between 1-2 % wb. Since macadamia nuts are rich in unsaturated fatty acid, subjecting the nut to heat would stimulate rancidity.

Morever, drying process, roasting and storage can alter the composition of lipid and volatile compounds and contribute to off flavour and colour in the dried nut. Effects of different drying techniques on quality of dried macadamia nut have been given little attention. The industrial drying process is employed for macadamia nuts has the disadvantage of requiring a very long drying period (>1 month). Heat pump drying has advantages of energy recovery result in lower energy consumed for each unit of water removed and ability to control temperature and humidity. Moreover, it is higher efficiency (60 %) than the general hot air oven. A high quality product should be characterized by high oil content. Therefore, there is a potential of using heat pump for drying macadamia nuts.

Based on many published studies on macadamia nuts, it appears that there is little information on changes of flavour and lipid composition under the influence of handling, drying, roasting and storage. The information available only refers to optimum conditions for processing of macadamia nuts related to physical properties such as their colour, texture and moisture content. Therefore the proposed research is aiming at providing the information on changes of flavour and lipid composition during processing of macadamia nuts. The outcome of this research is expected to be used as a set of guidelines for selecting the appropriate processing strategy for macadamia nuts in order to gain the maximum quality of the macadamia nuts. It is expected that the information obtained from this study can be used to develop procedures related to improvement of quality characteristics of macadamia nuts during processing.

Objectives

a) To study the effects of processing and storage on the composition of volatile compounds and lipids in macadamia nuts.

b) To study the effects of processing and storage on the quality attributes of macadamia nuts.

c) To study the effects of drying treatment, namely hot air drying and heat pump drying on quality of macadamia nuts.

Relevance of the research to the food industry

Baseline survey of key volatile compounds and lipids during processing and storage of macadamia nuts. The results will be used to develop guidelines for improve processing of macadamia nuts. Further aims were to provide information for the macadamia nut industry on how to improve their product quality.

CHAPTER 2

LITERATURE REVIEW

2.1 History

The macadamia nut was first described by Walter Hill, former director of the Brisbane Botanical Gardens and Ferdinand Mueller, Government Botanist of Victoria. They discovered the nut on the Pine river, near Brisbane over 100 years ago while investigating the flora of the Moreton district. Mueller named the macadamia after Dr John Macadam, president of the Philosophical Society of Victoria, Australia (Trochoulias, 1984).

The macadamia nut was first introduced into Hawaii by William PurviKukuiballe, between 1882 and 1885 and two American brothers, E. W. Jordan and R. A. Jordan in 1892. After a varietals selection programme involving the examination of many trees over many years, the macadamia industry became firmly established in Hawaii in the 1930s (Leverington, 1958). Today, the macadamia nut industry is one of the most established and profitable industries in Hawaii.

The first macadamia seed in Thailand came from Hawaii in 1953 (Trochoulias, 1984). Seed was germinated at Bangkok Noi experiment station and seedlings were sent to various experimental stations in Thailand. Reports showed that nut size from fruit bearing trees was variable and not as good as Hawaiian selection until in 1968 when the commercial clones were imported from Hawaii and were side grafted in many scions. In 1984 the varieties HAES 741 and HAES 800 were planted at Mae Chon Luang highland station, together with 300 D4 seedlings. A further lot of 800 D4 seedlings were planted at Khun Wang Royal highland station near Chiang Mai.

The Royal Thai government has recognized the need to diversify for a broader range of horticultural crops, particularly those which have export potential and require a large amount of labour. Beside large scale orchards, if it can be demonstrated that suitable varieties will produce good quality kernel in remote hilly area. These are the reason why the macadamia nut is the new target of the five to six year plan for national and social development as a crop with high agro-industrial input.

2.2 Classification

The macadamia nut is an evergreen tree, which belongs to the family Proteaceae. It is indigenous to the coastal rain forests of South Eastern Queensland and the Northern Rivers districts of New South Wales (Winterton, 1968).

There are ten species of the genus macadamia within this family. Only two species produce edible nuts, *Macadamia integrifolia* and *Macadamia tetraphylla.* Hybrids between these two edible species exist. However, most plantings of macadamia nuts are entirely of *M. integrifolia* cultivars (Cavaletto, 1983).

In Australia, *M. integrifolia* is the major species (Mason and McConachie, 1994). Among other eight species, four species exist in Australia, three in New Caledonia and one in Celebes. Kernels of these eight species are not of commercial value as they are predominantly small, bitter and rendered inedible due to the presence of cyanogenic glycoside (Vivien, 1989).

Macadamia fruit is a follicle with a husk that opens along one suture surrounding one seed, with an extremely hard seed coat. The coat is commonly known as the shell as shown in Figure 2.1.

Figure 2.1 Fresh macadamia nuts

Kernels are normally darker coloured and tend to have a light grey coloured upper half. Cooler growing climates are more tolerated by *M. tetraphylla* than by *M. integrifolia* and hence this species is sometimes selected for growth in cooler regions. *M. tetraphylla* kernels range in oil content between 67 g -75 g oil/100g nut and sugars between 6 g – 8 g /100g nut (Cavaletto, 1983). This variation renders inconsistent and unpredictable eating quality and roasting quality of the kernels. As a consequence of such disadvantages, *M. tetraphylla* is regarded as being inferior to *M. integrifolia* and is therefore not widely used in Australian production (Cavaletto, 1983).

Therefore, the macadamia (*M. integrifolia* and *M. tetraphylla*) is the only native tree so far developed as a commercial food crop and seedling to various countries including Thailand (Trochoulias, 1984).

2.3 Compositions

2.3.1 Proximate composition and nutritional value

The proximate composition of macadamia is shown in Table 2.1 and nutritional information is shown in Table 2.2.

Table 2.1 Proximate composition of macadamia nut

Composition	Per 100 g
Energy	3040 kJ (726.6 kcal)
Protein	9.20 g
Fat-Total	76.0 g
Saturated	9.50 g
Monounsaturated	63.84 g
Polyunsaturated	2.66 g
Dietary Fiber	7.0 g
Carbohydrates – Total	10.0 g
Sugars	4.6 g
Ash	1.8 g

Source : Cavaletto (1980)

Table 2.2 Nutritional information of macadamia nut

Nutrient information	Per 100 g
Thiamine (B1)	0.22 mg
Riboflavin (B2)	0.12 mg
Niacin	1.60 mg
Calcium	53.00 mg
Iron	2.00 mg
Phosphorus	241.00 mg
Magnesium	149.00 mg
Potassium	409.00 mg
Sodium	3.00 mg

Source : Grimwood (1971)

The kernel has a high fat content combined with relatively high protein and carbohydrate contents. In addition, the kernels can be regarded as a good source of the B-vitamins (thiamin, riboflavin and niacin) (Grimwood, 1971).

2.3.2 Oil composition

Macadamia nuts have about 69-78 % fat. The major fatty acids are oleic acid, palmitoleic acid and palmitic acid. Oleic acid accounts for 41 – 59 % of the total fatty acids. Macadamia oil has the most highly monounsaturated oil content (80%) among products olive oil (74%) and canola oil (58%). In addition, the oleic acid levels is higher than in other nuts as shown in Figure 2.2. The polyunsaturated fatty acid content is low, ranging from 3 – 5 % (Kaijser *et al.*, 2000). Although macadamia nuts are rich in fat, they are generally low in saturated fatty acid (SFA) and high in monounsaturated fatty acid (MUFA). There is evidence that MUFA rich diet can lower the risk of Coronary Heart Disease (CHD) and also has preventive effects on atherosclerosis (Curb *et al.*, 1992). This is believed to result from the particularly high oleic acid content of the nut oil. The popularity of macadamia nuts is increased substantially by processing into snack nuts, candies, confectionery, nut butters and oils.

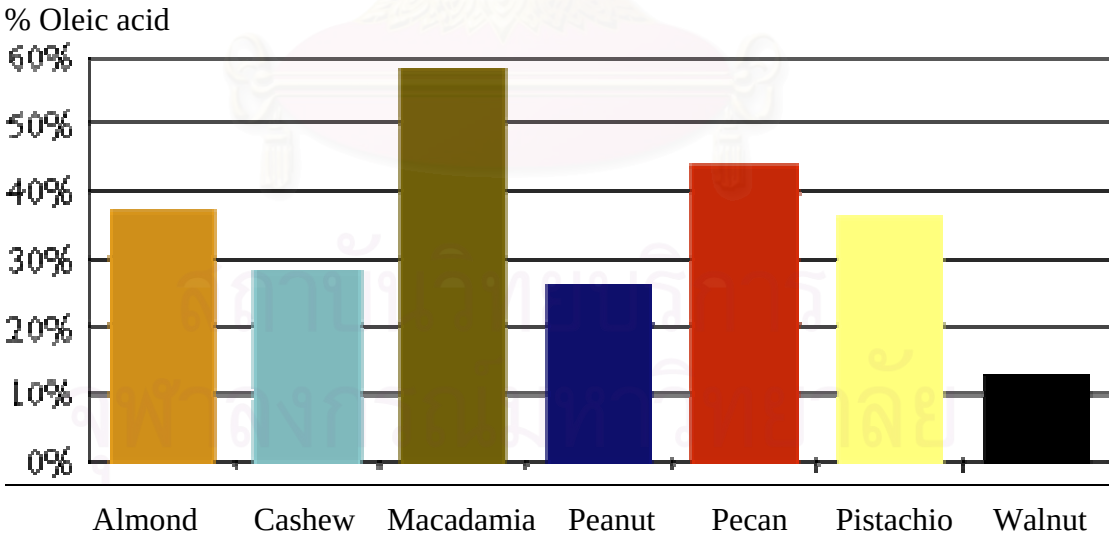


Figure 2.2 Monounsaturated fat content in different nuts

(Australian Macadamia Society, 2006)

However the cultivars, plantation area and weather can have major impact on the oil composition and quality of macadamia nuts as shown in Tables 2.3 and 2.4.

Table 2.3 Fatty acid composition of *Macadamia* spp.

Fatty acid	References (g/100g oil)									
	1	2	3	4	5	6	7	8	Mean*	Range*
C12:0				1.4	0.6				1.0	0.6-1.4
C14:0	0.8	0.7		0.8	0.8	1.0	0.6	1.8	0.8	0.6-1.0
C16:0	7.4	9.1	8.3	8.0	6.2	8.0	9.1	8.4	8.2	6.2-9.3
C16:1 (ω 7)	18.5	21.9	21.8	15.6	19.1	22.0	19.5	29.7	17.7	15.6-22.0
C18:0	2.8	2.2	2.1	3.4	1.6	2.0	3.5	1.7	2.6	1.6-3.5
C18:1 (ω 9)	65.0	59.9	56.4	64.2	67.1	60.0	59.3	47.3	61.9	59.3-67.1
C18:1 (ω 11)								3.1		
C18:2 (ω 6)	1.5	1.9	2.8	1.6	1.3	2.0	1.9	3.0	1.8	1.2-2.0
C18:3 (ω 3)						2.0		0.17		nd-2.0
C20:0	1.9	1.8	2.4	2.4	1.6	2.0	2.7	1.2	2.1	1.6-2.7
C20:1 (ω 9)	2.3	2.0	3.1	2.1	1.7	1.0	2.5	2.1	2.1	1.0-2.5
C22:0			0.8	0.5			0.8	0.4	0.7	0.5-0.8
C22:1 (ω 11)			0.3					0.2		
C24:0			0.5				0.1	0.1		

C12:0 =Lauric acid, C14:0 =Myristic acid, C16:0 =Palmitic acid, C16:1 =Palmitoleic acid, C18:0 =Stearic acid, C18:1 =Oleic acid, C18:2 =Linoleic acid, C18:3 =Linolenic acid, C20:0 =Arachidic acid, C20:1=Eicosenic acid, C22:0= Behenic acid , C22:1= Erucic acid, C24:0= Linoceric acid and nd = not detected

* Values not calculated on C18:3ω 3, C22:1ω11 and C24:0, excluding value of *M. tetraphylla* spp (8).

1. Caveletto *et al.* (1966) *M. integrifolia*
2. Saleeb *et al*. (1973) *M. integrifolia*
3. Beuchat and Worthington (1978) *M. tetraphylla*
4. Macfarlane and Harris (1981) *M. integrifolia* (1st Grade Kernel)
5. Cavaletto *et al.* (1983) *M. integrifolia*
6. McConachie (2001) *M. integrifolia*
7. Mason *et al.* (1998) *M. integrifolia* (cultivar 246)
8. Kaijser *et al.* (2000) *M. tetraphylla* (cultivar Jordan)

Table 2.4 The fatty acid composition of *Macadamia* spp. from Doi Saked District, Chiang Mai, Thailand

Fatty acid	Reference (g/100g oil)							
	1	2	3	4	5	6	Mean	Range
C12:0	0.06	0.04	0.06	0.06	0.04	0.05	0.05	0.04-0.06
C14:0	0.58	0.36	0.38	0.51	0.34	0.43	0.43	0.34-0.58
C16:0	8.07	7.12	7.05	7.69	7.55	7.32	7.47	7.05-8.07
C16:1 (ω 7)	16.52	16.35	15.11	14.87	13.83	14.24	15.15	13.83-16.52
C18:0	3.59	4.27	3.82	3.76	3.99	3.96	3.90	3.59-4.27
C18:1 (ω 9)	62.46	62.68	53.60	64.64	64.89	65.44	62.29	53.6-65.44
C18:1 (ω 7)	-	-	-	-	-	-	-	-
C18:2 (ω 6)	1.41	1.44	1.74	1.46	1.55	1.40	0.33	0.33-1.74
C18:3 (ω 3)	0.10	0.08	0.12	0.11	0.11	0.13	0.11	0.08-0.13
C20:0	3.02	3.33	3.32	3.02	3.31	3.17	3.20	3.02-3.33
C20:1 (ω 9)	2.49	2.69	2.95	2.47	2.69	2.46	2.63	2.46-2.95
C22:0	0.92	0.86	0.97	0.84	0.89	0.79	0.88	0.79-0.97
C22:1	0.24	0.26	0.31	0.19	0.24	0.22	0.24	0.19-0.31
C24:0	0.39	0.30	0.35	0.29	0.34	0.31	1.50	0.29-0.39

Source : Kungsadanaumpai *et al*., (2006)

C12:0 =Lauric acid, C14:0 =Myristic acid, C16:0 =Palmitic acid, C16:1 =Palmitoleic acid, C18:0 =Stearic acid, C18:1 =Oleic acid, C18:2 =Linoleic acid, C18:3 =Linolenic acid, C20:0= Arachidic acid, C20:1= Eicosenic acid, C22:0= Behenic acid, C22:1= Erucic acid and C24:0= Linoceric acid

1. Doi Saked Number 344
2. Doi Saked Number 800
3. Pongyang Number 344
4. Pongyang Number 508
5. Pongyang Number 660
6. Pongyang Number 741

The result showed that the fatty acid composition in macadamia oil involved 10 to 13 fatty acids from C12:0 to C24:0. The average proportion of saturated, unsaturated and polyunsaturated fatty acids from Tables 2.3 and 2.4, respectively can be calculated as followed:

Saturated oil 15.42 g /100g, 17.43 g /100g

Monounsaturated oil 81.7 g /100g, 80.66 g/100g

Polyunsaturated oil 3.88 g /100g, 0.44g/100g

An average ratio of unsaturated to saturated fatty acids is 5.54:1, 4.43:1.

Generally, the higher the ratio between unsaturated to saturated fatty acids, the more susceptible to oxidation due to increased oxidation potential of unsaturated fatty acids (Frankel, 1998). However, a high ratio is nutritionally beneficial, as this indicates a higher proportion of unsaturated fatty acids which have been shown to reduce the risk of some cancers and heart disease. Further, a diet rich in monounstaurated fatty acids has been shown to decrease serum cholesterol, low density lipoprotein cholesterol and triacylglycerols (Colquhoun *et al*., 1996). Macadamia oil has attracted most research attention. Changes of fatty acid compositions or oil content determine the quality of macadamia nut.

During the processes of drying and roasting, heat is supplied to the nut and that stimulates lipid oxidation which reduces the quality of the nut. Therefore, it is important to be able to control the oxidation process occur during drying, roasting and storage. Thus, a study of changes of fatty acids during macadamia nut processing should be carried out.

2.4. Market and production

Macadamia nut has a high nutritional value due to the large quantity of oleic acid. In addition macadamia nuts are hard, non-perishable and can be transported for long distances. The amount of macadamia nuts in terms of value added and marketing at the processor level in Australia is shown in Figure 2.3 and World macadamia nuts production is shown in Figure 2.4**.**

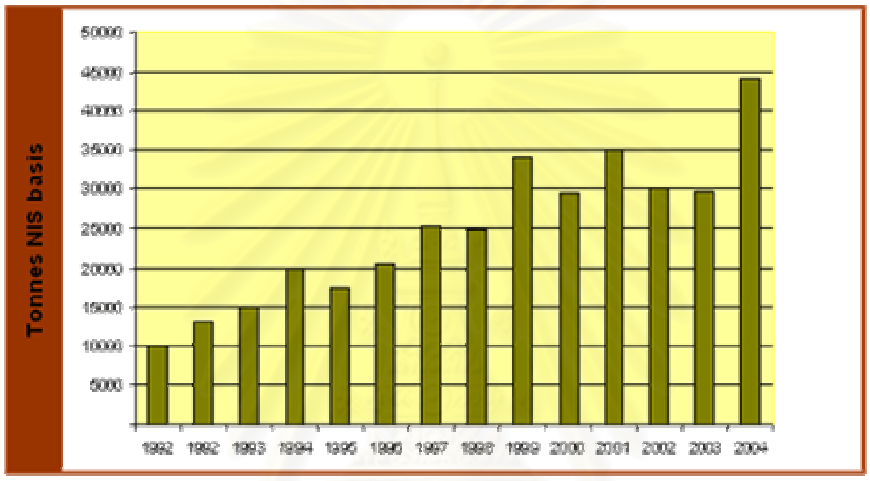


Figure 2.3 Australian macadamia nut production (Nambucca Macnuts Pty Ltd, 2006)

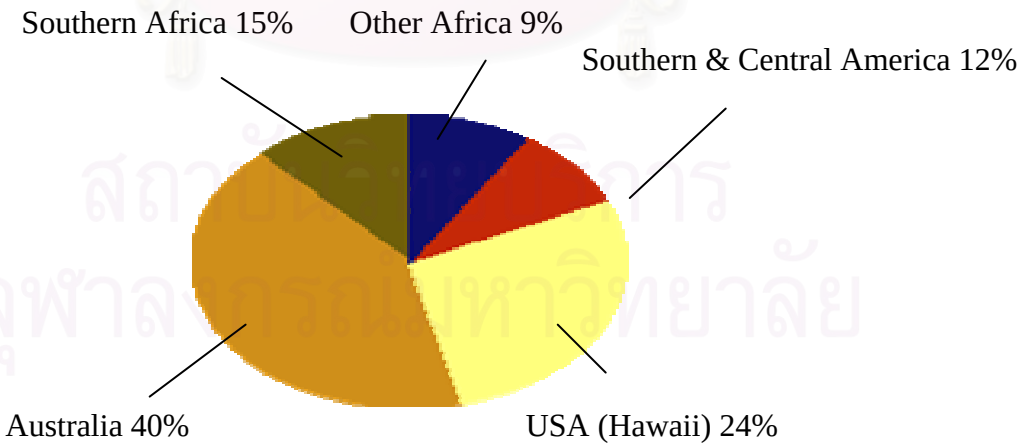


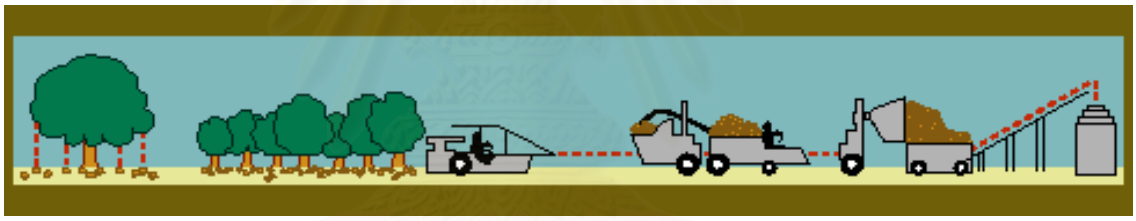
Figure 2.4 Macadamia World Production 2004 (USDA, 2004)

It is currently estimated that the Australian industry has about 3,250,000 trees covering an area of 120,000 hectares, of which 98 % are of the commercially preferred *M. integrifolia* species. Approximately 80 % of current production is based on Hawaiian cultivars with the remainder being new Australian releases. The age of the trees range from newly planted to in excess of 20 years old, with approximately 45 percent being mature, 30 percent in the early bearing stage and 25 percent not yet bearing (Anon, 2000).

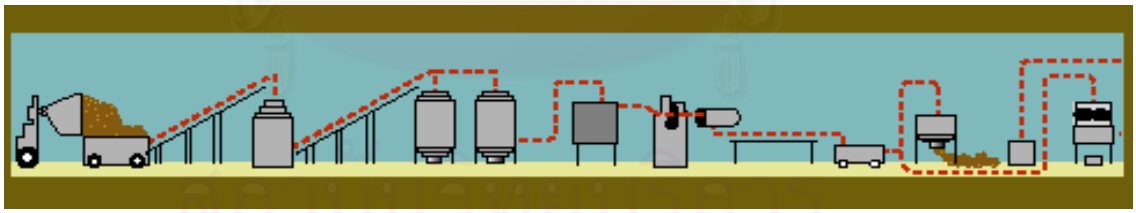
Hawaii established a macadamia processing industry in the 1950s which produces about 15,000 tonnes of nut in shell from 4,000 hectares (Leverington, 1958).

2.5 Processing

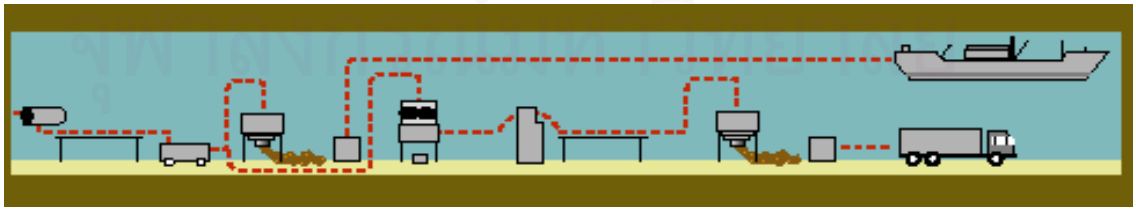
Steps of processing in macadamia nut in general are shown in Figure 2.5.



a **b** **c**



d **e** **f**



g **h**

Figure 2.5 Production stages of macadamia from farm to factory (Australian Macadamia Society, 2006)

a = Nuts fall, b = Harvesting, c = Dehusking, d = Pre-drying, e = Drying
f = Cracking and Sorting, g = Grading and h = Roasting and Packaging

2.5.1 Nuts fall

When the macadamia nuts become mature, they fall to the ground where they are gathered and harvested. Generally, the grasses planted around the macadamia nut trees (Figure 2.6) resulted in easy picking up by mechanical harvesting equipment as described in 2.5.2.

2.5.2 Harvesting

In Australia, the harvesting time usually extends from March to June as not all nuts drop at the one time (Anon, 2000). The macadamia nuts are gathered and harvested either mechanically or manually. Mechanical harvesting involves mechanical blowers and sweepers (Figure 2.7) and nuts are collected by the harvesting machine (Figure 2.8), while manual harvesting involves picking nut by hand. Although manual harvesting is time consuming and involves high labour costs, mechanical harvesting equipment is expensive and maintenance costs are high. However, both manual and mechanical harvesting are used in current commercial practice, depending on the size of the orchard or farm (Vivien, 1989).

Figure 2.6 Macadamia nut plantation

Figure 2.7 Macadamia nut sweeper and blower machine

Figure 2.8 Macadamia nuts harvesting machine

2.5.3 Dehusking

Freshly harvested macadamia nuts can have a moisture content up to 30% in the husk alone, and 25% in the rest of the nuts (Grimwood, 1971). Thus, the fibrous outer husk of the macadamia is removed within 24 hours (Figure 2.9) after harvesting

to reduce heat respiration and facilitate drying. Mechanical screw has also been used for dehusking. Furthermore, the high water content in the husk leads to mould development, especially during prolonged storage. The husk material is usually recycled as organic mulch leaving the “Nut in Shell” (NIS) to size grading machine (Figure 2.10).

The NIS is graded by diameter. Generally NIS should have diameter more than 18 mm. Smaller and defective NIS are sorted and discarded.

Figure 2.9 Dehusking machine of macadamia nuts

Figure 2.10 The size grading of macadamia nuts in shell

The NIS is furthered graded by floating in water (Figure 2.11) to determine specific gravity (SG) and graded when SG =1. Maturity and moisture content of NIS influence SG by making nut float or sink in water. The membrane between shell and kernel indicated maturity of macadamia nuts. The NIS may float on water, resulting from immature and low moisture content (10%wb). The food quality nut is the sinking one. It is then sent to an in-store dryer to reduce the moisture content to 9-10% wb before transfer to the factory. The floating one are removed and transferred to another silo for rewetting until the moisture content up to 14% wb. NIS is then returned to the water bath for floating. The second floated nut will be considered as immature nut and will be discarded.

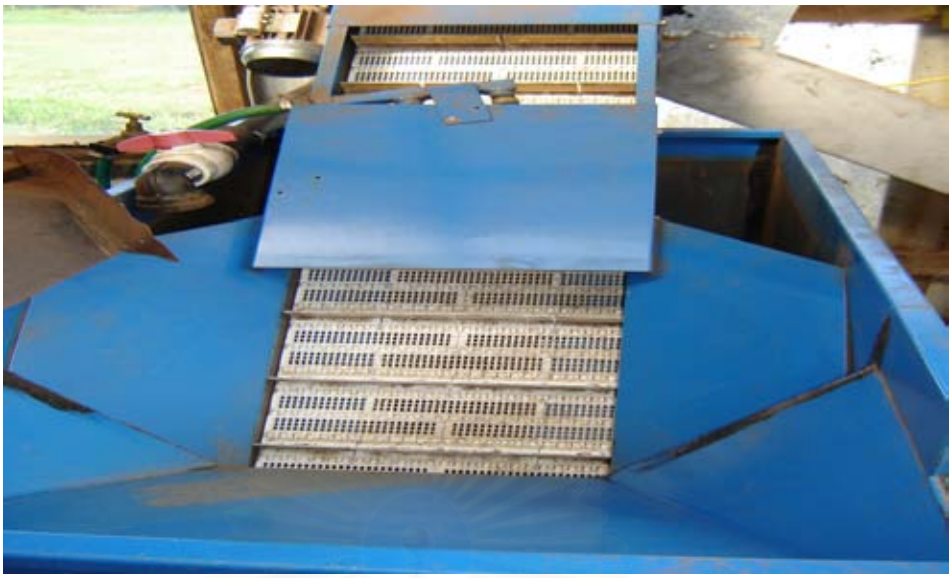


Figure 2.11 The nut in shell grading by floating

2.5.4 Pre-drying

This stage is aimed to reduce moisture content of fresh NIS to 9-10%wb. Drying to 10% wb with in store dryer (Figure 2.12) can take up to 6 weeks, depending on the initial moisture content and weather condition during drying. Cavaletto *et al*. (1968) suggested that 10% wb is acceptable for nut stability for transportation and short period storage prior to transfer to factory.

Figure 2.12 In store dryer

2.5.5 Drying

2.5.5.1 Drying in factory

Drying is a critical step in macadamia processing to maximise shelf life and quality of the end product. At harvest the nuts have a moisture content up to 30%wb. Drying can take up to three weeks and reduces the moisture content to around 1.5%wb. Long period of drying affects the quality of macadamia nut as will be described in 2.6.1. The kernel shrinks away from the inside of the shell and allows the shells to be cracked with minimal damage to the kernel.

Mason and Wills (2000) reported that drying is one of the most important stages in the processing of macadamia nuts. It is mandatory that the drying operation be started soon after harvesting to prevent any hydrolytic rancidity or mould

development. The same authors stated that kernels with a moisture content of about 1.5% db can be stored for up to one year without significant losses in quality.

2.5.5.2 Techniques of drying macadamia nut

The industrial drying process currently employed for macadamia nuts has the disadvantage of requiring a very long total drying cycle, extending for a period of more than one month. The first stage from pre-drying on farm described in 2.5.4 takes three to four weeks. After that, six days (144 hrs) are used for the second stage which generally uses the hot air convection starting at 40°C and finishing at 60°C until the kernel moisture is down to 1.5%wb (Silva *et al*., 2005). Therefore, the technique for drying macadamia nuts contributed to time saving and preserving its natural quality as compared to the quality obtained using the conventional drying process. The application of microwave to assist the hot air drying process by using hot air for drying fresh macadamia nut down to 10%wb and then using a microwave apparatus to dry the nuts further (Figure 2.13) until the moisture content is down to 1.5%wb was investigated by Silva *et al*., (2005).

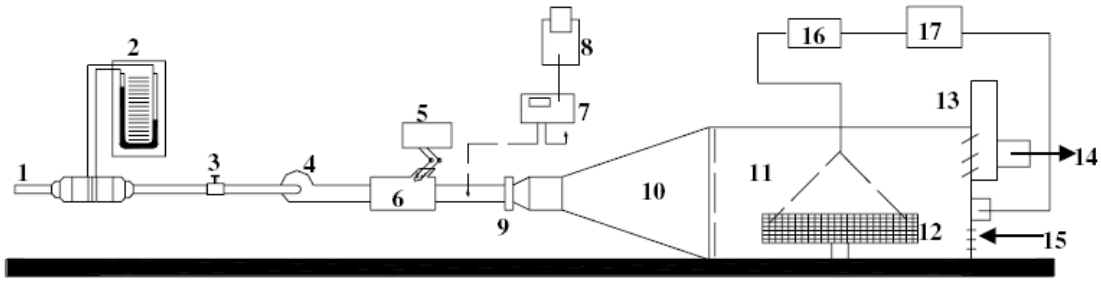


Figure 2.13 Microwave oven (Silva *et al*., 2005)

(1) air inlet; (2) pressure meter; (3) valve; (4) fan; (5) temperature controller; (6) electric heater; (7) two channel temperature meter; (8) two channel temperature recorder; (9) quick clamp connector; (10) air diffuser; (11) microwave cavity; (12) sample baskets; (13) exhausting window; (14); air outlet; (15) cooling air of the microwave generator; (16) infrared sensor signal; (17) set point temperature on the controller to switch the magnetron on/off.

The result showed that the combination of two methods reduced the drying time and increased the industrial yield and quality of the kernels as compared to those from conventional processes. The quality of the kernels was controlled for a period of six months after processing by determining the peroxide values, free fatty acid percentages and sensory acceptance evaluations. The product was very well accepted in the sensory evaluation.

Heat pump drying has main advantages in terms of energy recovery from the exhaust and ability to control temperature and humidity independently. Figure 2.14 shows a heat pump drying system. The inlet drying air passes through the drying chamber at point (1) and picks up moisture from the product. The moist air at point (2) is directed to an evaporator. The refrigerant changes from liquid to vapour in a direct expansion coil in order to cool and dehumidify. The compressor takes cold and low pressure refrigerant gas from the evaporator and compresses it to deliver hot and high

pressure gas to the condenser (from (3) to (4)). Heat is absorbed from the hot gas in the condenser by air passing through the condenser. The hot and high pressure gas is thus condensed to a hot and high pressure liquid. The hot and high pressure liquid is passed through an expansion valve and into the evaporator where, at low pressure and temperature, it evaporates to form cold and low pressure refrigerant gas, which passes back to the compressor hence completing the cycle. Heat is rejected from the refrigerant to the air of drying system by the condenser (from (4) to (1)) and is absorbed by the refrigerant from the air of drying system by the evaporator (from (2) to (3)). The warm and dry air leaving the condenser is circulated through the product being dried by a fan and evaporates moisture from the product (Chua *et al.*, 2002).

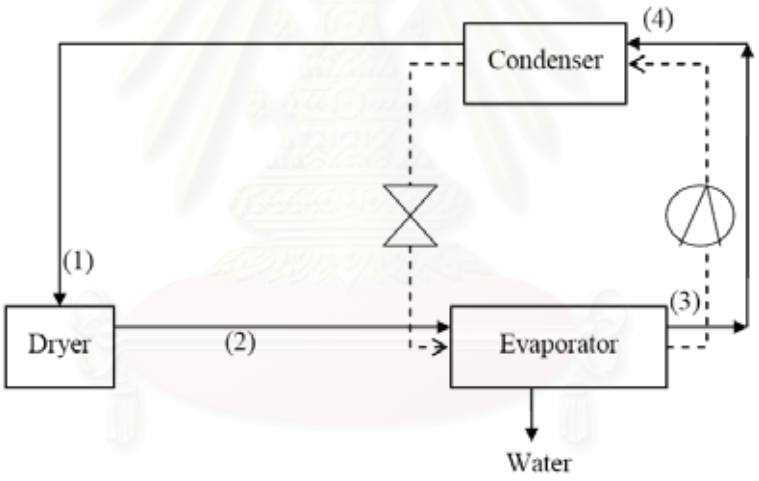


Figure 2.14 Schematic representation of general heat pump drying system

____, air path
------, refrigerant path

 Compressor

 Expansion value

Source: Adapted from Chua et al. (2002)

Heat pump dryers have many advantages. Higher energy efficiency with improved heat recovery result in lower energy consumed for each unit of water

removed. Better product quality is achieved with controlled temperature profile to meet product requirements. A wide range of drying conditions typically from -20°C to 100°C (with auxiliary heating) is feasible and determining the output of the product. Hence, there is a potential of using heat pump for drying macadamia. Many researches on heat pump drying of fruits and vegetable showed that heat pump dryer is able to preserve quality of product (Sunthonvit, 2005).

Sunthonvit (2005) studied the effects of different drying strategies on the volatile compounds in nectarines by using a cabinet dryer, tunnel dryer and heat pump dryer. The result showed that overall, the heat pump dryer was the best of the three types of dryers in terms of preservation of the fruit aroma.

2.5.6 Cracking and grading

The 1.5% wb dried macadamia nuts were cracked by the various methods. Cracked shells and kernels are shown in Figure 2.15 or their fragments must be separated. Usually separation is done by vibrating screens with or without blowers, which separate the nuts into whole or half kernels, broken kernels or chip. Mouldy or insect damaged kernels are removed by hand sorting (Leverington, 1971).

Typically, modern machines have been developed to crack the hard shell of the macadamia without damaging the precious kernel within. This results in the highest value of the kernels macadamia nuts as a whole nut. Whole macadamia kernel is graded into “Styles” numbered from 0 to 7 which represent the sizes of the kernel pieces. Style 0 is large whole kernel, style 2 is a mixture of wholes and halves, style 4 is primarily half kernels and higher numbers relate to various sizes of chips and small pieces. Once macadamia nut is sorted into styles, the kernels are vacuum packed into poly lined, foil bags then into cartons ready for sale to manufacturers of various value

added products such as chocolates, biscuits, snack packs and ice cream (Australian Macadamia Society, 2006).

Figure 2.15 Cracking the nuts from shell

2.5.7 Roasting and packaging

Moltzau and Ripperton (1939) advised that macadamia nuts should be stored at a moisture content of 1.5% wb or below before roasting, to provide the best quality product. High moisture kernels have a soft texture and brown more rapidly during roasting (Cavaletto et al., 1980). Dela *et al*. (1966) recommended that the moisture of the raw kernels be reduced to approximately to 1.5% wb before roasting in order to have a moisture content of 1% wb after roasting, since this moisture content proved to result in the most stable product. The lower the moisture content and storage temperature, the longer the shelf-life. Macadamia nuts should be packed as soon as possible after roasting to prevent absorption of moisture (Grimwood, 1971). Macadamia kernel is generally supplied in a nitrogen flushed, vacuum sealed laminated foil package to reduce oxygen concentration and prevent oxidative rancidity

ensuring a long storage life. Macadamias sold through retail outlets are packed in laminated foil pouches, metal cans, glass, PET jars or plastic film packs with a high oxygen barrier (Nambucca Macnuts Pty Ltd , 2006).

2.6 Quality

2.6.1 Moisture Content

Cavaletto *et al*. (1966) found that the most important factor for macadamia nut stability was moisture, and the kernel must be dried to approximately 1% moisture,
In fact, kernels of 1% moisture were the most stable. Dela *et al*. 1966 supported that because the enzymatic reactions responsible for kernel determination are dependent on moisture content, and at low moisture content, as recommended to prevent enzymatic reaction to take place to any significant degree during storage for more than one month under sealed airtight conditions.

Leverington (1971) proposed that drying the macadamia nut to a moisture content of 1.5 %wb would extend shelf life of nut for long period of time. Woodroof (1979) reported that the stability of the kernels decreased with increasing moisture and increasing storage temperature. At moisture of 1.4% wb only very small changes in flavour and chemical composition occurred after 18 months storage at room temperature. At higher moisture level and storage temperature, total sugars decrease and reducing sugar and free fatty acid increase as the moisture content increase to 1.5% to 4.5%. A temperature of -17.8°C was reported to maintain quality of macadamia nuts for 18 months. Light apparently has no effect on stability of raw or roasted macadamia kernels.

Colour and flavour of raw nuts can be maintained for 18 months at room temperature and moisture content of 1.4%wb. Storage at -17.8°C is recommended for moisture content of 4.3%wb.

2.6.2 Water activity

The water activity (a_w) of products is a measure of the water available for microbial growth, chemical, biochemical changes and is indirectly related to moisture content. Usually, the lower the a_w, the more stable a product will be during storage from a microbiological point of view.

Labuza (1971) suggested that the rate of lipid oxidation is high at low water activities (a_w = 0.1-0.2), then decreases as water activity increases (0.2-0.4), and then increases again above 0.4. Therefore, to incur oxidative rancidity in macadamias, a kernel a_w of above 0.3 to 0.4 is required. In addition, lipolytic enzymes must be present and active at these water activities. A a_w of 0.3 is equal to approximately 3.0% kernel moisture (Kowitz, 2000, cited by Himstedt, 2002).

2.6.3 Temperature

Storage temperature is very important for macadamia quality. The lower the storage temperature, the longer the shelf life of the products (Cavaletto *et al.*,1966). At a temperature of 1.5°C for vacuum packed raw kernels (2.3%wb) the shelflife was up to 16 months but storage at 37°C resulted in rapid deterioration of kernel quality and storage life of less than 8 months. Increasing storage temperature resulted in increasing darkening of the kernels during storage. Higher storage temperature and increasing moisture contents also resulted in less total sugars, but increasing reducing

sugar contents. The free fatty acids also increased with temperature and moisture content.

2.6.4 Grading

Oil grading process involves submersion of kernels in solutions with a known specific gravity (SG). The flotation behaviour of kernels in each of these solutions indicates the total oil content. Kernels that float in water (SG $\leq$ 1.000) are normally separated as a first or premium grade and sold for accordingly increased prices, compared with kernels that sink in water. These kernels that float in water have an oil content of 73 g /100 g or higher (Mason, 1982). Macadamias can also be submersed into other modified SG solutions to further separate kernels into more selective oil grade categories (such as those that contain 76 g /100 g oil or more, or those that contain between 67 g and 73 g /100g oil). For Australian industry wet processing is not applied to grade kernels (Anon, 2000). It is anticipated that indirect measuring techniques such as NIR offer substantial advantage over SG solutions to grade kernels. However, no information has been documented regarding the use of NIR for grading macadamias.

2.6.5 Sugar and Colour

Sucrose can undergo hydrolysis to yield glucose and fructose for Maillard reaction substrate resulting in browning of macadamia nuts (Wall and Gentry, 2006). Prichavudhi and Yamamoto (1987) investigated the effects of drying conditions on the chemical composition and quality of macadamia nuts. The result showed that the reducing sugar decreased during drying and kernel centers darkened slightly. This indicates that any glucose and fructose present in fresh kernels were used in browning

reactions during drying. However, the incremental drying process limited sucrose hydrolysis, minimizing the amount of glucose and fructose available for Maillard reactions. Therefore, the internal colour of dried kernels was only slightly darker than fresh kernels. After roasting, centers of roasted kernels were not darker than dried kernels.

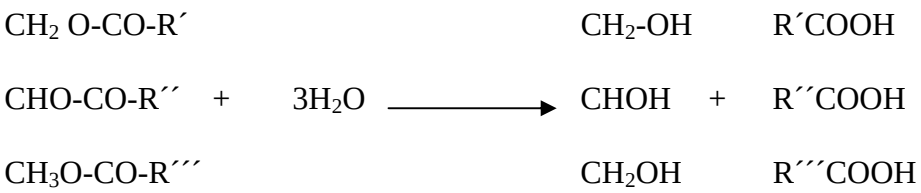
Wall and Gentry (2006) illustrated that the variability in sugar composition in fresh kernels had a minimal impact on colour quality when drying and roasting conditions were well controlled. Therefore, content of reducing sugar plays a significant role in colour quality under commercial processing conditions.

2.6.6 Rancidity

Rancidity is defined as the development of an off flavour in food containing fat. Rancidity is a very complex reaction and produced by either lipolysis or hydrolytic and oxidative reactions (Himstedt, 2002).

2.6.6.1 Lipolysis or Hydrolytic Rancidity

Hydrolysis occurs when fats and oils react with water and hydrolyse some of the fatty acids from the triacylglyderols as described in 2.6.6.2. The reaction promotes the formation of free fatty acids and consequently leads to a decrease in storage stability of fats and oils. Complete hydrolysis reaction of hydrolysis of a triglyceride is demonstrated as follows:



Where R´, R´´, R´´´ represent the same or many different hydrocarbon chains.

Free fatty acids help to increase the solubility of water in oil and promote further hydrolysis reactions. Therefore, hydrolysis which produces free fatty acid becomes autocatalytic (Himstedt, 2002).

Hydrolysis is encouraged by moisture and heat. Macadamia nuts become most susceptible to hydrolysis when stored at high moisture and temperature. Cavaletto *et al*. (1966) found that increasing content of free fatty acids in kernels happened at higher moisture content and higher storage temperature. Kernels of 2.3% wb and 4.3% wb at ambient temperature exhibited particularly large increase in free fatty acids as compared with nuts at 1% wb at ambient temperature and stored at -17.8°C.

2.6.6.2 Free Fatty Acid

The measurement of free fatty acids (usually expressed as a percent of oleic acid) in an oil provides an indication of the degree of hydrolytic rancidity. Free fatty acid content is not a good indicator for predicting flavour changes. However, oxidation is substantially increased when fatty acids are unattached to the triglyceride molecule. The technique is useful for evaluating the likely susceptibility of an oil undergoing subsequent oxidative deterioration (Lawson, 1995). Furthermore, as the hydrolysis process is stimulated in foods via storage at high moisture or temperatures, determination of the free fatty acid could provide information on quality and stability of macadamia.

2.6.6.3 Oxidative Rancidity

Oxidation is initiated by the presence of oxygen. This process is commonly referred as autoxidation (Frankel, 1998). Autoxidation is a series of free radical reactions initiated and propagated by free radicals reacting with the $-CH_2$ groups that are adjacent to double bonds. These free radicals (or unpaired electrons) are extremely unstable and reactive. The free radical theory of oxidation involves a three stage series of reactions that include the following:

1. Initation

The initiation reaction produces a small number of extremely reactive free radicals from the fatty acid molecule. The following formula depicts the process:

$$RH \xrightarrow[\text{Initiators}]{} R^{\bullet} + H^{\bullet} \qquad \text{Reaction 1}$$

In this reaction, a hydrogen radical ($H^{\bullet}$) and a lipid free radical are produced (otherwise known as alkyl radicals) ($R^{\bullet}$) in the presence of initiators such as heat, light, metals and oxygen.

2. Propagation

In the presence of oxygen, the alkyl radical rapidly reacts to form the peroxy radical ($ROO^{\bullet}$) (Reaction 2). This peroxy radical in turn reacts with more unsaturated lipids (RH) to form hydroperoxides (ROOH) (Reaction 3). Alkoxy radicals ($RO^{\bullet}$) can also remove the hydrogen bound to the unsaturated lipids to form further lipid free radicals (Reaction 4). During this stage, the level of free radical formation reaches a level where a chain reaction is sustained (Frankel, 1998). The fundamental primary products of this oxidation process are hydroperoxides (ROOH)

formation (Reaction 3). This reaction is slow, and hydrogen abstraction becomes selective for the most weakly bound hydrogen (Frankel, 1998). Hence, the rate of autoxidation increases with the degree of unsaturation. On the basis of oxygen uptake, linoleate has been shown to be approximately 40 times more reactive than the ester of oleic acid, and linolenate is approximately 100 times more reactive than oleate and 2.4 times more reactive than linoleate (Frankel, 1998).

$$R^{\bullet} + O_2 \longrightarrow ROO^{\bullet} \qquad \text{Reaction 2}$$

$$ROO^{\bullet} + RH \longrightarrow ROOH + R^{\bullet} \qquad \text{Reaction 3}$$

$$RO^{\bullet} + RH \longrightarrow R^{\bullet} + ROH \qquad \text{Reaction 4}$$

3. Termination

This stage occurs when the free radicals reach sufficiently high concentrations, they tend to react together in termination reactions. At this stage, the free radical (breakdown) reactions have ended and the end products are stable. At low oxygen pressures and elevated temperatures, alkyl and alkoxy radicals can combine to form carbon linked dimers (Reaction 5) and ether containing dimers (Reaction 6). At low temperatures, peroxy radicals can condense and combine to form peroxy-linked dimers and oxygen (Reaction 7) (Frankel, 1998).

$$R^{\bullet} + R^{\bullet} \longrightarrow RR \qquad \text{Reaction 5}$$

$$R^{\bullet} + RO^{\bullet} \longrightarrow ROR \qquad \text{Reaction 6}$$

$$ROO^{\bullet} + ROO^{\bullet} \longrightarrow ROOR + O_2 \qquad \text{Reaction 7}$$

Hydroperoxides are the major initial reaction products of fatty acids from autoxidation. In the presence of heat, moisture, oxygen and even at ambient temperature, hydroperoxides easily break down and produce a variety of hydrocarbons, aldehydes and ketones. These oxidation products are responsible for the flavour and odour in rancid fatty acids. (Petterson, 1989).

2.6.6.4 Peroxide Value

The most common method used for measure degree of oxidation is peroxide value. The value indicates the level of peroxides in a fat or oil that have developed as a result of oxidation. Gunstone and Norris (1983) showed that changes in peroxide levels can be used to predict rancidity in nuts instead of using a trained sensory panel.

Kaijser *et al*. (2000) used the peroxide value to indicate the oxidative of oil from four cultivars of New Zealand macadamia. The results showed that the polyunsaturated fatty acid in GT 207 cultivar had the highest level of peroxides. However, the macadamia nuts had very low concentration of polyunsaturated fatty acid. Thus, generally the levels of peroxides in macadamia oil are quite low.

2.6.7 Volatile Compounds

2.6.7.1 Volatiles compounds in macadamia nuts

There are only few studies on volatile compounds in macadamia nuts, among the work by Crain and Tang (1975) and Chitundu (1994).

Crain and Tang (1975) have evaluated volatile components of roasted macadamia nuts. They used both head space analysis and fractionation into neutral and basic subfraction. Of the 41 compounds identified by the two techniques, it was found that the basic compositions of the volatile substances consist of a number of pyrazines which are common in heat-treated foods whereas most of the compounds identified in the neutral fraction come from the autoxidation of unsaturated lipids. Moreover, they have found that methyl sulfide as shown in Table 2.5 is the major component among the highly volatile compounds found in macadamia nuts. Even though it is present in lesser quantities, it may play an important role in determining overall flavour.

Table 2.5 Compounds identified from the headspace analysis of roasted macadamias and other nuts.

Compound found in macadamias	Size of peak[a]	Present in peanuts[b]	Present in hazelnuts[c]	Present in pecans[d]
Headspace				
n-Hexane	S		X	
n-Heptane	M		X	
Methyl Sulphide	L			
n-Octane	M		X	
2-methylpropanol	L	X	X	
Methylfuran	S		X	
2-methylbutanal	L	X	X	
3-methylbutanal	L		X	
Benzene [d]	S	X	X	
Toluene [d]	S	X	X	
Methyl Disulphide	S	X	X	
Essence extract-Neutral				
n-hexanal	M	X	X	X
n-heptanal	M	X	X	X
n-pentanol	S	X	X	X
2-pentylfuran	S	X	X	
n-octanal	M	X	X	X
n-hexanol	M	X	X	X
n-nonanal	L	X	X	
n-heptanol	M	X	X	X
2-furfural	S	X	X	X
Benzaldehyde	S	X	X	
n-octanol	M		X	X
Phenylacetaldehyde	L	X	X	
Essence extract-Basic				
2-methylpyrazine	S	X	X	X
2,5-dimethylpyrazine	L	X	X	X
2,3-dimethylpyrazine	S	X	X	X
2-ethyl-5-methyl-pyrazine	S	X	X	X
2,3,5-trimethyl-pyrazine	M	X	X	X
2-ethyl-3,6-dimethyl-pyrazine	L	X	X	X
2,5-diethyl-3-methyl-pyrazine	S		X	

[a] Estimate of peak size relative to other peaks in the same group. (Symbols S, M, L, X indicate small, medium, large peak and present, respectively).
[b] Walradt *et al*. (1971)
[c] Kinlin, *et al*. (1972)
[d] Wang and Oell (1972)

2.6.7.2 Lipid Volatile Formation

Macadamia nuts have high contents of oleic acid and linoleic acid. Therefore, another factor that affects macadamia flavour is their lipid oxidation.

Hydroperoxide, which are a product of lipid oxidation can decompose and lead to aldehyde formation such as hexanal (Figures 2.16 and Table 2.6) that resulted in rancidity and the free radicals.

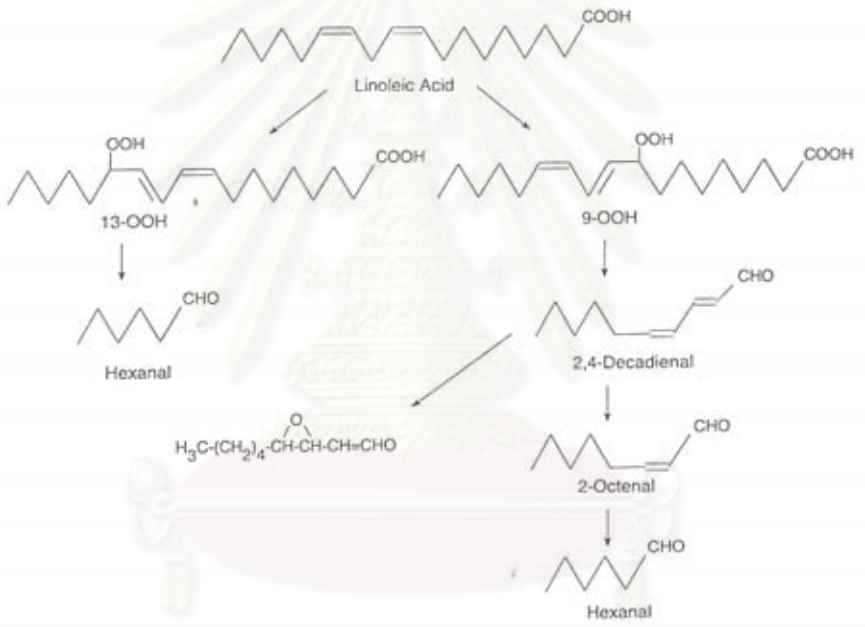


Figure 2.16 Oxidation of linoleic acid and formation of 2-octenal and hexanal from 2,4-decadienal (Shahidi, 2001)

Table 2.6 Some volatile aldehydes obtained from oxidation of unsaturated fatty acids (Himstedt, 2002)

Fatty acid	Hydroperoxide	Aldehyde formed	
		Name	Off-flavour
Oleic acid (18:1)	8-OOH	2-undecenal	
		decanal	
	9-OOH	2-decenal	
		Nonanal	
	10-OOH	Nonanal	
	11-OOH	Octanal	
Linoleic acid (18:2)	9-OOH	2,4-decadienal	fatty, waxy
		3-nonenal	
	11-OOH	2-Octenal	
	13-OOH	hexanal	green
Linolenic acid (18:3)	9-OOH	2,4,7-decatrienal	painty, fishy
		3,6-nonadienal	soapy
	12-OOH	2,4-heptadienal	
		3-hexenal	green bean
	13-OOH	3-hexenal	green bean
	16-OOH	Propanal	
Arachidonic acid (20:4)	8-OOH	2,4,7-tridecartrienal	
		3,6-dodecadienal	
	9-OOH	3,6-dodecadienal	
	11-OOH	2,4-decadienal	
		3-Nonenal	
	12-OOH	3-Nonenal	
	15-OOH	Hexanal	

Chitundu (1993) identified a small quantity of oxidative volatiles from raw macadamia kernels stored for up to 4 months at 30°C. The following compounds were identified as the major volatiles: pentanal, heptane, 4-methylpentan-2-one, pentanol, hexanal, octane, hexanol, heptanol, heptanal, octanal, octanol and nonanal. Also, hexanal was highlighted as a possible measure to indicate rancidity and they conclude that macadamia were deemed rancid at a hexanal concentration of 0.393 ppm in the sample headspace which was compared with sensory testing. Thus, determining hexanal levels was the most accurate method for determining the extent of oxidation in macadamia nut (Himstedt, 2002).

Kinderlerer and Johnson (1992) found the formation of hexanal and octanal from linoleate and oleate in oxidised hazelnuts. Their study showed that the reactions involving oxidation of linoleic acid occur more than the reactions involving oxidation of oleic acid, despite significantly larger quantities of oleic acid present in hazelnut oil. They concluded that the higher levels of linoleic acid can produce a more rancid flavour during storage of macadamia oil.

Therefore, the quality deterioration of macadamia nuts during processing and storage were monitored by the changes of lipid volatiles due to factors such as hexanal.

2.6.7.3 Isolation of volatile compounds

As the volatile compounds in food are complex and present in very small quantities, isolation and separation processes are often difficult. Flavours are usually distributed in food matrices, which can cause problems of artifacts formation and emulsification. Instability of compounds, such as heat degradation, oxidation in air or loss with extreme pH is also considered. There is no universal method for volatiles isolation, thus the selection of methods suitable for each food is important. In general, more than one method is employed which usually gives the best result.

Simultaneous steam distillation and extraction

One of the most popular and valuable techniques in the flavour analysis field is the simultaneous steam distillation and extraction (SDE) first described by Likens and Nickerson (1966). The apparatus used (Figure 2.17) provides for the simultaneous condensation of the steam distillate and of an immiscible organic solvent. Both liquids are continuously recycled, and thus the steam distillable-solvent soluble compounds are transferred from the aqueous phase to the solvent.

The apparatus has a number of advantages, including that the volatile compounds can be concentrated thousand fold in aqueous media in a single operation. Also, only a small quantity of organic solvent is used, minimising the possibility of artifact buildup as the solvent is concentrated. Likens-Nickerson apparatus is simple and convenient to use, with a high recovery of aroma compounds. It can be used under either atmospheric steam distillation condition or under reduced pressure to reduce thermal decomposition (Parliment, 1997).

Simultaneous steam distillation and extraction involves a thermal effect which contributes to some degree of degradation of compounds of interest. Nevertheless, it has been employed by most research groups regardless of the effect.

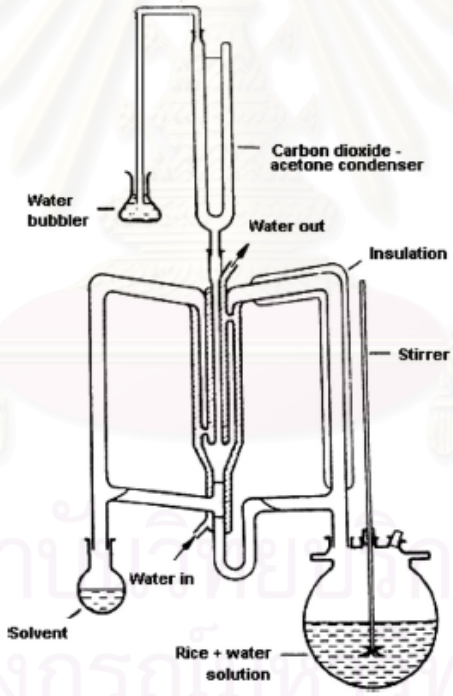


Figure 2.17 Likens-Nickerson apparatus (Sunthonvit, 2005)

2.6.7.4 The GC-MS technique (gas chromatography and mass spectrometry)

Gas chromatography/mass spectrometry is an analytical technique that is used for identification of chemical compounds. Due to its high resolution, sensitivity, precision and accuracy along with fast performance, GC-MS has become a popular method for analysis of volatile compounds in food.

GC-MS is the combination of two techniques. As the name implies, it consists of gas chromatography, which is an essential technique for separating a mixture of chemicals into individual components. Another technique is mass spectrometry, which measures the mass to charge ratio of ions and generates a mass spectrum that is a powerful tool for characterisation of separated components.

MS is one of the most specific and sensitive methods available. It is suitable for identifying very small amounts of compounds. It is based on an ionisation of a molecule under vacuum producing a characteristic group of ions of different mass to charge ratio (m/z). The result is shown as a plot of relative intensity of the ions versus their mass to charge ratio (Merritt and Robertson, 1982). Generally, MS data are analysed by computer matching with the spectra in a database. Commercial libraries, such as Wiley provide many individual mass spectra, with multiple entries for a selected compound. Unknown compounds that are not matching those in the library require knowledge of chemistry and experience to interpret (Huston, 1997).

2.6.8 Fatty acids

The results of the high oil content in macadamia nut, namely, the highest contents of monounsaturated fatty acids of all plant materials found in this nut were described in 2.3.2. Changes to fatty acid composition can occur via lipid oxidation.

Furthermore, the monitoring of fatty acids during drying and storage have not found before. Therefore, a study of changes of fatty acids during macadamia nut processing should be undertaken.

The fatty acid composition, or fatty acid profile, of a food product is determined by quantifying the kind and amount of fatty acids that are present, usually by extracting the lipids and analyzing them using capillary gas chromatography. Typically, triacyglycerols and phospholipids are saponified and the fatty acids thus liberated are esterified to form fatty acid methyl ester as described in 2.6.8.1.

2.6.8.1 Derivative preparation of fatty acids

Generally, the polarity of fatty acid is quite low and the higher the molecular weight the greater the difficulty in properly identifying them by gas chromatography. Therefore, the fatty acids were converted into more volatile non-polar derivative forms. In addition, this method is faster, simpler to prepare (2 minutes) and the sample is ready to inject GC-FID. Sodium methoxide is the catalyst in trans esterification (Figure 2.18) to fatty acid methyl ester before injecting to GC-FID (Bannon *et al*., 1982).

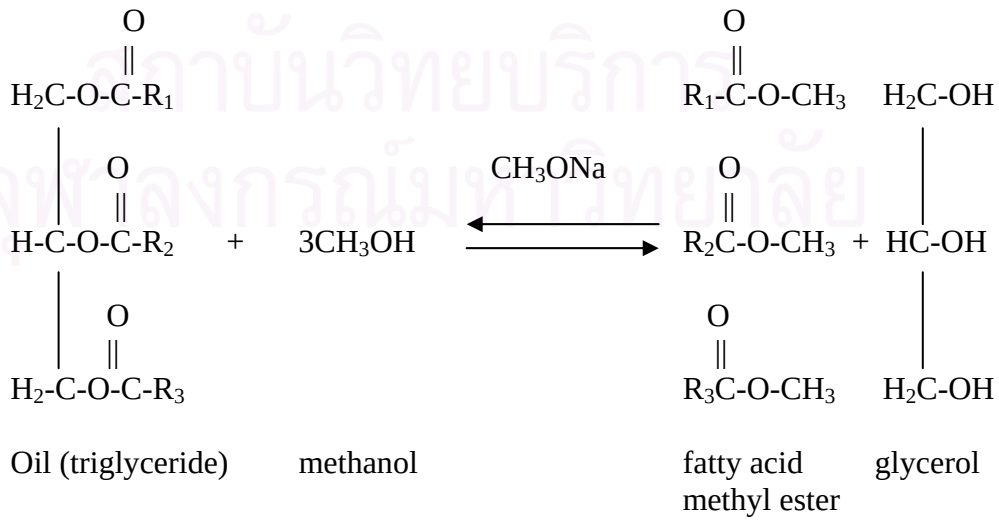


Figure 2.18 The reaction of methylation of fatty acid

2.6.9 Gas chromatography (GC)

The GC principle is based on the distribution of an analyte between a stationary phase which is either solid or liquid, and a mobile gas phase. A sample is introduced into the mobile phase through an injection system. Once the mobile phase moves along the column, the sample components partition between the stationary phase and the mobile phase. This causes a particular analyte either to diffuse in stationary phase or to move along with the carrier gas. The process occurs repeatedly until the analyte emerges from the end of the column. It then enters a detector in which the signal, the strength and duration are measured in relation to the amount and nature of an analyte. The signal is generally passed to a computer by which the chromatogram and further quantification of the analyte is obtained.

2.6.9.1 Carrier gas source

The carrier gas source consists of a high pressure cylinder containing a carrier gas. The carrier gas must be inert in order to prevent interacting with the analytes and other components in GC system. It must be compatible with the detectors and must not be hazardous. Helium is widely used for capillary GC.

The GC system usually equipped with a flow controller and flow meter to obtain a constant flow when there is a situation of change in pressure or pressure drops through the column.

2.6.9.2 GC injection/inlet

The injection chamber is a part that serves as a heating chamber in order to vaporise the sample components. Sample is injected into a heated chamber port and undergoes flash vaporisation. The vaporised sample is then carried by an inert

gas through the column. There are three types of injection technique involved with gas chromatography:

Splitless injection

Splitless injection was developed by Kurt Grob in 1969. It has become the technique predominantly used for trace analysis. At the first 30-90 seconds after injection, the purge valve is kept closed in order to transfer as much of the sample to the column as possible. The carrier gas enters at the top and applies pressure on the volatiles, driving them into the top of the column. Then, the purge valve is re-opened to purge the remaining sample material from the injector and the oven temperature program is initiated. The recommended purge flow rate is 10-20 ml/min. The steps of splitless injection are shown in Figure 2.19..

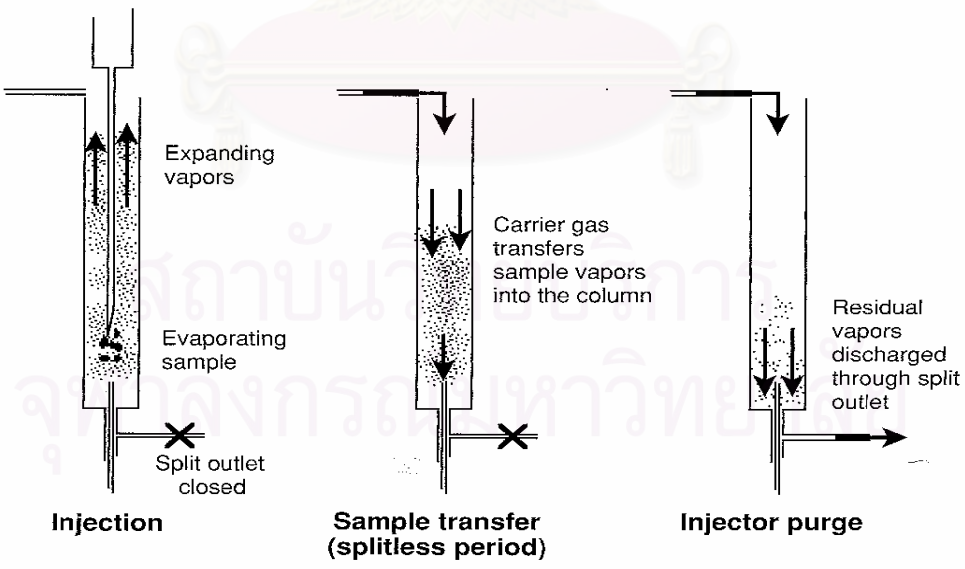


Figure 2.19 Steps of splitless injection (Grob, 2001)

Split injection

Split injection is used for widely differing applications. Two major purposes of splitting are to reduce the amount of material reaching the column and to achieve sharp initial bands. The injected sample undergoes flash vaporization in the injection region and is then split into unequal portions. A small portion from the split enters the column by the flow of carrier gas, while the large portion is vented out of the system through the split exit (Grob, 2001). The ratio of gas flow through the inlet and gas flow through the column for large bore capillary column is 10:1 to 50:1. In a narrow bore column the ratio is generally 50:1 to 500:1. The small fraction of the sample to the column leads to minimal initial solute zone spreading which allows large injected sample volume without overloading the column (Cronin and Caplan, 1987). Basic design of the split injector is shown in Figure 2.20.

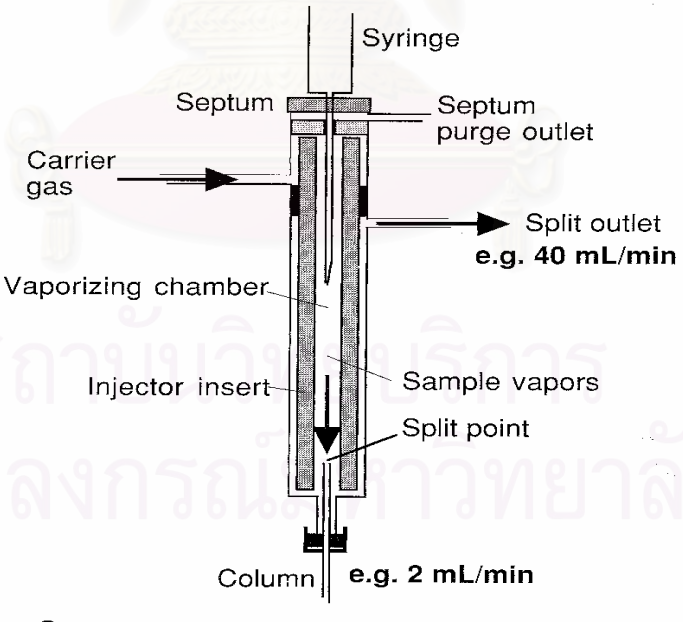


Figure 2.20 Basic design of the split injector (Grob, 2001)

Direct injection

Direct injection involves the use of an injector without a split outlet, allowing neither split injection nor rinsing of the vapourisation chamber after splitless sample transfer. The vapourising chamber is connected directly to the column. The main advantage of direct injection is that the exclusive discharge from the liner into the column ensures complete transfer of vaporised sample into the column.

2.6.9.3 Column

Column of GC can be seen as a long tube with small diameter. It is the most important part of the instrument, as the volatiles components are separated here. Two types of columns are generally classified, capillary or open tubular and packed column. The packed column is 2-4 mm. i.d. and 1-4 m, packed with a suitable adsorbent. The capillary column is smaller in i.d. which ranges from 100-500 µm with a length of 10-100 m. The stationary phase of capillary column is a very thin film, 0.2-1µm, coated on the internal wall of the column (Dickes and Nicholas, 1976).

2.6.9.4 Detector

Generally, a good detector must provide high sensitivity, wide linear range and small cell volume so that the peaks are not distorted. There are three types of detectors that are widely used, including the flame ionisation detector (FID), mass selective detector (MSD) and thermal conductivity detectors (TCD). Among these, FID seems to be the most popular due to its high sensitivity for variety of organic compounds, simplicity and capability of response within a wide range of linearity (Dickes and Nicholas, 1976). However, when the characterisation of the eluted components is required, the MSD is the detector of choice.

FID detector

FID detector is one of the most widely used detectors. This is because of many advantages including the high sensitivity to virtually all organic compounds, the modest changes in flow, pressure, or temperature having a minimal effect on its response characteristics. Furthermore, FID does not respond to common carrier gas impurities such as water or carbon dioxide, has a stable baseline when properly installed and a wide linear range (about 10^8) (Henrich, 1995).

The detector consists of a small hydrogen-air diffusion flame burning at the end of a jet (Figure 2.21). When organic compounds are introduced into the flame from the column effluent, electrical charges are formed. These are collected at an electrode and produce an increase in current proportional to the amount of carbon in the flame. The resulting current is amplified by an electrometer (Henrich, 1995).

The FID is actually a highly specific detector. It detects only compounds that have C-H bonds. As almost all organic compounds have some C-H bonds, it appears to be a general detector.

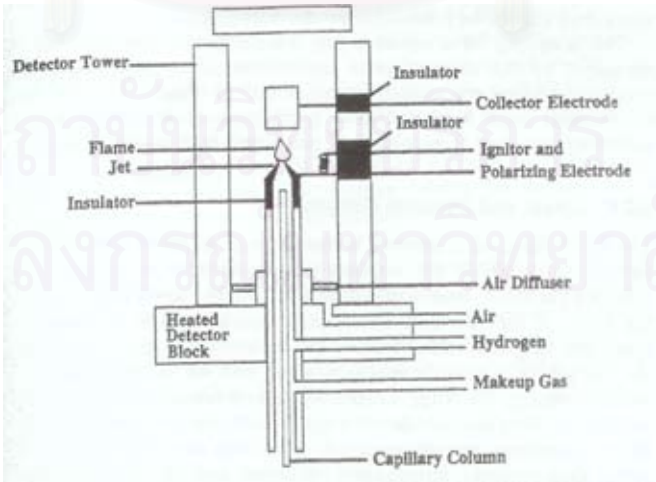


Figure 2.21 Schematic diagram of a flame ionisation detector (Henrich, 1995)

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials and Equipment

3.1.1 Raw materials

The macadamia nuts from Denham Plantation, Knockrow, Northern New South Wales, Sydney. The initial moisture content of macadamia nuts was 10% wb NIS. Fresh product is kept frozen at – 18°C until the further processing.

3.1.2 List of chemicals

Table 3.1 Chemical reagents used in the project

Chemical reagents	Supplier name/grade
Ethanol	Ajax Finechem Analytical grade
Dichloromethane	Lab-Scan HPLC grade
Diethyl ether	Ajax Finechem 99.0% pure
Dry ice pellets (CO_2)	Air Liquide (Botany, Sydney)
n-tetradecane	Sigma 99% pure
Petroleum ether	Ajax Finechem BP: 40-60°C Analytical grade
Phenolphthalein	Asia Pacific Analytical grade
2,2,4-trimethyl-pentane	Mallinckrodt HPLC grade
Methanol	Lab-Scan HPLC grade
Sodium Lumps	Riedel-Dehoeh
Sodium chloride	Ajax Finechem Analytical grade
Sodium hydroxide	Jaeger chemicals
Sodium sulfate anhydrous	Ajax Chemicals 99.0% pure
Acetic acid	APS Analytical grade
Tetradecane	Sigma-Aldrich 99.0% pure

Chemical reagents	Supplier name/grade
Potassium sulphate	Ajax chemical Analytical grade
Potassium dichromate	Ajax chemical Analytical grade
Chloroform	ChromaAR HPLC Grade
Potassium chloride	Ajax Finechem Analytical grade
Methyl red	Fluka, Italy
Methylene blue	Merck, Germany
Sulfuric acid	Merck, Germany
Boric acid	Merck, Germany
Kjeldahl-Tablet	Merck, Germany
Oleic acid	Sigma HPLC Grade
Palmitoleic acid methyl ester	Sigma HPLC Grade
Standard mixture of methyl esters of	
Octanoic acid C 8:0	
Nonanoic acid C9:0	Sigma HPLC Grade
Decanoic acid C10:0	
Undecanoic acid C11:0	
Lauric acid C12:0	
Standard mixture of methyl esters of	
Tridecanoic acid C13:0	
Myristic C14:0	Sigma HPLC Grade
Pentadecanoic acidC15:0	
Palmitic acid C16:0	
Heptadecanoic acid C17:0	
Standard mixture of methyl esters of	
Palmitic acid C16:0	
Stearic acid C18:0	Sigma HPLC Grade
Oleic acid C18:1	
Linoleic acid C18:2	
Linolenic acid C18:3	

Chemical reagents	Supplier name/grade
Standard mixture of methyl esters of	
Stearic acid C18:0	
Nonadecanoic acid C19:0	Sigma HPLC Grade
Arachidic acid C20:0	
Heneicosanoic acid C21:0	
Behenic acid 22:0	

3.1.3 List of equipments

Table 3.2 List of equipment used in this study

Equipment	Specification
Hot air oven	Contherm, Australia
Tunnel dryer	Custom design, UNSW
	Food Engineering, Australia
Heat pump dryer	Greenhalgh, Australia
Colorimeter	Minolta, Japan
Soxhlet apparatus	Labec, Australia
Hot plate	Belle, China
Analytical balance	Mettler H80, PE 3600, Switzerland
Evaporator and rotavapor	Buchi, Switzerland
Digestion unit	Buchi, Switzerland
Distillation unit	Buchi, Switzerland
Plastic sealer	Helix, Australia, Diary farm, Australia
Plastic film	Ethylene vinyl alcohol copolymer (EVOH)
Likens and Nickerson apparatus	UNSW, Australia
GC-MS	Agilent Technologies, USA.
GC-FID	Shimadzu, Japan

3.2 Experimental Design

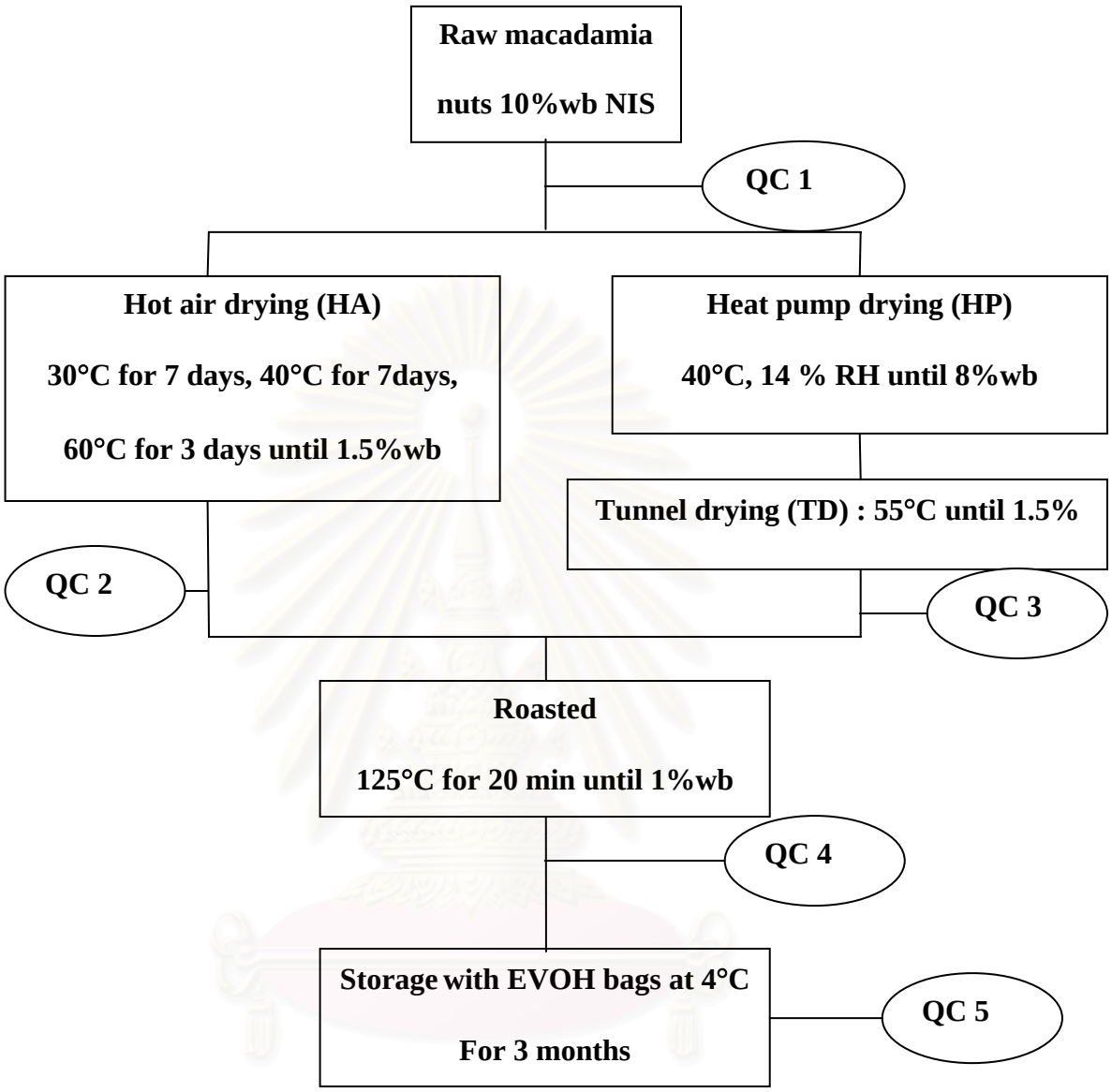


Figure 3.1 The overview of the experiments

QC = Quality checking

3.2.1 Hot air drying (Wall and Gentry, 2006)

The nuts in shells of macadamia nuts were dried in a hot air dryer (Contherm, Australia) at temperature of 30°C for 7 days then 40°C for 7 days and 60°C for 3 days until the final kernel moisture content was down to 1.5% wb.

3.2.2 Heat pump drying

Heat pump dryer (Figure 3.2 and 3.3) used in the experiment was manufactured by Greenhalgh Refrigeration Pty. Ltd., Caloundra, Queensland, Australia. It is an MK series medium temperature heat pump dryer with two temperature ranges: low temperature (10-35°C) and medium temperature (35–50°C). It is fitted with a 0.75 kW radial fan (Figure3.3b indicating MCF) and a refrigeration unit (Lohachoompol, 2007).

The dryer has an air-bypass provision by no recuperators. Approximately 10% of the recirculation air was used for air-bypass for dehumidification. The average air velocity (measured using a hotwire anemometer, Veloc"Calc, TSI model 8350-1) inside the drying chamber was 1.71 m/s. The dryer has built-in temperature and humidity controls (Lohachoompol, 2007).

Macadamia nuts were placed on a perforated tray. The temperature was set at 38-40°C, 14 % relative humidity. A data logger was connected which enabled recording of drying our temperature and relative humidity inside the dryer. The weight of samples was recorded every 15 minutes until macadamia nut dried to 8% wb. The dried sample were then taken to the tunnel dryer.

Figure 3.2 Heat pump dryer (UNSW, Australia)

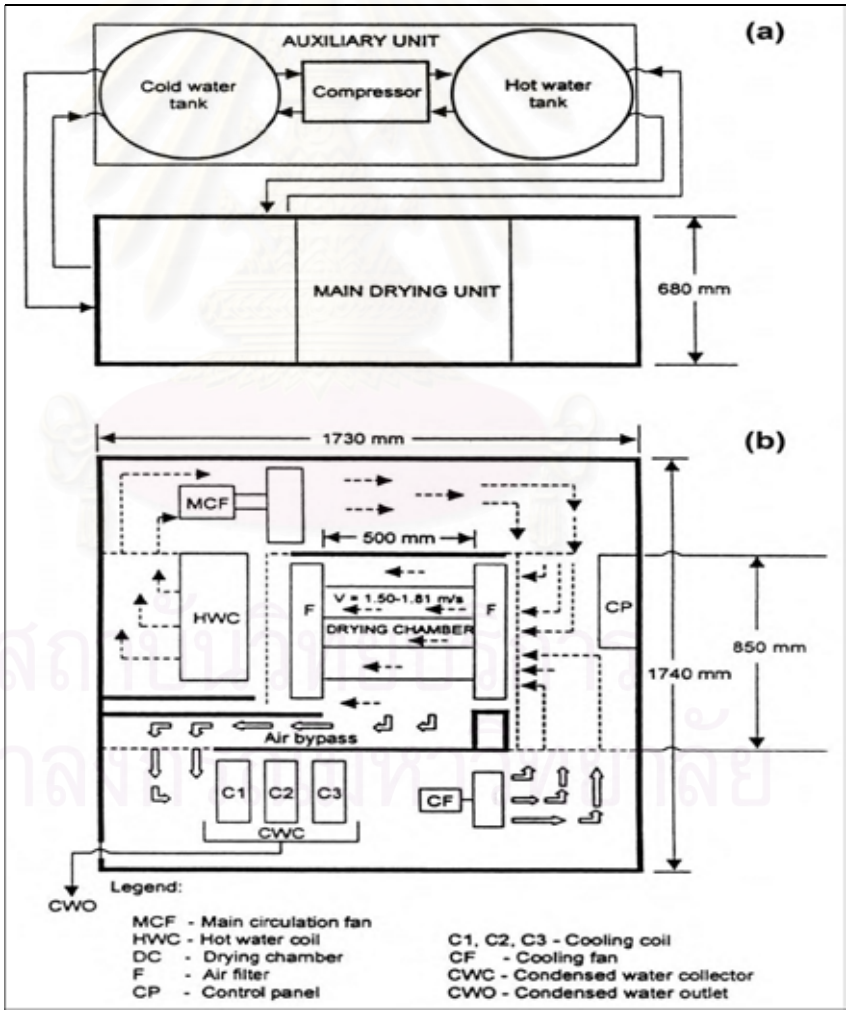


Figure 3.3 Schematic diagram of the heat pump dryer (Lohachoompol, 2007)

(a) Top view
(b) Side view

3.2.3 Tunnel drying

A laboratory-scale tunnel dryer was used in the experiments. The system included a fan, a heating section, a steam injection system and a drying chamber. The drying chamber walls were made from heavy gauge aluminium, filled with 5 cm thick fibre glass insulation. The drying chamber (50 cm wide, 30 cm deep and 70 cm long) was made from 2.5 cm thick plywood, painted both sides with water proof paint and an aluminium panel fitted on top (Figure 3.4).

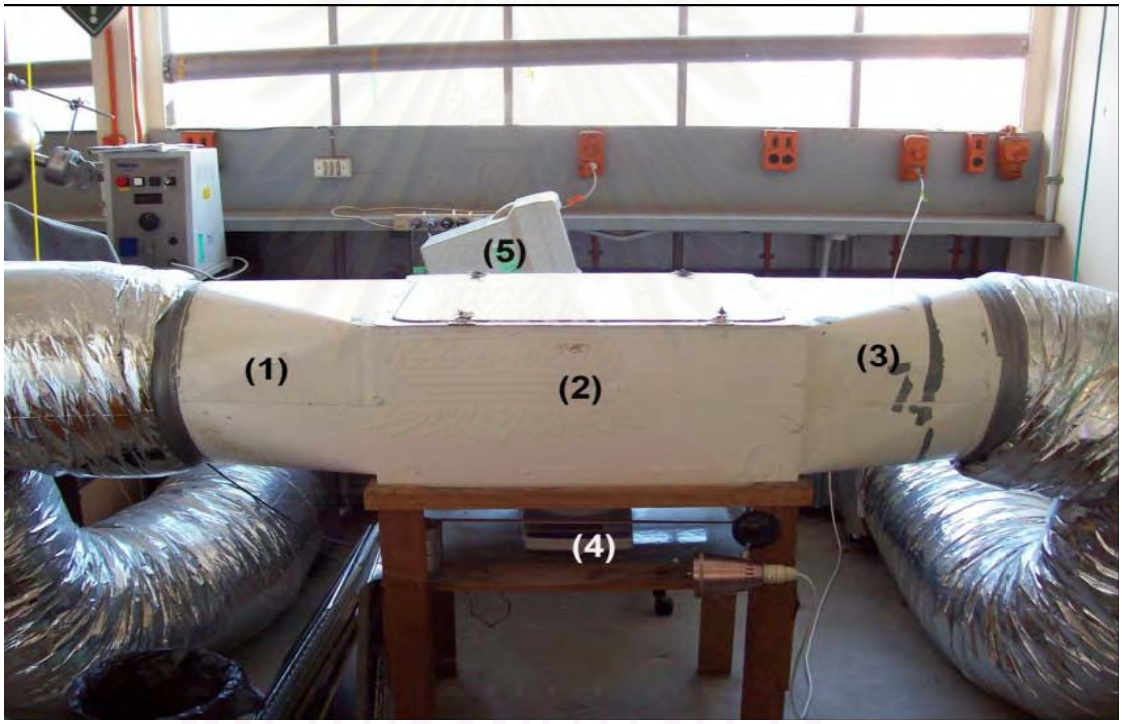


Figure 3.4 Laboratory-scale tunnel dryer (UNSW, Australia)

(1) air inlet
(2) drying chamber
(3) air outlet
(4) digital balance
(5) computer

A perforated tray was placed parallel to the air flow and supported with four rods, which passed through the bottom of the drying chamber to a Mettler Toledo

PB3002-S balance connected to a computer. The temperature set at 55°C dried until moisture content was reduced to 1.5% wb. During drying process, moisture content of macadamia nut were determined by weight of sample at each time interval that recorded by the two computers.

3.2.4 Macadamia nut roasting

Dried macadamia nut (1.5% db) nuts were cracked and roasted for 20 min in the convection oven at 125°C until 1% kernel moisture content is reached (Wall and Gentry, 2006).

3.2.5 Storage conditions

After roasting macadamia nuts were packed in semi-vacuum sealed EVOH bags and stored at 4 °C for 3 months (Department of Agriculture, 2004).

From the flow chart each stage of processing and storage were taken to determine the following;

3.2.6 Quality checking 1

Fresh macadamia nut was taken to determine the proximate analysis, physical and chemical qualities in term of protein content, lipid content, a_w, moisture content, colour, free fatty acid content, peroxide value as described in Appendix A. Determination of volatile compounds and fatty acid composition are described in section 3.3 and 3.4.

3.2.7 Quality checking 2 - 4

Dried and roasted macadamia nuts were taken to determine their quality in term of a_w, moisture content, colour, free fatty acid content, peroxide value, volatile compounds and fatty acid composition.

3.2.8 Quality checking 5

Every month of storage, a sample was collected to determine a_w, moisture content, colour, free fatty acid, peroxide value, volatile compounds and fatty acid composition.

3.2.9 Statistical Analysis

The experiment follows a completely randomized design, with 3 replications for each analysis. Statistical evaluation by SPSS with the analysis of difference of means by Duncan's Multiple Range Test.

3.3 Isolation of volatiles compounds from macadamia nut

3.3.1 Sample preparation

The fresh and dried macadamia nuts were cracked then chopped into small pieces on the day of the experiment. Fifty g of finely macadamia kernels were weighed and mixed with 150 ml of volatile-free distilled water, which was prepared by boiling distilled water from 1500 ml down to 1000 ml.

3.3.2 Likens-Nickerson simultaneous distillation and extraction (LNSDE)

The 50 g of finely macadamia nut sample and 150 ml volatile free distilled water were placed in a 2 l round bottom flask. Distilled dichloromethane (40 ml) was placed in another 500 ml round bottom flask as a solvent. The purification of solvent will be described in 3.3.4. These two flasks were attached to different sides of the Likens-Nickerson apparatus is shown in Figure 3.5. Both flasks were heated separately with heating mantles to their respective boiling points. Sample vapour and solvent vapour mixed and condensed together in the cooled central collector. Two liquid

phases separated due to their density differences and returned to their respective flasks. The steam removes volatile components from the sample and the solvent extracts the volatile components from the condensed steam. Dry ice-acetone condenser was employed beyond the main condensation area to prevent the loss of volatile compounds. It is situated at the top of the apparatus with a water bubbler in order to eliminate contamination from laboratory atmosphere that may diffuse back in the vapour. The process was carried out at atmospheric pressure for 2 hours.

The cooled extract was collected in a pear shaped flask and internal standard (10 µl) was added to the extract before freezing out the remaining water in dry ice for 3 minutes. The liquid phase was then transferred to another pear shaped flask and further dried with volatile-free anhydrous sodium sulphate which was used for absorb the remaining of water before to analyse with GC-MS. The purification of anhydrous sodium sulphate will be described in 3.3.5. The removal of sodium sulphate was done by transferring only the extract to a new flask while leaving the sodium sulphate in the original flask. The volatile-containing solvent then underwent a concentration process. This process was performed by using steam of nitrogen gas under room temperature to concentrate the extract down to 0.25 ml for further analysis with GC-MS.

Figure 3.5 Modified Likens-Nickerson simultaneous distillation and extraction apparatus

3.3.3 Internal standard

Internal standard was prepared by adding 0.025 g of C_{14} hydrocarbon into a 25 ml volumetric flask and making up the solution to volume with distilled dichloromethane.

3.3.4 Solvent purification

Dichloromethane (300 ml) was put in a 1 l round bottom flask which was heated by the heating mantle as shown in Figure 3.6. The temperature was maintained at 40°C which is the boiling point of dichloromethane. A thermometer was situated at the top of a fractionating column to monitor changes in temperature. Dichloromethane

vapour through fractionating column and condensed in the condenser area. Purified dichloromethane was collected into another round bottom flask.

Figure 3.6 Solvent purification apparatus

3.3.5 Anhydrous sodium sulphate purification

Anhydrous sodium sulphate (50 g) was put in a 1 l round bottom flask and was then connected to the rotary evaporator equipment. The evaporation of volatiles that may be present was done under vacuum and at ambient temperature. After 1 hour the run was stopped. Purified anhydrous sodium sulphate was taken and kept in closed vial.

3.3.6 Analysis of macadamia volatiles by gas chromatography and mass spectrometry (GC-MS)

3.3.6.1 Apparatus

The concentrated extract was analysed by gas chromatography and mass spectrometry (Agilent 6890 N and 5975 inert mass selective detector, Agilent Technologies, USA.) are shown in Figure 3.7 and equipped with a fused silica capillary column with 5% phenylpolysiloxane as the non polar stationary phase

(60.0 m long × 220 µm i.d × 0.25 µm film thickness). The column is manufactured by SGE, USA.

3.3.6.2 GC-MS Conditions

Injector information

Injection mode	:	Splitless
Solvent delay	:	5.4 min
Injection volume	:	5 µl

Inlet condition

Heater	:	150ºC
Pressure	:	165.4 kPa
Total flow	:	104 ml/min

Column conditions

Detector	:	MSD
Pressure	:	165.5 kPa
Flow rate	:	1.0 ml/min
Average velocity	:	26 cm/sec

Oven conditions

Initial temperature	:	30ºC (hold for 1 minute)
Ramp 1	:	50ºC/min to 60ºC
Ramp 2	:	3ºC/min to 250ºC

Scan mass range : 30-220 atomic mass unit

Run time : 74.93 min

Figure 3.7 Gas chromatography – Mass spectrometry

3.3.6.3 Qualitative analysis

The mass spectra of the peaks were compared with those in mass spectral library (WILEY275) of the ChemStation software to assign the identification. Peak integration was conducted on the ChemStation integrator set to integrate mode.

3.3.6.4 Quantitative analysis

The concentration of the compounds of interest was determined by using the formula:

$$Conc(C) = \frac{A(C)}{W(S)} \times \frac{W(IS)}{A(IS)} \quad \ldots (3.1)$$

where:

Conc(C) = the concentration of compound C (µg/kg, ppb)

A(C) = the peak area of compound C

A(IS) = the area of the internal standard

W(S) = the weight of the sample (kg)

W(IS) = the weight of the internal standard (µg)

3.4 Isolation of macadamia fatty acid

3.4.1 Methylation (Bannon *et al*., 1982)

A sample of 10 drops macadamia oil or approximately 100 mg was transferred to a dry, 50 ml volumetric flask fitted with a B14 ground-glass joint. The mixture was boiled under reflux for 30 sec with 5 ml of 0.25 M sodium methoxide in methanol-diethyl ether (1:1). The flask was removed from the heat source, 3 ml of isooctane and approximately 15 ml of saturated sodium chloride were added and the flask was stoppered and shaken vigorously for 15 sec while tepid. The liquid level was brought to the neck of the flask with more sodium chloride solution and the phases were allowed to separate. Approximately 2.5 µl of the upper layer were injected into the Gas chromatograph- Flame ionisation detector (GC-FID) is shown in Figure 3.8.

Figure 3.8 Gas chromatography – Flame ionisation detector

3.4.2 Apparatus

The sample was analysed by gas chromatography and flame ionisation detector (GC-FID) (Varian Corporation Model 1700 version 3, USA) equipped with a DB-23 capillary column with (60.0 m long × 0.25 µm i.d × 0.25 µm film thickness). The column is manufactured by J&W Scientific, USA.

3.4.3 GC-FID Conditions

Injector information

Injection mode	:	Split
Pressure	:	100 kPa
Measure flow	:	3 ml/min
Injection volume	:	1 µl

Inlet condition

Heater	:	250ºC
Pressure	:	157 kPa

Column conditions

Detector	:	FID
Total Flow	:	157ml/min
Velocity	:	4 cm/sec
Split ratio	:	100:1
Split flow	:	150 ml/min
Column flow	:	1.5 ml/min

Oven conditions

Isothermal	:	180 ºC, 40 min

Detector condition

Make up flow	:	2 ml/min
Hydrogen flow	:	1 ml/min
Air flow	:	10 ml/min

Run time : 40 min

3.4.4 Qualitative analysis

Fatty acids were identified by Stat integration software (Version 4.5 Varian associate Inc., Japan). The peak of fatty acid methyl ester were compared with the retention time from standard mixture fatty acid methyl ester (Sigma Chemical Company, USA) (Appendix D).

3.4.5 Quantitative analysis

3.4.5.1 Theoretical relative response factor (TRF) for fatty acid methyl ester

The concept of using a theoretical relative response factor enables conversion of raw peak area to corrected areas when analysing fatty acid methyl esters (FAME) by gas chromatography. Because of the factor is not determined by analysis of a standard sample but by calculation of the theoretical relative response from a considered amount of carbon in the FAME that is bonded to one or more hydrogen atoms relative to the amount bonded in 18:0. Therefore, the peak area of FAME from the sample were converted with TRF factors to the corrected peak area (see the TRF of FAME (Table D. 2 in Appendix D).

3.4.5.2 Standard curve of palmitoleic acid preparation

Standard curve of palmitoleic acid was constructed by plotting correct palmitoleic acid concentration correspond to its corrected peak area (Figure D.3 in Appeendix D). The corrected peak area of palmitoleic acid from a sample was compared with the standard curve to obtain its concentration. Peak area of fatty acid sample was then compared to peak area of palmitoleic acid in the sample. Therefore, the concentration of sample fatty acid can be calculated. In another word, palmitoleic acid is used as internal standard (Appendix D).

CHAPTER 4

RESULTS AND DISCUSSION

This study focuses on quality changes of macadamia nut during processing and storage. The quality attributes are fatty acids, volatile compounds, free fatty acids, peroxide value, colour, moisture content and water activity. Two drying methods will be compared in this study in order to select the most appropriate drying method for macadamia nut leading to production of superior quality of the nut.

4.1 Raw material analysis

4.1.1 Proximate analysis, physical and chemical analysis

Macadamia nuts predried to about 10% wb nut in shell (NIS), were supplied by Denham Plantation, Knockrow, Northern New South Wales, Australia. The nut samples were taken for proximate, physical and chemical analysis. The results are shown in Table 4.1.

Table 4.1 Proximate analysis, physical and chemical analysis of raw macadamia nuts

Analysed parameters	Value
Oil content	72.83 ± 0.88 (% dry basis)
*Specific gravity	≥72 g/100 g oil
Water activity (a_w)	0.71 ± 0.02
Moisture content	10.54 ± 0.16 (% wet basis) (NIS)
	Kernel 5.33 ± 0.24 (% wet basis)
	Shell 15.78 ± 0.32 (% wet basis)

Table 4.1 Proximate analysis, physical and chemical analysis of raw macadamia nuts (continued)

Analysed parameters	Value
Protein	7.53 ± 0.08 (% wet basis)
Peroxide value (meq O_2/kg oil)	0.37 ± 0.00
Free fatty acid (% oleic acid)	0.042 ± 0.00
Colour	
L value	90.27 ± 0.48
a value	0.60 ± 0.30
b value	24.34 ± 4.67

Each figure represents mean value from 3 replications.

* If all 20 kernels of macadamia nut were floated in water SG=1

The kernels that float in water usually have oil content of 72 g /100g oil or higher, while kernels that sink in water will contain less than 72 g /100 g oil (Mason, 1982). The results show that macadamia nuts used in this study have an oil content of about 72.83 g/100g of kernel and oil grading with specific gravity is ≥72 g/100g oil.

The comparison of chemical properties of raw macadamia oil grown in Australia and Thailand is shown on Table 4.2.

Table 4.2 Comparison of chemical properties of raw macadamia oil from nuts grown in Australia and Thailand

Properties of macadamia oil	Average value	
	Australia	**Thailand***
Oil content (% dry basis)	72.10-73.81	72.55-78.05
Peroxide value (meq O_2/kg oil)	0.37-0.38	nd-0.42
Free fatty acid (% oleic acid)	0.041-0.043	0.17-0.27

* Kungsadanaumpai *et al.* (2006), nd = not detected

4.1.2 Fatty acid composition

The fatty acid composition of raw macadamia nuts investigated in this study before processing is shown in Table 4.3.

Table 4.3 The fatty acid composition of raw macadamia nuts

Fatty acid	Average concentration (mg/100g dry basis)
Lauric acid (C12:0)	0.14±0.01
Myristic acid (C14:0)	1.00±0.16
Palmitic acid (C16:0)	8.5±0.84
Palmitoleic acid (C16:1)	18.1±0.84
Stearic acid (C18:0)	3.5±0.93
Oleic acid (C18:1)	61.4±1.46
Linoleic acid (C18:2)	2.3±0.71
Linolenic acid (C18:3)	0.1±0.01
Arachidic acid (C20:0)	2.7±0.09
Eicosenic acid (C20:1)	2.2±0.35
Behenic acid (C22:0)	0.8±0.07

Values are means ± SD. Six replications for each fatty acid

The chromatogram of standard oil used in this experiment can be seen in Appendix D. A sample of the chromatogram for extracted macadamia nut oil is shown in Appendix E. In the raw material, over 60% of the extracted macadamia oil consisted of the unsaturated fatty acid. This content is similar to published data on fatty acid composition of macadamia nuts by Winterton (1966) and Kaijser *et al*. (2000).

Eleven fatty acids were found in macadamia nuts including lauric acid C12:0, myristic acid C14:0, palmitic acid C16:0, palmitoleic acid C16:1, stearic acid C18:0, oleic acid C18:1, linoleic acid C18:2, linolenic acid C18:3, arachidic acid C20:0, eicosenic acid C20:1 and behenic acid C22:0.

Major fatty acids are oleic acid (59.36-62.36 mg/100g db), palmitoleic acid (17.71-18.79 mg/100g db) and palmitic acid (6.27-8.13 mg/100g db).

Average proportion of saturated, monounsaturated and polyunsaturated fatty acids in nuts grown in Australia was found to be as follows:

Saturated oil 14.05 -16.94 mg /100g db.

Monounsaturated oil 78.92 – 83 mg/100g db.

Polyunsaturated oil 2.34 – 2.52 mg/100g db.

The average ratio of unsaturated to saturated fatty acids is 5.78:1 – 5.04:1. Therefore, these figures indicate that the oil of macadamia nut in Australia is more susceptible to oxidation due to increased oxidation potential of unsaturated fatty acids (Frankel, 1998).

However, a difference in qualitative and quantitative composition of fatty acids can occur as postulated by Kaijser *et al*. (2000) who have concluded that cultivars, plantation area and weather can have a major impact on the fatty acid composition of macadamia nuts.

4.1.3 Volatile compounds in raw macadamia nuts

The flavour of foods is an integrated response primarily composed of sensations from aroma and taste. It is the odour or aroma which is the single most important contributor to the flavour of most foods (Cronin, 1982).

Flavours in nuts and nut products have been widely researched and reveal a vast array of volatile compounds, largely depending on whether the nuts are raw or roasted. In raw kernels, these volatiles are characteristic of natural processes such as enzymatic and/or lipid oxidation. Roasted nuts contain compounds such as pyrazines and pyridines, which are characteristic formation products from temperature sensitive reactions such as Maillard reaction (Waltking and Goetz, 1983). However, there are only few studies on volatile compounds in macadamia nuts.

Volatile compounds in raw macadamia nut that were used in this study were extracted by Likens-Nickerson method and analysed by GC-MS. The results are shown in Table 4.4.

Table 4.4 Volatile compounds in raw macadamia nuts

Volatile compound	**Concentration (ppb)**
Aldehydes	
Hexanal	72.76
n Heptanal	127.64
Octanal	72.42
Benzeneacetaldehyde	29.66
Nonyl aldehyde	275.12
Others	
Dimethyl disulfide	25.20
Pyridine	48.29

A study on volatile compounds found in roasted macadamia nuts was published by Crain and Tang in (1975). They used the steam distillation of samples followed by solvent extraction to analyse the volatiles of roasted macadamia nuts. This method uses a large number of solvents to extract volatiles and showed pieces of equipment. The method is also time consuming.

The results show that the aldehydes were present in raw nut. They might be formed by the autoxidation of unsaturated fatty acid via enzymatic reaction. Moreover, the presence of pyridine and dimethyl disulfide in raw nuts is due to heat-induced interaction of amino acids and sugars (Maillard reaction) that occurred during distillation at high temperature with Likens-Nickerson apparatus which will be discussed in section 4.3.

4.2 Effect of processing and storage on quality of macadamia nuts

4.2.1 Oil content and composition

The factors that indicate the quality of macadamia oil include many parameters such as fatty acids, peroxide value and free fatty acids.

Table 4.5 summarized effects of processing and storage on quality in term of fatty acids of macadamia nuts. Statistical analysis was done separately in each drying method since raw materials of each batch were not the same.

Table 4.5 Fatty acid composition (mg/100 g dry basis) in macadamia nut oil from extracted samples subjected to different drying methods and storage duration

Stage	C12:0	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2 ns	C18:3	C20:0	C20:1 ns	C22:0
Raw	0.13±0.01^{b}	0.90±0.09^{d}	8.13±1.00^{b}	17.71±0.95^{b}	3.39±1.04^{b}	59.36±1.00^{e}	2.39±1.11	0.13±0.01^{d}	2.64±0.00^{d}	1.85±0.03	0.72±0.08^{cd}
HA	0.12±0.00^{b}	0.72±0.02^{c}	6.98±0.09^{ab}	12.71±0.95^{a}	1.66±0.88 ^{a}	54.27±0.95^{d}	2.35±1.06	0.12±0.01^{cd}	2.35±0.01^{c}	1.61±0.94	0.22 ±0.01^{b}
Roasting	0.15±0.01^{c}	0.38±0.11^{a}	6.61±0.95^{a}	11.74±0.55^{a}	2.69±0.97^{ab}	50.58±0.72^{c}	1.62±1.22	0.11±0.01^{bc}	1.97±0.01^{b}	2.25±0.02	0.12±0.01 ^{a}
Storage 1 mo	0.05±0.01^{a}	0.45±0.04^{ab}	6.24±0.01^{a}	11.59±0.14^{a}	2.3±0.00 ^{ab}	47.63±1.09^{b}	1.62±0.17	0.09±0.00^{ab}	2.03±0.06^{b}	1.82±0.02	0.13±0.01 ^{a}
Storage 2mo	0.04±0.01^{a}	0.54±0.02 ^{b}	6.40±0.94 ^{a}	12.59±0.98^{a}	2.93±1.01^{ab}	45.68±0.30^{a}	1.78±0.9	0.09±0.01^{ab}	1.76±0.09^{a}	2.22±0.01	0.68±0.04^{c}
Storage 3 mo	0.04±0.00^{a}	0.45±0.1^{ab}	6.34 ±1.04 ^{a}	12.42±1.10^{a}	3.02±0.03^{ab}	45.53±1.15^{a}	1.73±0.95	0.08±0.00 ^{a}	1.68±0.01^{a}	2.25±0.01	0.77 ±0.04^{d}

Stage	C12:0	C14:0 ns	C16:0	C16:1	C18:0 ns	C18:1	C18:2 ns	C18:3	C20:0 ns	C20:1 ns	C22:0
Raw	0.14±0.01^{c}	1.09±0.17	8.94±0.53^{b}	18.79±0.95^{c}	3.6±1.03	62.36±1.00^{bc}	2.21±0.12	0.13±0.01^{b}	2.73±0.13	2.50±0.06	0.80±0.04^{c}
HP+TD	0.11±0.01^{a}	0.97±0.05	7.92±0.21^{ab}	15.83±0.07^{ab}	3.12±0.12	62.25±0.22^{bc}	1.95±0.07	0.12±0.01^{b}	2.67±0.07	2.53±0.02	0.27±0.02^{b}
Roasting	0.13±0.00^{bc}	1.18±1.02	8.13±0.05^{ab}	15.51±1.07^{ab}	3.51±1.07	62.79±0.24 ^{c}	2.07±0.06	0.12±0.01^{b}	2.50±0.14	2.35±1.05	0.24±0.02^{ab}
Storage 1mo	0.15±0.00^{d}	1.15±0.01	8.38±0.06^{ab}	14.8±0.18^{a}	3.24±0.04	63.06±1.03 ^{c}	2.01±0.08	0.12±0.01^{b}	2.84±1.05	2.39±1.00	0.35 ±0.01^{c}
Storage 2mo	0.12±0.01^{ab}	1.02±0.01	8.61±0.95^{ab}	16.3±0.75^{b}	4.24±1.02	60.89±1.01^{b}	1.73±1.07	0.12±0.01^{b}	2.44±1.05	2.83±0.94	0.22±0.01^{a}
Storage 3mo	0.11±0.01^{ab}	1.04±1.00	7.40 ±1.06 ^{a}	16.63±1.23^{b}	2.89±1.02	57.37±0.86 ^{a}	1.46±0.89	0.09±0.00^{a}	2.50±0.95	2.36±1.07	0.21±0.01^{a}

Mean separation within columns based on probability of significant difference ($p \leq 0.05$)

ns Nonsignificant difference ($p \leq 0.05$)

C12:0 = lauric acid, C14:0 = myristic acid, C16:0 = palmitic acid, C16:1 = palmitoleic acid, C18:0 = stearic acid, C18:1 = oleic acid, C18:2 = linoleic acid, C18:3 = linolenic acid, C20:0 = arachidic acid, C20:1 = eicosenic acid and C22:0 = behenic acid

(a) After drying

The results show that the fatty acids such as C14:0, C16:1, C18:0, C18:1, C20:0 and C22:0 decreased significantly ($p \leq 0.05$) after drying with HA drying. In contrast, the fatty acids of C12:0, C16:1 and C22:0 decreased significantly ($p \leq 0.05$) after drying with HP+TD drying as shown in Table 4.5.

Generally, the decomposition of fatty acid was the result of autoxidation and hydrolytic rancidity. Oxidative rancidity might be initiated by the presence of oxygen which reacts adjacent to the double bond positions of unsaturated fatty acids. The results show that the decrease of fatty acid content involved the unsaturated fatty acids such as C16:1 and C18:1. The latter decreased significantly ($p \leq 0.05$) in macadamia nuts after drying with HA drying. Similarly the content of C16:1 decreased after drying with HP+TD drying seen in Tables 4.5. However, the content of oleic acid C18:1 was not significantly different ($p > 0.05$) from that in the raw macadamia nuts after drying with HP+TD drying. These results indicate that the longer exposure to oxygen during HA drying (17 days) influenced the decomposition of unsaturated fatty acid via lipid oxidation, especially C16:1 and C18:1, which generally resulted in an overall higher content of fatty acids in macadamia oil. Thus, the contents of C16:1 and C 18:1 after drying with HA drying were reduced from 17.71 to 12.71 mg/100g db and from 59.36 to 54.27 mg/100 g db, respectively. In contrast, the contents of C16:1 and C18:1 decreased from 18.79 to 15.83 mg/100 g db, respectively and were not significantly changed after drying with HP+TD drying.

An extensive survey of the published studies on macadamia nuts revealed only measurement of fatty acid composition of raw kernels, roasted kernels and the various composition changes of the fatty acids during maturity.

However, the decomposition of fatty acids could also occur by hydrolytic activity due to enzymes such as lipase, the presence of moisture or high temperatures which do not require oxygen to hydrolyse triacylglycerols molecules to produce free fatty acids (Frankel, 1998). The results of the experiments show that the content of saturated fatty acid decreased similar to the decrease of unsaturated fatty acids after drying with HA drying and HP+TD drying. In addition, the decreasing of C14:0, C18:0, C20:0 and C22:0 after drying with HA drying and HP+TD drying such as C12:0 and C22:0 decreased significantly ($p \leq 0.05$).

Moreover, the presence of free fatty acids provides an indication of the degree of hydrolytic rancidity as indicated in the discussion in 4.2.2. Fatty acids exist in an unbound state in foods, they are much more reactive and more liable to be broken down to volatile by-products (see discussion in 4.3).

Therefore, HP+TD drying can reduce drying time from 17 days (hot air dryer) down to 3 days to achieve 1.5 % wb. Colquhoun *et al*. (1996) showed that C16:1 and C18:1 may have a role in preventing cardiovascular disease. Consequently, it can be concluded that HP+TD drying can reduce the decomposition of fatty acids via oxidation and hydrolytic rancidity during drying in comparison with HA drying.

(b) Roasting and storage

The decomposition of fatty acids occurred continuously as a result of lipid oxidation and hydrolytic rancidity which occurred during processing seen in Table 4.5. The decomposition happened during HA drying more than HP+TD drying. The factors that influenced the decomposition of fatty acids during storage are materials and conditions of packaging. This result can be explained by increasing moisture content (>1%wb) which can be seen in the results (Table 4.6). Dela *et al*. (1966)

recommended that the moisture content of 1% wb after roasting gives the most stable product. However, the lipid oxidation rate should be low as a result of water activity being lower than 0.4 during storage. However, other factors will affect the quality of the nuts such as the remaining oxygen in packaging, material of packaging since water and oxygen can cause deterioration during storage. Roasted macadamia nuts were stored at 4°C in EVOH plastic bag sealed under semi-vacuum. The barrier properties can be indicated by the value of oxygen transmission rate (OTR) cm^3/m^2 d bar and water vapour transmission rate (WVTR) g / m^2 d. The adequate barrier values of plastic in terms of OTR and WVTR are less than 1 cm^3/m^2 d bar and less than 0.5 g / m^2 d, respectively. The OTR and WVTR values of EVOH plastic are 0.15 cm^3/m^2 d and 22 g / m^2 d. (Hahtamaki Pty Ltd. 2004). Thus, this plastic film is a good oxygen barrier but water sensitive. It could be that some remaining oxygen contributed to oxidative rancidity and the increasing contributed moisture content to hydrolytic rancidity during storage.

The results show that the fatty acids such as C18:1, C20:0 and C22:0 decreased significantly ($p \leq 0.05$) during storage after drying with HA drying as well as the decreasing of fatty acids such as C12:0, C16:1, C18:1, C18:3 and C22:0 during storage after HP+TD drying as shown in Table 4.5.

According to Vivien (1989) studied the storage and temperature conditions of macadamia nuts and found that the oleic acid (C18:1), linolenic acid (C18:3) and arachidic acid (C20:0) decreased during storage at 30°C for 2 months.

Therefore, the decomposition of fatty acids during processing and storage can be shown in Figure 4.1 and 4.2. The fatty acids of C16:1 and C18:1 are useful properties preventing cardiovascular disease (Colquhoun *et al*., 1996). The results show that heat pump combined with tunnel drying can maintain the content of

palmitoleic acid and oleic acid more than hot air drying as shown in Figure 4.1 and 4.2.

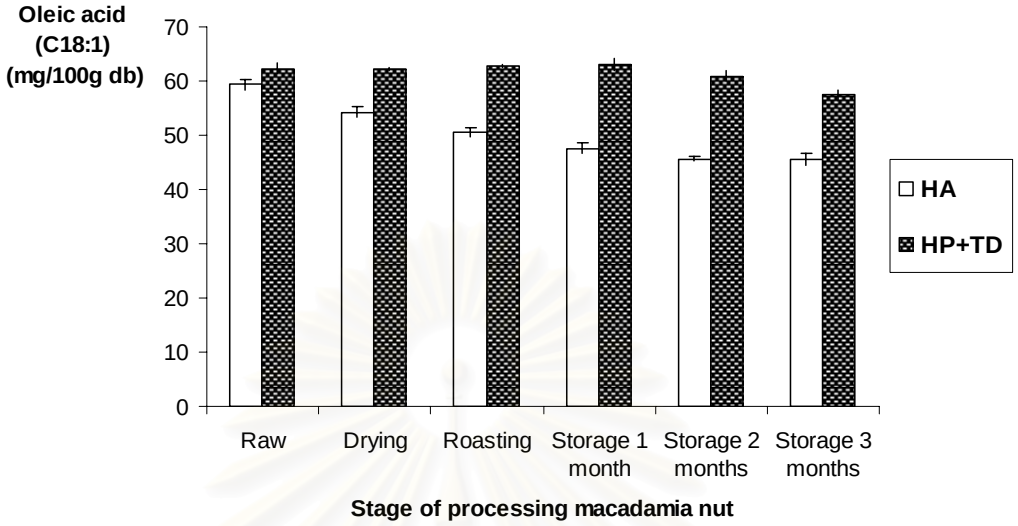


Figure 4.1 Content of palmitoleic acid (C16:1) during different stages of processing and drying

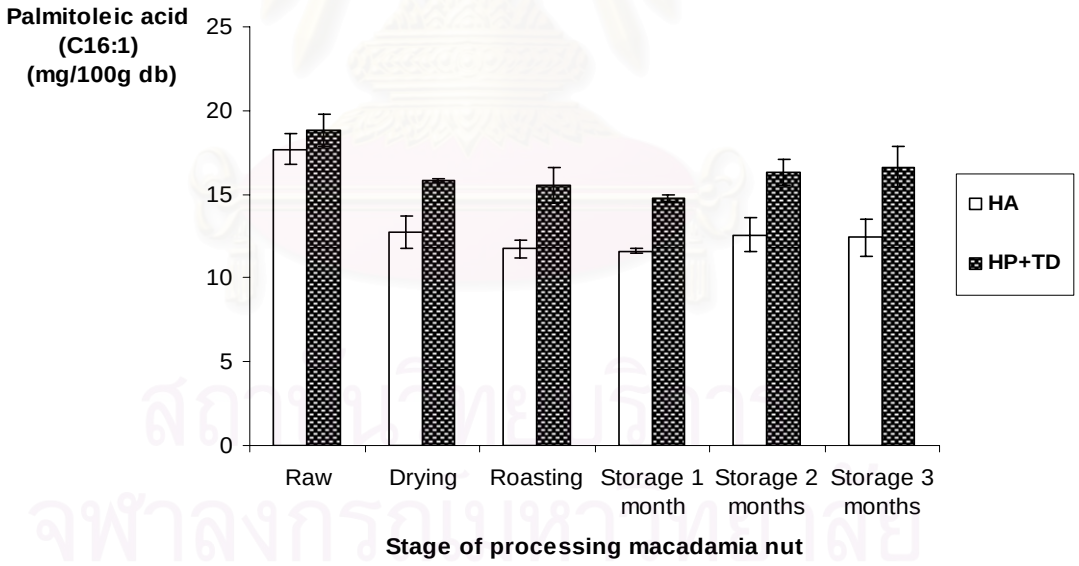


Figure 4.2 Content of oleic acid (C18:1) during different stages of processing and drying

4.2.2 Peroxide value, free fatty acid, moisture content and water activity

The deterioration of macadamia nuts during processing and storage can be rapidly characterised by the changes in moisture content, water activity, peroxide value and free fatty acids as shown in Table 4.6.

Table 4.6 The quality of macadamia nuts during processing and storage

Stage	Moisture content (% wb) kernel	Peroxide value (meq O_2/kg oil)	Free fatty acid (% oleic acid)	Water activity (a_w)
Raw	5.33±0.05 [f]	0.37±0.00 [c]	0.042 [a]	0.709 ± 0.02
HA	1.43 ±0.02 [e]	0.40±0.00 [b]	0.050 [b]	0.373 ± 0.01
Roasting	0.94 ±0.02 [a]	0.29±0.00 [a]	0.061[c]	0.342 ± 0.04
Storage 1 mo	1.06±0.07 [b]	0.36±0.00 [c]	0.064 [d]	0.356± 0.02
Storage 2 mo	1.31 ±0.01 [d]	0.43±0.00 [d]	0.095[e]	0.375± 0.01
Storage 3 mo	1.25±0.02 [c]	0.62±0.00 [e]	0.132 [f]	0.381± 0.01
Raw	5.39±0.03 [e]	0.37±0.00 [a]	0.041 [a]	0.709 ± 0.02
HP+TD	1.43±0.01 [d]	0.39±0.00 [b]	0.044 [a]	0.377± 0.03
Roasting	1.07±0.01 [b]	0.45±0.00 [c]	0.041[a]	0.348± 0.04
Storage 1 mo	1.03±0.02 [a]	0.46±0.01 [c]	0.055 [b]	0.359± 0.01
Storage 2 mo	1.16±0.03[c]	0.51±0.01 [d]	0.061[b]	0.366± 0.01
Storage 3 mo	1.16±0.15 [c]	0.60±0.01 [e]	0.063 [c]	0.378± 0.03

Values are means ± SD. Statistical analysis was done separately in each drying method since raw materials of each batch were not the same. Mean separation within columns based on probability of significant difference ($p \leq 0.05$)

4.2.2.1 Peroxide value

(a) After drying

Peroxide values (PV) is the most commonly used parameter to measure the extent of oxidation in oils. The measurement of peroxides is generally a good technique for determining the onset of oxidation as it is a measure of the hydroperoxides formed in the initial stages of oxidation as shown in Figure 4.3.

The peroxide value has changed significantly ($p \leq 0.05$) as shown in Table 4.6 during processing and storage. The maximum peroxide value that was detected was not more than 0.62 meq/kg oil which was within acceptable value. In addition, a value of 3.0 meq/kg is generally regarded as the maximum peroxide limit and beyond 5.0 meq/kg, macadamias have a noticeably rancid flavour (McConachie, 2001 cited by Himstedt, 2002).

The peroxide value increased significantly ($p \leq 0.05$) in raw macadamia nut after drying with HA and HP+TD drying. The results show that the peroxide value after drying with HA was slightly higher than the peroxide value after drying with HP+TD drying. The peroxide value increased from 0.37 to 0.40 meq O_2/kg oil after drying with HA and from 0.37 to 0.39 meq O_2/kg oil after drying with HP+TD drying as shown in Table 4.6. These results are due to the decomposition of unsaturated fatty acids in the HA drying more than in the HP+TD drying via oxidative rancidity which are described in section 4.2.1 and Figure 4.3.

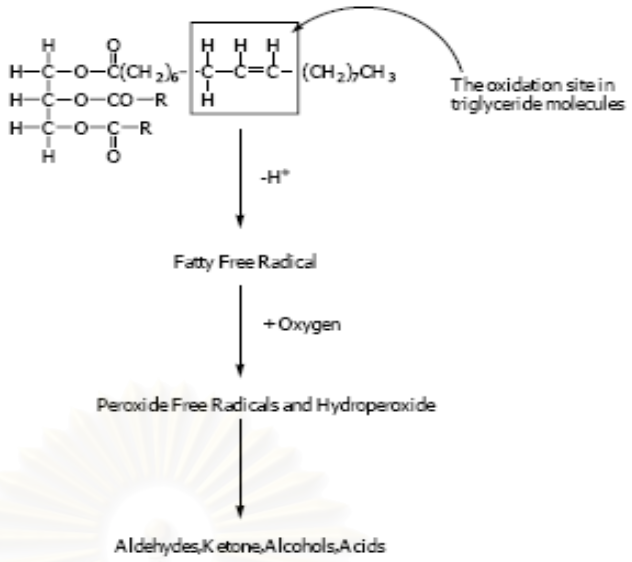


Figure 4.3 Formation of oxidative rancidity (Frankel, 1998)

(b) Roasting and storage

Hydroperoxides are active oxidants and have a tendency to react with other compounds during storage and processing to form carbonyl compounds such as mono-carboxylic acids, aldehydes, ketones, hydrocarbons, esters and lactones (Ozdemir *et al*., 2001). Moreover, the decreasing peroxide value after roasting with hot air dryer is a result of the formation of hydroperoxides are that not heat stable and able to decompose to carbonyl and hydroxyl acids under heat treatment. Therefore, peroxide fluctuates during processing or storage. Moreover, some Australian macadamia processors currently do not use a standard peroxide value to determine an oxidation limit for goods sold.

Macadamia kernels are generally sold in a nitrogen flushed, vacuum sealed laminated foil package to reduce oxygen concentration and prevent oxidative rancidity, thus ensuring a long storage life. The peroxide value during storage increased significantly ($p \leq 0.05$) (Table 4.6) This result can be seen in section 4.2.1 (b).

According to Chitundu (1994) the peroxide value increased from 0.278 to 0.606 meqO_2/kg oil during 3 months storage at 30°C.

The peroxide value of roasted macadamia nuts during drying in a hot air dryer was higher than that of nuts dried in a heat pump dryer. This is due to the decomposition of fatty acid via oxidative rancidity and hydrolytic rancidity which was described in section 4.2.1. Moreover, the increase in free fatty acids during storage was influenced by the higher peroxide value. The free fatty acids are particularly amenable to further degradation by oxidative rancidity.

4.2.2.2 Free fatty acid

Free fatty acid values are used to measure the amount of free fatty acids liberated by the breakdown of triacylglycerols (Frankel, 1998) which is referred to as hydrolytic or lipolytic rancidity. The macadamia industry generally accepts free fatty acid values of 0.2 to 0.6 g /100g expressed as oleic acid to represent a product of good quality (McConachie, 2001 cited by Himstedt, 2002). However, the maximum value of free fatty acid was detected in this study was not more than 0.132 g/100 g expressed as oleic acid which is below the acceptable value.

The free fatty acids changed significantly ($p \leq 0.05$) during processing. The results show that the increase in free fatty acid content during hot air drying was more pronounced than during heat pump drying. This results from the higher decomposition of fatty acids during hot air drying via hydrolytic rancidity as described in 4.2.1. Moreover, the fatty acids during storage increased significantly ($p \leq 0.05$). According to Frankel (1998) the lipase and lipoxygenase involve in hydrolytic and oxidative rancidity, respectively. These enzymes were inactivated during roasting. Thus, the increase of free fatty acid content occurred through increasing of moisture

content during storage which indicated that the higher moisture content during storage might result in a higher free fatty acid content via hydrolytic rancidity.

Moreover, the free fatty acids present in macadamia nut resulted from hydrolytic rancidity after drying. The free fatty acids induced lipid oxidation during storage if oxygen was present. The results show that the decrease in oleic acid content after hot air drying was higher than after heat pump drying.

4.2.2.3 Moisture content and water activity

The moisture content of macadamia nut is the most important factor for macadamia nut stability. A higher moisture can stimulate the hydrolytic and oxidative rancidity. The suitable moisture contents during processing and storage are 1.5% and 1% wb, respectively (Dela *et al*., 1966). From the results, the moisture content increased during storage may be caused by the packaging materials and storage conditions as described in 4.2.1 (b). Grimwood (1971) mentioned that free fatty acids also increased with increasing temperature and moisture content. The kernels with a moisture content of 1 % wb should be stored at low temperature or kept frozen, it the moisture content is higher.

Labuza (1971) has suggested that the rate of lipid oxidation was decreased when water activity was in the range of 0.2-0.4. The water activity during processing and storage in this study shown in Table 4.6 were not higher than 0.4. Therefore, lipid oxidation may slowly occur during processing and storage.

4.2.3 Colour evaluation

The kernels were separated on the basic of colour. The colour changes of macadamia nut during processing and storage are shown in Table 4.7. From the study of Wall and Gentry (2006), good quality macadamia nut should have cream coloured kernel with internal and external L values of 74.3 and 71.1. Generally, kernels with internal browning (L = 59.9) or external browning (L = 55.3) had higher reducing sugar concentrations (0.24-0.27 g/100g db).

Table 4.7 summarized effects of processing and storage on quality in term of colour of macadamia nuts. Statistical analysis was done separately in each drying method since raw materials of each batch were not the same.

Table 4.7 Colour changes of macadamia nut during processing and storage

Stage	Colour			
	L		*a [ns]	*b [ns]
	External	Internal		
Raw	89.41 ±0.39 [e]	91.09 ±0.08 [d]	0.60±0.3	24.34±4.67
HA	83.20 ±0.22 [c]	87.08 ±0.07 [c]	1.65±0.88	26.5± 4.71
Roasting	82.52± 0.38 [b]	85.52 ± 0.15 [a]	1.65±0.94	27.96± 5.62
Storage 1 mo	84.30± 0.35 [d]	86.35± 0.07 [b]	1.64 ±0.95	27.12± 6.89
Storage 2 mo	83.32± 0.28 [c]	87.10± 0.10 [c]	1.91±0.99	27.1± 5.82
Storage 3 mo	81.18± 0.16 [a]	85.42± 0.16 [a]	2.01± 1.10	27.85± 4.76
Raw	89.32 ±0.39 [e]	91.16± 0.14 [e]	0.60± 0.30	24.34 ±4.67
HP+TD	85.32±0.40 [d]	87.35± 0.32 [d]	1.08± 1.50	26.49 ±5.24
Roasting	82.47± 0.43 [b]	86.52± 0.26 [c]	1.09 ±1.48	27.34± 6.44
Storage 1 mo	83.29±0.27 [c]	86.22± 0.12 [c]	1.44 ±1.04	27.44± 3.50
Storage 2 mo	81.79±0.21 [a]	83.32± 0.18 [b]	1.22 ±0.92	26.64± 4.87
Storage 3 mo	81.32± 0.28 [a]	82.72± 0.24 [a]	1.23 ±0.90	26.37± 4.49

Values are mean ±SD. Mean separation within columns based on significant differences ($p \leq 0.05$). * Each replication consisted of 20 kernels with average value of internal and external colour.
[ns] Non significant difference ($p > 0.05$)
L = lightness (0 = black, 100 = white) a = red components, b = yellow components

External and internal colour of macadamia kernel centre were slightly darker after drying. The result showed that the L value after drying with hot air drying and heat pump drying combined with tunnel drying decreased significantly ($p \leq 0.05$). This agrees with published literature on colour quality of macadamia kernels at different

stages of processing by Wall and Gentry (2006). Glucose and fructose present in fresh kernels were used in browning reaction during drying. However, drying temperatures used in the study were between 30 and 60°C and the drying process may limit sucrose hydrolysis, minimising the amount of glucose and fructose available for Maillard reaction. Therefore, the external and internal colour of dried kernels obtained was not much different from raw kernels and the roasted kernels were not darker than dried kernels. Moreover, the average minimum internal and external L value during processing and storage in both drying treatments were 82.72 and 81.18 (Table 4.7) which was higher than the L value of the cream coloured kernels which was 74.3 and 71.1 for internal and external colour of kernel (Wall and Gentry, 2006).

4.3 Effect of processing and storage on volatile compounds of macadamia nuts

The simultaneous steam distillation and extraction (Likens-Nickerson apparatus) was used to analyse the volatile compounds of macadamia nuts in this study and was followed by identification by gas chromatography-mass spectrometry (GC-MS). The volatile compounds macadamia nuts found during processing and storage are shown in Tables 4.8 and 4.9.

Table 4.8 Concentration (µg/kg, ppb) of volatile compounds after HA drying of macadamia nuts

Volatile compounds	Stage					
	Raw	**Hot air drying**	**Roasting**	**Storage 1 mo**	**Storage 2 mo**	**Storage 3 mo**
Alcohol						
Isopentanol				154.11	212.44	323.38
1-Ethoxy-2-propanol			98.75	113.25	154.09	182.77
1-Pentanol			193.34	330.79	453.89	721.19
1-Hexanol		213.22	467.40	615.92	874.54	1756.15
1-Heptanol		122.21	400.78	528.32	723.23	1623.96
2-Ethylhexanol				73.06	121.32	207.51
1-Dodecanol,3,7,11-trimethyl				30.77		
1-Octanol		156.34		441.14	652.32	1189.09
1-Nonanol				61.78	76.3	151.23
Total		**491.87**	**1160.07**	**2369.14**	**3268.13**	**6155.27**
Aldehyde						
Hexanal	72.76	243.26	700.08	1208.66	1433.2	2035.99
n Heptanal	127.64	287.4	375.43	477.07	665.44	1453.27
Heptenal			104.32	111.47	155.98	263.87
Benzaldehyde		34.56	86.53	114.67	175.43	283.96
Octanal	72.42	187.3	386.06	443.43	524.87	949.35
Benzeneacetaldehyde	29.66	132.43	232.45	136.45	200.4	310.93
2 Octenal		145.6	189.32	227.76	326.94	622.75
Nonyl aldehyde	275.12	301.31	467.00	501.48	784.23	1529.64
2-Decenal			153.33			793.34
Trans-2-undecenal		25.9	46.78	66.66	342.1	1860.79
Trans-2-tridecenal						82.49
Total	**577.6**	**1357.76**	**2741.3**	**3287.65**	**4608.59**	**10186.38**
Hydrocarbon						
Toluene		111.26	144.21	222.12	341.22	380.17
Octane		78.23	87.5	182.66	278.54	353.13
Dodecane				40.70		
Total		**189.49**	**231.71**	**445.48**	**619.76**	**733.3**
Miscellaneous						
Dimethyl Disulfide	25.2	311.23	300.19	287.33	251.44	216.17
Pyridine	48.9					

Table 4.9 Concentration (µg/kg, ppb) of volatile compounds after HP+TD drying of macadamia nuts

Volatile compounds	Stage					
	Raw	**Heat pump drying**	**Roasting**	**Storage 1 mo**	**Storage 2 mo**	**Storage 3 mo**
Alcohol						
1-Ethoxy-2-propanol			171.48	146.74	350.27	202.39
1-Pentanol			165.66	111.38	243.09	305.07
1-Hexanol			313.16	197.35	468.09	628.56
1-Heptanol			339.66	266.98	539.68	813.55
2-Ethylhexanol						
1-Dodecanol,3,7,11-trimethyl						
1-Octanol		77.34		166.19	473.59	680.34
1-Nonanol						
Total		**77.34**	**989.96**	**888.64**	**2074.72**	**2629.91**
Aldehyde						
Hexanal	72.76	144.67	639.5	508.94	1001.24	1110.90
n Heptanal	127.64	244.54	476.75	312.79	805.02	972.26
Heptenal			339.66		119.83	117.33
Octanal	72.42		350.57		662.49	
Benzeneacetaldehyde	29.66	143.23	303.29	495.52	547.09	730.53
2 Octenal		165.4	243.31		291.10	
Nonyl aldehyde	275.12	301.4	391.47	348.47		826.63
2-Decenal				122.21	304.29	359.77
Trans-2-undecenal					163.20	180.44
Trans-2-tridecenal			53.41		304.29	359.77
Total	**577.6**	**736.74**	**2979.07**	**1941.53**	**4674.97**	**5114.3**
Hydrocarbon						
Toluene		67.43	138.76	119.08	292.05	148.11
Octane		122.23	436.84	363.83	673.94	848.07
Dodecane						
Total		**189.66**	**575.6**	**482.91**	**965.99**	**996.18**
Miscellaneous						
Dimethyl Disulfide	25.20	27.24	214.22	37.23	221.34	233.67
Pyridine	48.29	48.2		57.20		

Lipid degradation is one of the most common sources of off-flavours in food products. The lipid degradation basically can be classified into two routes namely autoxidation and enzymatically induced degradation (Nijssen, 1991).

The unsaturated compounds can undergo further oxidation or secondary reactions resulting in the production of compounds such as aldehydes, ketones, acids, alcohols, hydrocarbon, lactones and esters. The types of compounds formed depend on type of hydroperoxide (Table 2.6), temperature and availability of oxygen (Joseph, 1991).

Two types of enzymes are involved in lipid degradation, namely lipase and lipoxygenase. In contrast to autoxidation, a relatively high moisture content is needed for the propagation of this pathway (Nijssen, 1991).

The decomposition of fatty acids after drying was described in section 4.2.1. as lipid oxidation and hydrolytic rancidity were the main causes of degradation of fatty acids. The volatile compounds such as hexanal, alcohols, hydrocarbons increased during processing and storage as shown in Tables 4.8 and 4.9. Moreover, the hexanal content, total alcohols and total hydrocarbons in samples dried with hot air were higher than in samples with heat pump dryer.

The decomposition of fatty acids, especially oleic acid (C18:1) in macadamia oil after drying was accompanied by the increase of volatile compounds which were derived from oleic hydroperoxide such as 2-undecenal, decanal, nonanal, 2-decenal and octanal (see Table 2.6). Presence of these derivatives can be seen in Tables 4.8 and 4.9.

According to Chitundu (1994) hexanal in macadamia nut kernels was increased during 3 months storage at 30°C in incubators maintaining a relative

humidity of 50% over a saturated solution of magnesium nitrate. The hexanal content increased during storage from 0.259 to 1.546 ppm.

The decomposition of fatty acids during hot air drying was more pronounced than during heat pump drying. From Tables 4.8 and 4.9, it can be seen that the increases of hexanal, total alcohol, aldehyde and hydrocarbon content after hot air drying were higher than after heat pump drying.

Moreover, the dimethyl disulfide and pyridine were found in every stage of processing and storage. These compounds can be found in thermal degradation from the processing such as during roasting via Maillard reaction (Figure 4.4). According to Arctander (1969), dimethyl sulfide is characterised by a strong, sweet burnt onion or cabbage odour which is described as repulsive at high concentrations. Crain and Tang (1975) identified dimethyl disulfide as a major compound in roasted macadamias and postulated that it contributes to the characteristic flavour of macadamias. However, the dimethyl disulfide and pyridine found in raw macadamia nut resulted from high temperature used during extraction the volatile compounds with Likens-Nickerson apparatus as high temperature affects on decomposition of proteins (Parliment, 1997). Pyrazine is generally found in other nuts such as in roasted peanuts where these compounds are thought to arise from the thermal interaction of sugars and amino acids (Mason *et al.*, 1966). The presence of pyridine and pyrazine via Maillard reaction is shown in Figure 4.4. However, this compound was not present in macadamia nut in this study. Crain and Tang (1975) found pyrazines in macadamia nuts roasted at 177°C.

The presence of dimethyl disulfide and pyridine are difficult to monitor as the peak areas of both compounds were difficult to separate and retention times were close to each other as can be seen in Appendix B. The overlap of peak areas occurred which

can be avoided by optimization the condition of GC-MS. However, optimization of GC-MS condition which is suitable to separate each volatile compound in macadamia nuts would require longer experimental time.

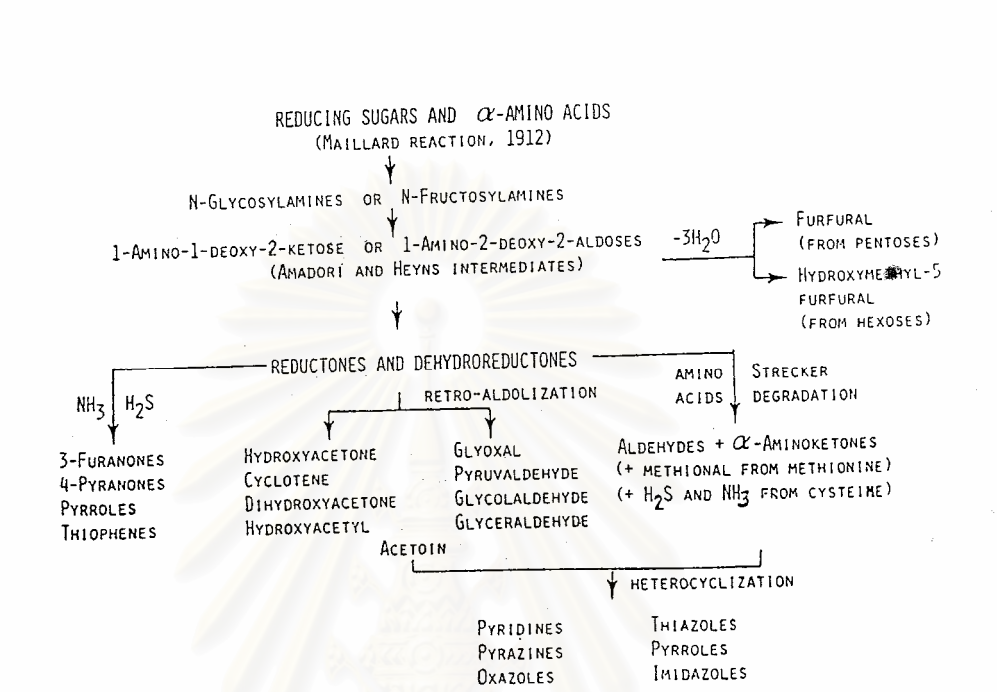


Figure 4.4 A summary of Maillard reaction (Parliment, 1989)

It does not appear that minor quantities of linolenic acid in macadamia oil as shown in Tables 4.5 contribute to any rancid flavour compounds. In the review of literature 2,4,7-decatrienal, 3,6-nonadienal, 2,4-heptadienal, 3-hexenal, 2-pentanal and propanal were shown as the main volatiles derived from the decomposition of linolenic acid (See Table 2.6) (Himstedt, 2002). If linolenic acid (C18:3) was broken down as a result of autoxidation, it would occur more rapidly than the decomposition of linoleic acid (C18:2) due to its high level of unsaturation. Therefore most of the linolenic compounds should be detected in the macadamia samples. Alternatively, linolenic acid did undergo autoxidation, however due to its low concentration in macadamias, this would not cause large amounts of volatiles to be produced.

During storage the results showed that increase of concentration of total alcohols, aldehydes and hydrocarbons occurred continuously as a result of lipid oxidation after drying. The packaging and conditions of storage macadamia nut could not protect macadamia nut from water and oxygen enough to delay the oxidation reactions. Moreover, the results showed that the decomposition of fatty acids occurred continuously during storage as described in section 4.2.1.

The comparison of volatile compounds in macadamia nuts that were found in this study agreed with previous studies as shown in Table 4.10.

Table 4.10 List of volatile compounds found in macadamia nuts in this study and in the literature

Volatile compound	Compound name	Sources
Alcohols	1-Ethoxy-2-propanol	4
	1-Pentanol	1, 2, 3, 4
	1-Hexanol	1, 2, 3, 4
	1-Heptanol	1, 2, 3, 4
	2-Ethylhexanol	4
	1-Dodecanol,3,7,11-trimethyl	4
	1-Octanol	1, 2, 3, 4
	1-Nonanol	4
Aldehydes	Hexanal	1, 2, 3, 4
	n Heptanal	1, 2, 3, 4
	Heptenal	4
	Benzaldehyde	1, 4
	Octanal	1, 2, 3, 4
	Benzeneacetaldehyde	1, 4
	2-Octenal	4
	Nonyl aldehyde (Nonanal)	1, 2, 3, 4
	Nonenal	4
	2-Decenal	4
	Trans-2-undecenal	4
	Trans-2-tridecenal	4
Hydrocarbons	Toluene	1, 4
	Octane	1, 3, 4
	Dodecane	4
Miscellaneous	Dimethyl Disulfide	1, 3, 4
	Pyridine	4

1 Crain and Tang (1975)

2 Chitundu (1994)

3 Himstedt (2002)

4 Present study

1, 2, 3 Identified using Headspace analysis by gas chromatography (GC-MS)

4 using Liken-Nickerson analysis and identified by gas chromatography (GC-MS)

Generally, the volatile compounds found in this study were similar to those found in previous research (Crain and Tang, 1975, Chitundu, 1994 and Himstedt, 2002) though the methods that were used to determine the volatile compounds were different. The advantage of Likens-Nickerson was the use of a small volume of solvent to extract the volatile aromas and then concentrate them in a single operation. In previous studies, it was concluded that factors affect quantity of volatile compounds are lipid and thermal degradation via Maillard reaction (Waltking and Goetz, 1983).

However, difficulties in identification of compounds were found with Likens-Nickerson and GC-MS. The reasons are as follow:

i) The different stage of macadamia nut such as drying or roasting can generate new volatile compounds. The same conditions of gas chromatography are not efficient enough to analyse or separate the new volatiles.

ii) The thermal degradation of proteins and sugars occurred in Likens-Nickerson apparatus which resulted in volatile compounds being detected that might not be from the original sample. Thus, the development of techniques such as vacuum distillation with heat sensitive samples before Liken-Nickerson apparatus can avoid the formation of new compounds as described above (Parliment, 1997).

Chapter 5

CONCLUSIONS

Macadamia nuts have about 72.83 % fat and the oil grading by specific gravity is ≥72 g/100g oil. Although macadamia nuts are rich in fat, they are generally low in saturated fatty acid (SFA) and high in monounsaturated fatty acid (MUFA). There were eleven fatty acids found in macadamia nuts used in this study. Among them, oleic acid had the highest content (60.97%), followed by palmitoleic acid (18.19 %) and palmitic acid (8.35%). The ratio of unsaturated fatty acid to saturated fatty acid was 5.78:1. Therefore, macadamia nuts are highly susceptible to oxidation due to high proportion of unsaturated fatty acid.

Volatile compounds found in raw macadamia nut were determined by Likens-Nickerson method. They included hexanal, heptanal, octanal, benzeneacetaldehyde, dimethyl disulfide and pyridine. The aldehydes were present in raw nut. They might be formed by the autoxidation of unsaturated fatty acid via enzymatic reaction. Further compounds, such as pyridine and dimethyl disulfide, were derived from the heat-induced interaction of amino acids and sugars (Maillard reaction) that occurred at high temperature during distillation and extraction with Likens-Nickerson apparatus.

Generally, after each stage of processing and storage, the content of fatty acids, peroxide value and free fatty acids changed significantly ($p \leq 0.05$). The drying treatments can affect differently the quality of macadamia nuts. Heat pump drying combined with tunnel drying can reduce decomposition of fatty acids vs. hot air drying. This affects particularly, the oleic and palmitoleic acids, which are claimed to prevent cardiovascular disease. Moreover, the heat pump drying can reduce the

degradation of fatty acid due to hydrolytic rancidity as a result of increasing concentration of free fatty acids during processing. Subsequently, the peroxide value and free fatty acid value after drying with heat pump drying combined with tunnel drying were less than those hot air drying.

The degradation of fatty acids during roasting and storage decreased continuously while free fatty acid and peroxide value were increased due to lipid oxidation and hydrolytic rancidity which occurred during drying. However, a small change was found during roasting and storage as less moisture contents was present after roasting (1% wb). In addition, the final peroxide value and free fatty acid that were detected when storing nuts at 4°C for 3 months were not more than 0.62 meq/kg oil and 0.132 g/100 g (% oleic acid) and thus within acceptable levels. The peroxide value and free fatty acid of macadamia oil should be less than 3 meq/kg oil and 0.2 to 0.6 g /100 g (% oleic acid).

The decomposition of fatty acids contributes to increase of total aldehydes, total alcohols and total hydrocarbons as results of lipid oxidation and hydrolytic rancidity. In particular, these were the volatile compounds which were derived from oleic hydroperoxide such as 2-undecenal, decanal, nonanal, 2-decenal and octanal. Therefore, the decomposition of fatty acids during hot air drying was more pronounced than in heat pump drying. The concentration of hexanal, total alcohol, aldehyde and hydrocarbon after hot air drying were higher than after heat pump dryer combined with tunnel drying.

The changes of volatile compounds were more pronounced after roasting as a result of high temperature treatment. During drying, the changes of volatile compounds occurred mostly due to lipid oxidation. Again, small changes of volatile compounds were observed after heat pump drying. Macadamia nut dried using

traditional hot air drying method (30°C for 7 days, 40°C for 7days, 60°C for 3 days) were found to be subjected to higher level of deterioration of fatty acid content and colour of the nut kernel due to long period of drying (17 days). Meanwhile, heat pump drying took 3 days to complete drying process and to reduce moisture content of the nut in shell to 1.5 % wb.

Colour value (L) of macadamia kernel centre after roasting and drying with heat pump dryer combined with tunnel drying and hot air drying was not significantly different ($p>0.05$). This resulted from the using incremental drying by both drying methods which were limiting sucrose hydrolysis and reducing the amount of glucose and fructose available for Maillard reaction. Moreover, the average minimum internal and external L value during processing and storage for both drying treatments was 82.06 which was higher than the L value of the cream coloured kernels which was 72.7 for colour of kernel.

Finally, heat pump dryer combined with tunnel drying showed advantages of time saving and quality preservation of the macadamia nuts more than hot air drying. The changes of fatty acids, volatile compounds, colour and rancidity after drying and roasting can continue during storage of macadamia nuts. At this point the packaging material and the condition of storage did affect the quality of macadamia nuts. However, the final value of peroxide values, free fatty acid and colour were still within acceptable limits.

Recommendations

1. Sensory test on rancidity along with amount of off flavour detected in this study should be developed to obtain the cut off limit for consumers in order to reject deteriorated nut.

2. Vacuum distillation (at ambient temperature) should be used before Likens-Nickerson process to avoid the thermal degradation of protein and sugar from long exposure to heat during Likens-Nickerson process.

3. Different stages of processing such as drying or roasting can generate new volatile compounds. The standard conditions of gas chromatography used in this study were not efficient enough to analyse or separate the new volatiles. Therefore, optimisation of the conditions of GC-MS appropriate to analyse each key volatile compound should be considered in further research.

REFERENCES

AOAC, 2000. Official Methods of Analysis. Association of official analytical chemist. 15 th ed. Washington D.C.: Association of official analytical chemists.

Anon. 2000. Australian Macadamia Society Researchers Meeting, Transcontinental Hotel, Brisbane. March 10.

Arctander, S. 1969. Perfume and Flavour Chemicals (Aroma Chemicals). USA: Steffen Arctander, publisher.

Australian Macadamia Society. 2006. The macadamia story. [online]. Australia. Source: http://www.macadamias.org. [15 November 2007].

Bannon, C.D., Breen, G.J., Craske, J.D., Ngo Trong Hai, Harper, N.L. and O'Rourke, K.L. 1982. Analysis of fatty acid methyl esters with high accuracy and reliability III. Journal of Chromatography. 247: 71-89.

Bannon, C.D., Craske, J.D. and Hilliker, A.E. 1986. Analysis of fatty acid methyl ester with high accuracy and reliability V. American oil Chemical Society. 63: 105-110.

Beuchat, L. and Worthinton, R.E. 1978. Fatty acid composition of tree nut oils. Food Technology. 13(4): 355-358.

Cavaletto, C.G., Dela Cruz, A., Ross, E., and Yamamoto, H.Y. 1966. Factors affecting macadamia nut stability. 1. Raw kernels. Food Technology. 20(8): 1084-1087.

Cavaletto, C.G., Ross, E., and Yamamoto, H.Y. 1968. In-Shell storage effects on quality of processed macadamia nuts. Food Technology. 22(3): 172-174.

Cavaletto, C.G., Dela, C.A., Ross, E. and Yamamoto. 1978. Composition, flavour and nutritive value of Macadamia nuts. American Oil Chemical Society. 176.

Cavaletto, C.G. 1980. Macadamia Nuts. In Tropical and Subtropical Fruits – Composition, Properties and Uses, Nagy, S. and Shaw, P.E. (ed). Australia: AVI publisher. 542-561.

Cavaletto, C.G. 1983. Macadamia nuts. In Handbook of Tropical Foods, Chan, H. T. (ed.). New York, U.S.A: Marcel Dekker, Inc. 361–397.

Chitundu, E.N. 1994. Studies on shelf life of macadamia nuts. Master's Thesis, School of Food Sciences, Faculty of Science and Technology, University of Western Sydney-Hawkesbury, Richmond, New South Wales. Australia

Chua, K.J., Chou, S.K., Ho, J.C. and Hawlader, M.N.A. 2002. Heat pump drying: recent developments and future trends. Drying Technology. 20(8): 1579-1610.

Colquhoun, D.M., Humphries, J. A., Moores, D., and Somerset, S.M. 1996. Effects of a macadamia nut enriched diet on serum lipids and lipoproteins compared to a low fat diet. Food Australia. 48(5): 216-222.

Crain, W.O. and Tang, C.S. 1975. Volatile components of roasted macadamia nuts. Journal of Food Science. 40: 207-208.

Cronin, D.A. 1982. Techniques in analysis of flavours. In Food Flavours, Morton, I.D. and MacLeod, A.J. (ed). Amsterdam: Elsevier Science publisher. 15-33.

Cronin, D.A. and Caplan, P.J. 1987. Application of GC/MS to identification of flavour compounds in foods. In Application of Mass Spectrometry in Food Science. Gilbert, J. (ed). London: Elsevier Science publisher. 1-71.

Curb, J.D., Wergowske, G. and Jean Hankin, J.A. 1992. The effect of dietary supplementation with macadamia kernels on serum lipid levels in human. Proceedings of the first International Macadamia Research Conference. Hawaii: Kona Hilton, Kailua-Kona. 129 -136.

Dela, C.A., Cavaletto, C., Yamamoto, H.Y. and Ross, E. 1966. Factors affecting macadamia nut stability. II Roasted kernels. Food Technology. 20: 1217-1218.

Department of Agriculture. 2004. Question and Answer of Agriculture [online]. Chiangmai. Source. http://www.doa.go.th/public/agro-tourism/chaingmai/chaingmai.htm.[17 November 2006].

Dickes, G.J. and Nicholas, P.V. 1976. Gas Chromatography in Food Analysis. Butterworth Co. Ltd. London.

Fourie, P.C. and Basson, D.S. 1989. Predicting rancidity in stored nuts by means of chemical analyses. Lebansm Wiss. Food Technology. 22: 251-253.

Frankel, E.N. 1998. Lipid Oxidation. 2nd edition. England: The Oily Press.

Grimwood, B.E. 1971. The processing of macadamia nuts. The Tropical Products Institute. London. 94-100.

Grob, K. 2001. Split and Splitless Injection For Quantitative Gas Chromatography. WILEY-VCH. Germany.

Gunstone, F. and Norris, F.A. 1983. Lipids in Foods - Chemistry, Biochemistry and Technology. Oxford, England: Pergamon Press.

Hahtamaki Pty Ltd. 2004. Active barrier packaging. [online]. Japan. Source: http://www.plastech.pl/search. [10 December 2007].

Hamilton, R.A. and Lto, P.J. 1976. Development of macadamia nut cultivars in Hawaii. California Macadamia Society Yearbook. 21: 94-100.

Henrich, L.H. 1995. Detectors and data handling. In Modern practice of gas chromatography, Grob, R.L. (ed), 3rd edition. Canada: John Wiley and Sons publisher.

Himstedt, S.R. 2002. Oil content and other components as indicators of quality and shelf life of macadamia kernels. Doctoral dissertation, The School of Land and Food, University of Queensland, Australia.

Huston, C.K. 1997. Ion trap mass spectrometry for food aroma analysis. In Techniques For Analyzing Food Aroma, Marsili, R. (ed), New York: Marcel Dekker, Inc. 209-233.

Joseph, A.M. 1991. Nuts. In Volatile Compounds in Food and Beverages. Maarse, H. (ed). New York: Marcel Dekker Inc. 689-723.

Kaijser, A., Dutta, P. and Savage, G. 2000. Oxidative stability and lipid composition of macadamia nuts grown in New Zealand. Food Chemistry. 71: 67-70.

Kinderlerer, J.L. and Johnson, S. 1992. Rancidity in hazelnuts due to volatile aliphatic aldehydes. Journal of the Science of Food and Agriculture. 58: 89-93.

Kinlin, T.E., Muralidhara, R., Pittet, A.O., Sanderson, A. and Walradt, J.P. 1972. Volatile components of roasted filberts. Journal of Agriculture and Food Chemistry. 20(5): 1021-1028.

Kowitz, T.K. 2000. Personal communication. The University of Queensland, Gatton, Australia.

Kungsadanaumpai, K., Supachaiweerakun, J and Wiwatbudsiri, S. 2006. Study of Lipid Composition in Macadamia Nut of Different Varieties Grown in Thailand. Bachelor's Thesis, Department of Food Technology, Faculty of Science, Chulalongkorn University.

Labuza, T.P. 1971. Kinetics of lipid oxidation in foods. CRC Critical Reviews in Food Science and Nutrition. 2: 355-405.

Lawson, H. 1995. Food Oils and Fats - Technology, Utilisation and Nutrition. New York, USA: Chapman and Hall publisher.

Leverington, R.E. 1958. The macadamia nut. Food Technology in Australia. 10(5): 243-251.

Leverington, R.E. 1971. The macadamia nut industry. Food Research Quarterly. 31(2): 57-65.

Lohachoompol, V. 2005. Effects of drying on anthocyanins in blueberries. Doctoral dissertation, Food Science and Technology, Faculty of Engineering University of New South Wales.

Macfarlane, N. and Harris, R.V. 1981. Macadamia nuts as an edible oil source. In New Sources of Fats and Oils, Pryde, E.H., Princen, L.H., and Mulherjee, K.D. (ed). American Chemical Society. Champaign. 103-108.

McConachie, I. 2001. Personal Communication. Suncoast Gold Macadamias Pty. Ltd., Gympie, Queensland, Australia.

Mason, M. E., Johnson, B. and Hamming, M. 1966. Flavour components of roasted peanuts. Some low molecular weight pyrazines and a pyrrole. Food chemistry. 14: 454-460.

Mason, R.L. 1982. The relationship between specific gravity and quality in macadamia nuts. Master's Thesis, The University of New South Wales, Australia.

Mason, R.L. and McConachie, I. 1994. A hard nut to crack. Food Australia. 46(10): 466-471.

Mason, R.L., Kowitz, T.J., Bowden, R.P. and Isaacs, A.R. 1998. Storage of macadamia nut-in-shell. Horticulture Australia Ltd. Sydney, Australia.

Mason, R. L. and Wills, R. B. H. 2000. Macadamia nut quality research: The processing challenge. Food Australia. 52(9): 416–419.

Merritt, J.R.C. and Robertson, D.H. 1982. Gas chromatography and mass spectrometry. In Food flavours, Morton, I.D. and Macleod, A.J. (ed). Netherlands. 49-73.

Moltzau, R.H. and Ripperton, J.Co. 1939. Processing of the macadamias. Hawaii Agricultural Experiment Station. Bull. 83.

Nambucca Macnuts Pty Ltd. 2006. The Australia macadamia nut industry. [online]. Australia. Source. http://www.macnuts.com.au/industry.htm. [21 November 2006].

Nickerson, G.B. and Likens, S.T. 1966. Gas chromatography evidence for the occurrence of hop oil components in beer. Journal of Chromatography. 21: 1-3.

Nijssen, B. 1991. Off-flavours. In Volatile Compounds in Food and Beverages, Maarse, H. (ed). New York: Marcel Dekker Inc. 689-723.

Ozdemir, M., Ackurt, F., Yildiz, M., Biringen, G., Gurcan, T. and Loker, M. 2001. Effect of roasting on some nutrients of hazelnuts. Food Chemistry. 73: 185-190.

Parliment, T.H. 1989. Thermal generation of aromas. In Thermal generation of aroma, Parliment, T.H., McGorrin, R.J. and Ho, C.T. (ed). Washington, DC: American Chemical Society. 2-11.

Parliment, T.H. 1997. Solvent Extraction and Distillation Techniques. In Techniques for Analyzing Food Aroma, Marsili, R (ed). New York: Marcel Dekker Inc. 1-25.

Petterson, H.B.W. 1989. Handling and Storage of Oilseeds, Oils, Fats and Meal. London and New York : Elsevier Applied Science.

Prichavudhi, K. and Yamamoto, H.Y. 1987. Effects of drying temperatures on chemical composition and quality of macadamia nuts. Food Technology. 19(5): 129-132.

Saleeb, W.F., Yermanos, D.M., Huszar, C.K., Story. W.B., and Labanauskas, C.K. 1973. The oil and protein in nuts of *Macadamia tetraphylla* L. Johnson, *Macadamia integrifolia* Maiden and Betche, and their F_1 hybrids. American Society for Horticultural Science. 98(5): 453-456.

Shahidi, F. 2001. Lipids in flavor formation. In Flavor Chemistry: Industrial and academic research, Risch, S.J. and Ho, C.T. (ed). Washington DC:.American Chemical Society.

Silva, F.A., Marsaioli, A.J., Maximo, G.J., Silva, M.A.A.P. and Goncalves, L.A.G. 2005. Microwave assisted drying of macadamia nuts. Journal of Food Engineering. 77: 550-558.

Somsong, P. 2007. Comparison method analysis of 2AP in fragrant rice. Bachelor's Thesis, Food Science and Technology, Faculty of Engineering, University of New South Wales.

Sunthonvit, N., Srzednicki, G, Craske., J.D. 2005. Effect of high temperature drying on the flavour components in Thai fragrant rice. Drying Technology 23: 1407-1418.

Sunthonvit, N. 2005. Effects of drying strategies on volatile compounds in stone fruit. Doctoral dissertation, Food Science and Technology, Faculty of Engineering. University of New South Wales.

Trochoulias, T. 1984. The Potential for Macadamia in Thailand. Department of Agriculture, New South Wales, Australia.

USDA Economic Research Service. 2004. Fruit and Tree Nuts. Publication 290. October. United States Department of Agriculture.

Vivien, H. L. 1989. Storage stability of macadamia nuts. Bachelor's Thesis, Food Technology, Faculty of Applied Science, School of Applied Bioscience Food Science and Technology, University of New South Wales.

Waldradt, J.P., Pittet, A.O., Kinlin, T.E., Muralidhara, R. and Sanderson, A. 1971. Volatile components of roasted peanuts. Journal of Agriculture and Food Chemistry. 19(5): 972-979.

Wall, M.M. and Gentry, T.S. 2006. Carbohydrate composition and color development during drying and roasting of macadamia nuts. Lebensmittel –Wissenschaft and Technologies. 23: 241-247.

Waltking, A.E. and Goetz, A.G. 1983. Instrumental determination of flavour stability of fatty foods and its correlation with sensory flavour responses. CRC Critical Reviews in Food Science and Nutrition. 19(2): 99-132.

Wang, P.S. and Odell, G.V. 1972. Characterisation of some volatile constituents of roasted pecans. Journal of Agriculture and Food Chemistry. 20(2): 206-210.

Winterton, D. 1966. Macadamia nut roasting studies. Food Technology in Australia. 16: 74-77.

Winterton, D. 1968. The macadamia nut industry- problems and prospects. Food Technology in Australia. 20: 119-121.

Woodroof, J.G. 1979. Tree Nuts: Production, Processing, Products. 2th edition. Westport, Australia: AVI publisher.

APPENDICES

APPENDIX A

Analytical Methods

A.1 Colour evaluation method (Wall and Gentry, 2006)

The colour of dried products was measured using a Hunter LAB meter (Figure A.1) (Minolta, model CR-300 Series equipped with data processor DP-301). The meter was calibrated with white plate no. 13233086 prior to measurement. A set of 20 kernels of macadamia was measured on inside and outside of each kernel for L, a, b value.

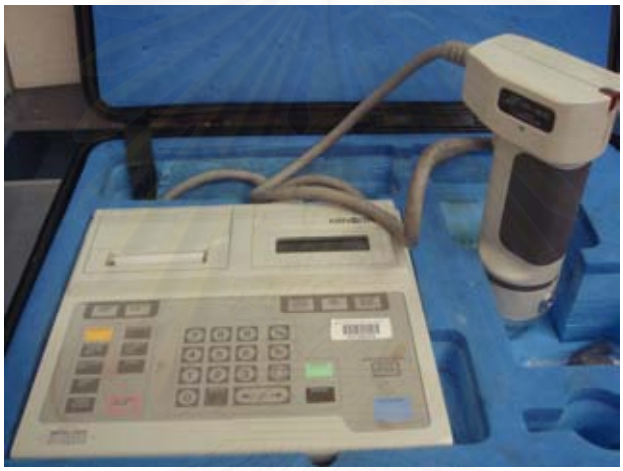


Figure A.1 Colour meter equipment

A.2 Protein Analyses (AOAC, 2000) Method 950.48

The protein content of macadamias was determined in duplicate using the Kjeldahl method as described in AOAC method 950.48 (AOAC, 2000). The digestion unit B-426 and the distillation unit B-324 were used for digestion and distillation (Buchi, Switzerland).

Place weight test portion in digestion flask. Add a tablet of K_2SO_4 and add 15 ml of H_2SO_4. Place flask in digestion unit and heat gently until frothing ceases; boil briskly until solution clears. Then cool and add methyl red 1 drop into the distillation unit. The NaOH solution was added to sample flask to make contents strongly

alkaline. The mixed indicator 3-4 drops were added in a receiver flask. Then heat until all NH_3 has distilled (≥150 ml distillate). Remove receiver flask, wash tip of condenser, and titrate the ammonium salt in distillate with standard 0.1 N H_2SO_4 solution until the gray colour was appeared. Correct for blank determination on reagents.

The protein conversion factor used was 5.30 (AOAC, 2000). Oil present in macadamia nuts for kjeldahl analysis causes inaccurate and false results. Therefore, the sample were defatted by using Soxhlet apparatus prior to tested. The 0.4 g of defatted sample was used in this study. Protein content is calculated according to equation (A.1) and (A.2)

$$Total \quad nitrogen \quad (\%) = \frac{(Va - Vb) \times 1.4007 \times N}{W(g)} \qquad \text{..... (A.1)}$$

$$\%\ \text{Protein} = \%\ \text{Total nitrogen} \times \text{F} \qquad \text{.... (A.2)}$$

Where:

Va	=	ml of standard H_2SO_4 titrated with sample
Vb	=	ml of standard H_2SO_4 titrated with blank
N	=	Normality of H_2SO_4 (N)
1.4007	=	milliequivalent weight of nitrogen x 100
W	=	weight of sample(g)
F	=	Conversion factor of macadamia nut

A.3 Determination of Lipid content

Macadamia nuts were cracked and shelled, then chopped into small pieces. Crude oil was obtained from finely chopped nuts, approximately 2 g extracted with petroleum ether b.p 40-60 °C in a Soxhlet (LABEC, Australia) (Figure A.2). The remaining solvent was removed by rotary evaporator (Figure A.3) (Buchi, Switzerland).This process was carried out for 6 hours. Then the flask was taken off and left in the hot air oven to remove residue traces of petroleum ether and the sample was weighed to calculate lipid content by using the following equation.

$$\text{Lipid (\% dry basis)} = \frac{(\text{Final wt of rotary flask} - \text{Initial wt of rotary flask})}{\text{weight of sample (db)}} \times 100 \ldots (A.3)$$

Figure A.2 Soxhlet extraction apparatus

Figure A.3 Rotary evaporation apparatus

A.4 Determination of moisture content

Nut in shell were cracked manually using a vice (Figure A.4). The kernels and shell were separately and chopped in small pieces. A 10 g of sample were placed into different dishes then dried in hot air oven at 103°C for 48 hours and the dried sample were cooled in a desiccator before weighing.

$$\text{MC (\% wb)} = \frac{(\text{Water loss from kernel} + \text{Water loss from shell})}{(\text{weight of kernel} + \text{weight of shell})} \times 100 \qquad \text{…. (A.4)}$$

Figure A.4 Vice for cracking machine nuts

A.5 Determination of Water activity (a_w)

The kernels were chopped in small pieces. Water was used to calibrate before the sample were analysed. A 3 g sample was placed into sample dish then put in a water activity analyser (Aqua lab, USA) The water activity of a product can be determined from the air surrounding the sample when the air and the sample are at equilibrium. Then, the data were recorded.

A.6 Determination of Peroxide value (AOAC, 2000) Method 965.33

Peroxide value (PV) was determined from oil extracted from kernels for all treatments using the AOAC (2000) method.

Macadamia oil (5.00±0.05) was weighed into a 250 ml Erlenmeyer flask and 30 ml of acetic acid-chloroform solution (3:2 v/v) was added. The oil was swirled to dissolve in the solvent and 0.5 ml of saturated potassium iodine solution was pipetted into the sample which was immediately placed in the dark to allow maximum colour development for exactly 1 min. Water (30 ml) was added to the sample immediately after 1 min. Starch solution (0.5 ml, 1%) was then added and the solution slowly titrated with 0.01 N $Na_2S_2O_3$ with vigorous shaking to release all iodine from the chloroform layer, until the blue colour is disappeared. The volume of $Na_2S_2O_3$ was recorded (a). A blank test was carried out in the same way. Volume of $Na_2S_2O_3$ used in blank was recorded as (b).

Peroxide value was calculated according to following equation and expressed as milliequivalents peroxide/kg sample:

$$PV(meq/kg\ \ oil) = \frac{N \times (a-b) \times 1000}{g(oil)} \qquad \text{....(A.5)}$$

Where:

N = the normality of the sodium thiosulphate standard solution

a = volume (ml) of the sodium thiosulphate solution obtained from the sample.

b = volume (ml) of the sodium thiosulphate solution obtained from the blank.

A.7 Determination of Free fatty acid (AOAC, 2000) Method 940.28

Weigh 7.00±0.05 g of macadamia oil into 250 ml flask. Add 50 ml ethanol, previously neutralized by adding 2 ml phenolphthalein solution and enough 0.1 N NaOH to produce faint permanent pink. Titrate with 0.25 N NaOH with vigorous shaking until permanent faint pink appears and persists more than 1 min. Free fatty acid is reported as % oleic acid by ml of 0.25 N NaOH used in corresponded titration.

A standard curve of concentration % oleic acid and volume of 0.25 N NaOH was used to titrate (ml) are shown in Figure A.5.

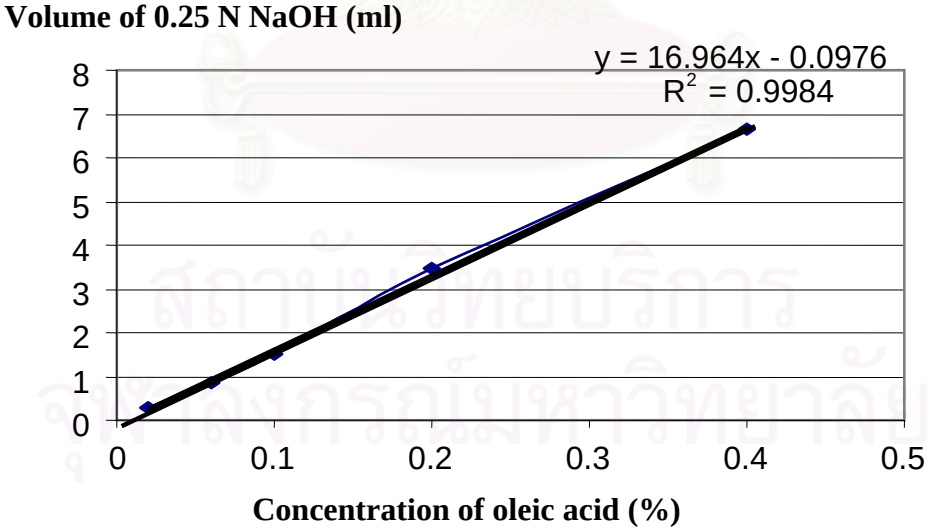


Figure A.5 Standard curve of concentration oleic acid and volume titrate of NaOH

The equation of the standard curve is

$$y = 16.964x - 0.0976 \quad \text{....(A.6)}$$

Where:

y = Volume of 0.25 NaOH (ml)

x = Concentration of oleic acid (%)

Determination of Specific gravity (SG)

Nut in shell were cracked manually using nut cracker (Figure A.4). The 20 kernels were floated in water (SG = 1). If all 20 kernels are floating in water, it can be concluded that the oil contents of kernels is more than ≥ 72g/100g oil.

Generally, the nut in shell were grading in the previous step

APPENDIX B

Chromatograms of volatile compounds from GC-MS

B.1 The chromatogram of purified tetradecane

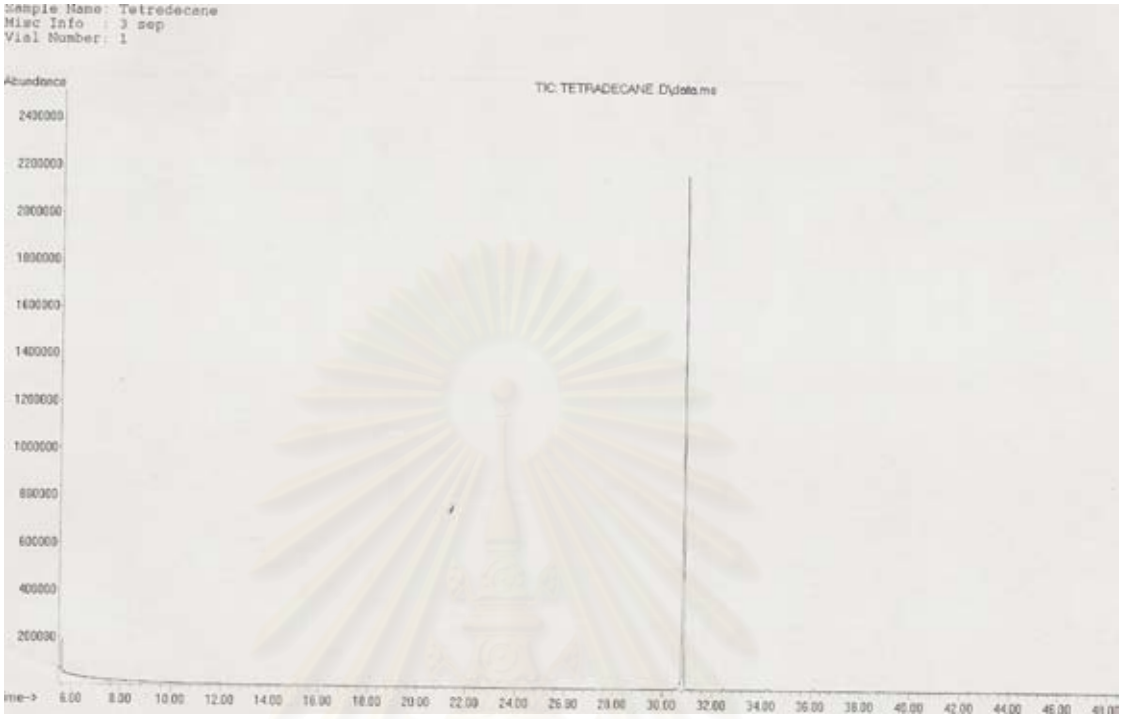


Figure B.1 The chromatogram of purified tetradecane (Internal standard)

The chromatograms of volatile compounds in macadamia nut subjected to different drying methods and storage duration

B.2 The chromatograms of volatile compounds in macadamia nut subjected to hot air drying treatment are shown below.

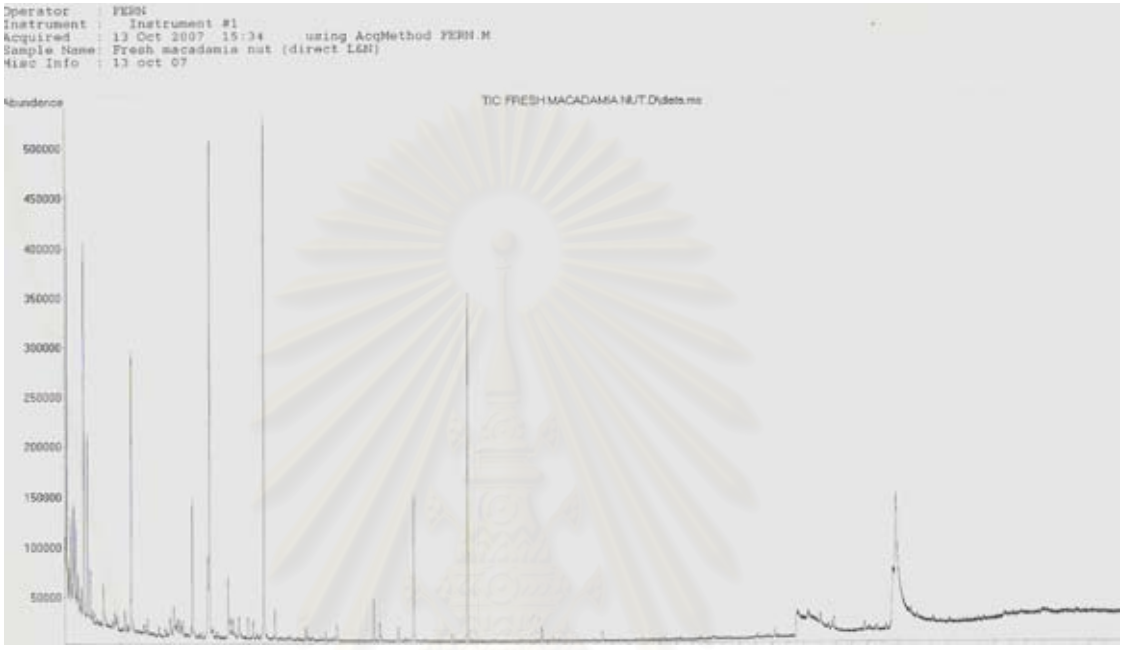


Figure B.2 The chromatogram of volatile compounds in fresh macadamia nuts

Figure B.3 The chromatogram of volatile compounds in dried macadamia nuts

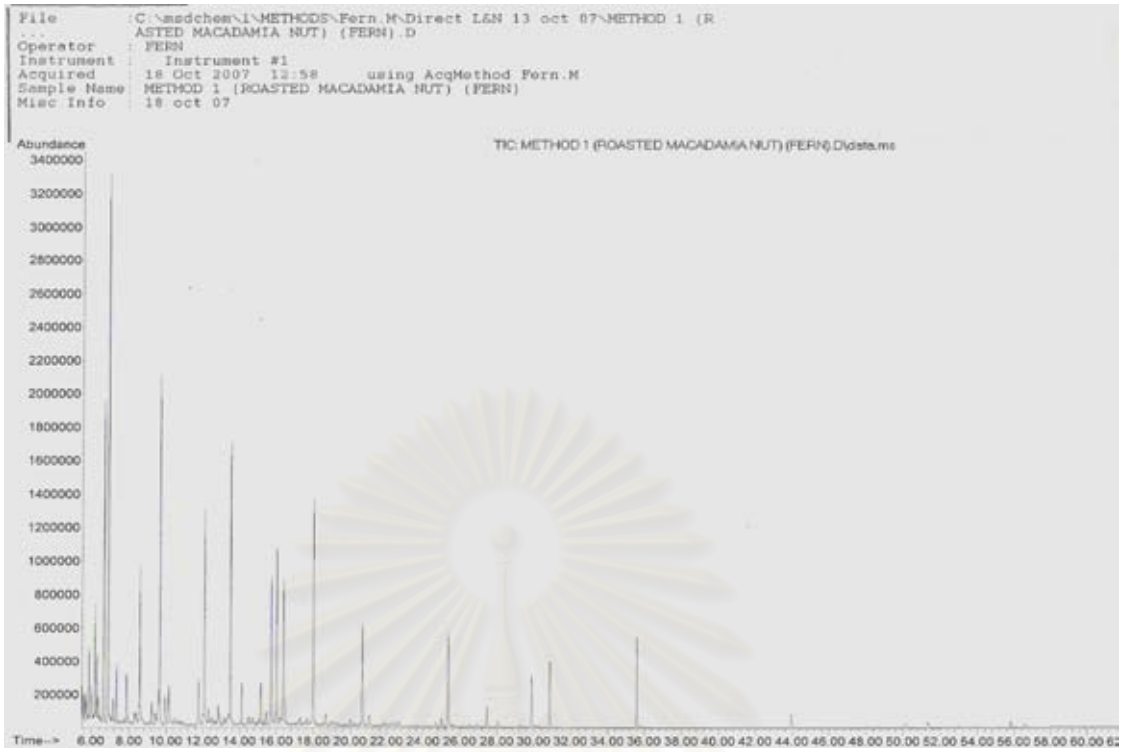


Figure B.4 The chromatogram of volatile compounds in roasted macadamia nuts

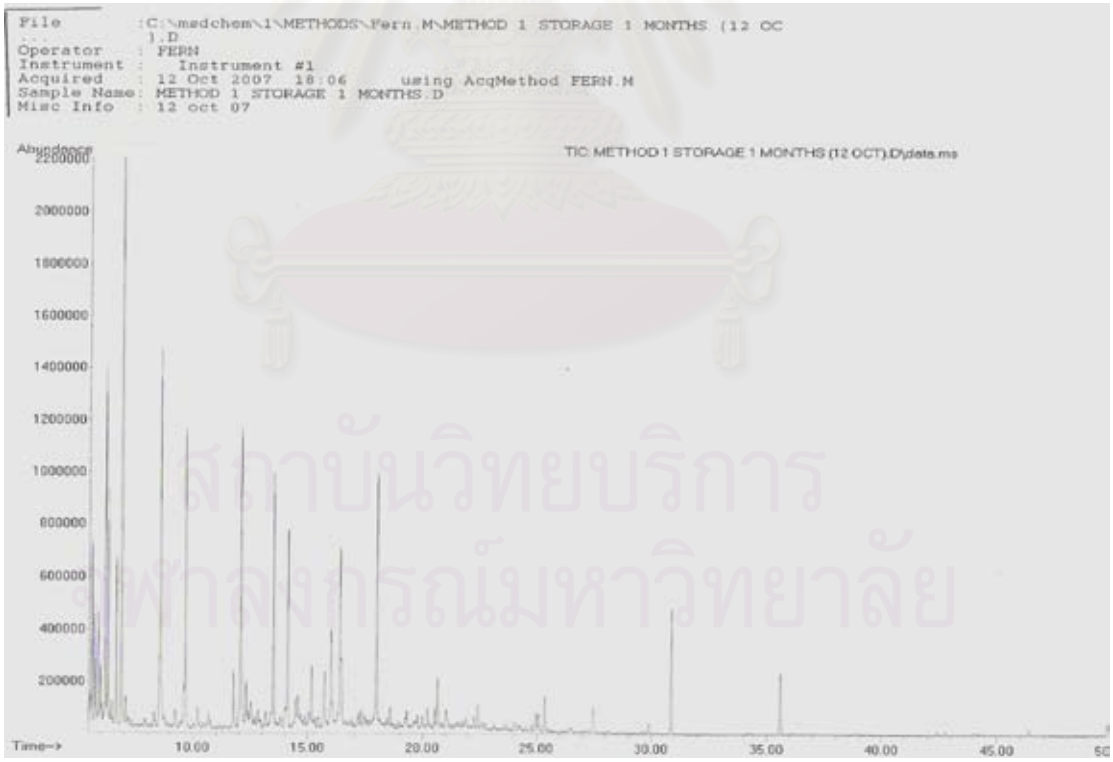


Figure B.5 The chromatogram of volatile compounds in macadamia nuts after 1 month storage

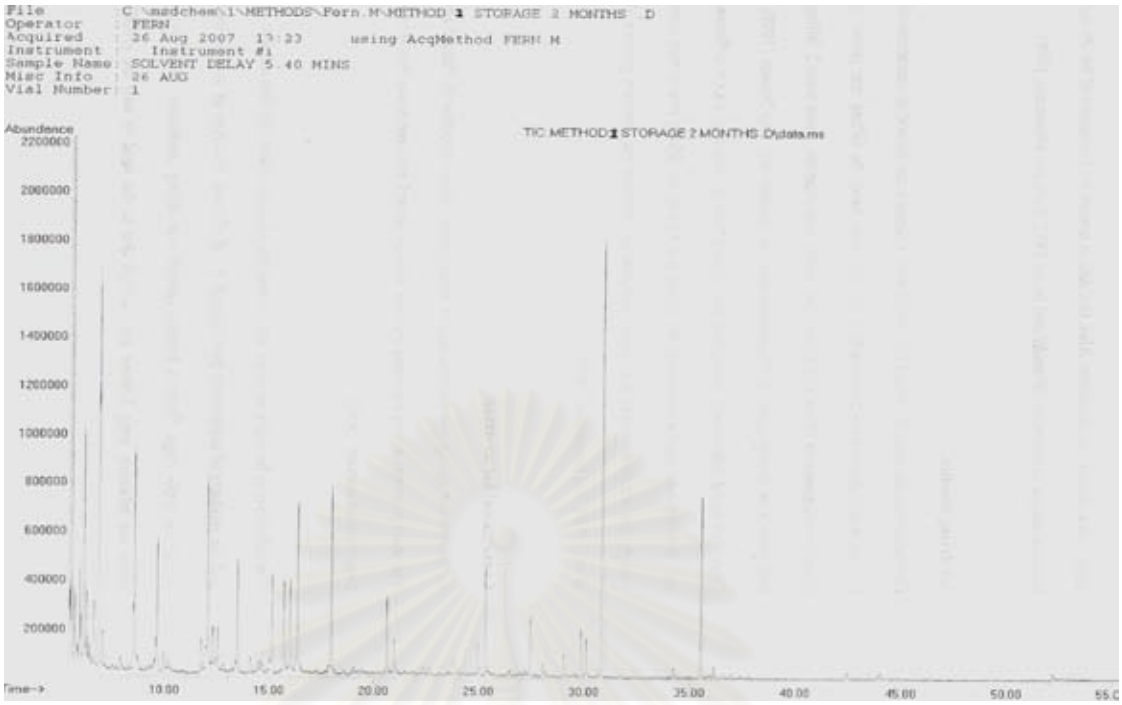


Figure B.6 The chromatogram of volatile compounds in macadamia nuts after 2 months storage

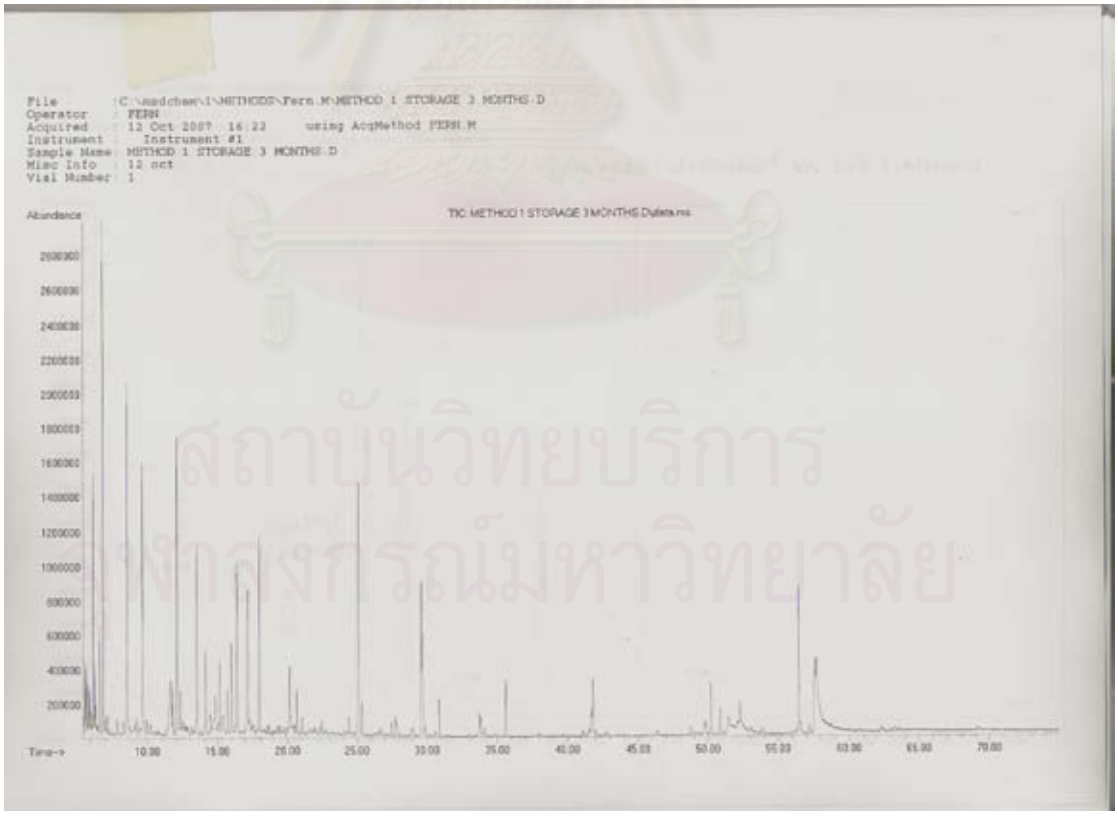


Figure B.7 The chromatogram of volatile compounds in macadamia nuts after 3 months storage

B.3 The chromatograms of volatile compounds in macadamia nut subjected to heat pump dryer combined with tunnel drying treatment are shown below.

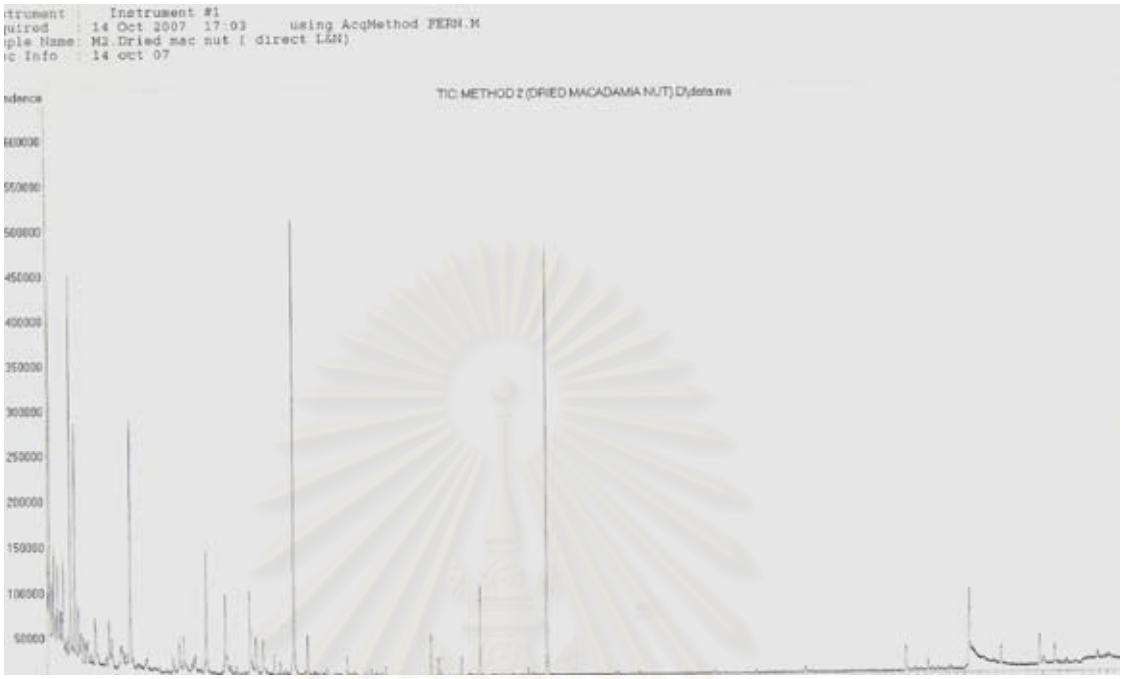


Figure B.8 The chromatogram of volatile compounds in dried macadamia nuts

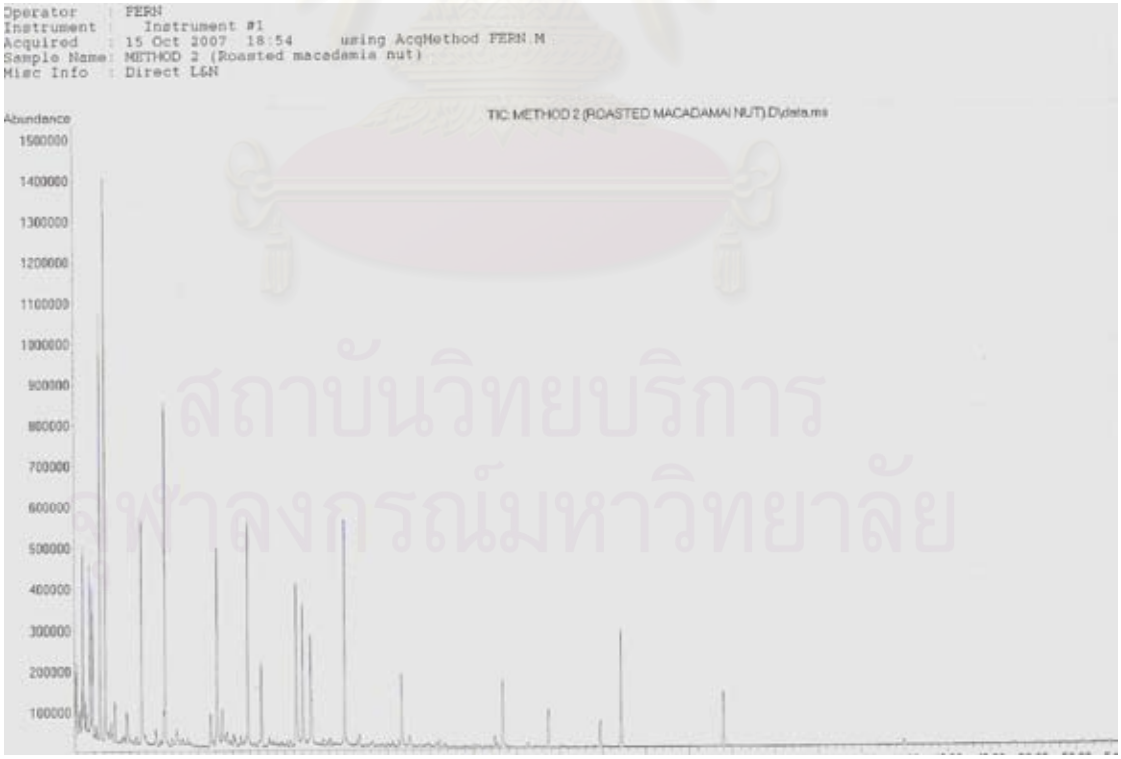


Figure B.9 The chromatogram of volatile compounds in roasted macadamia nuts

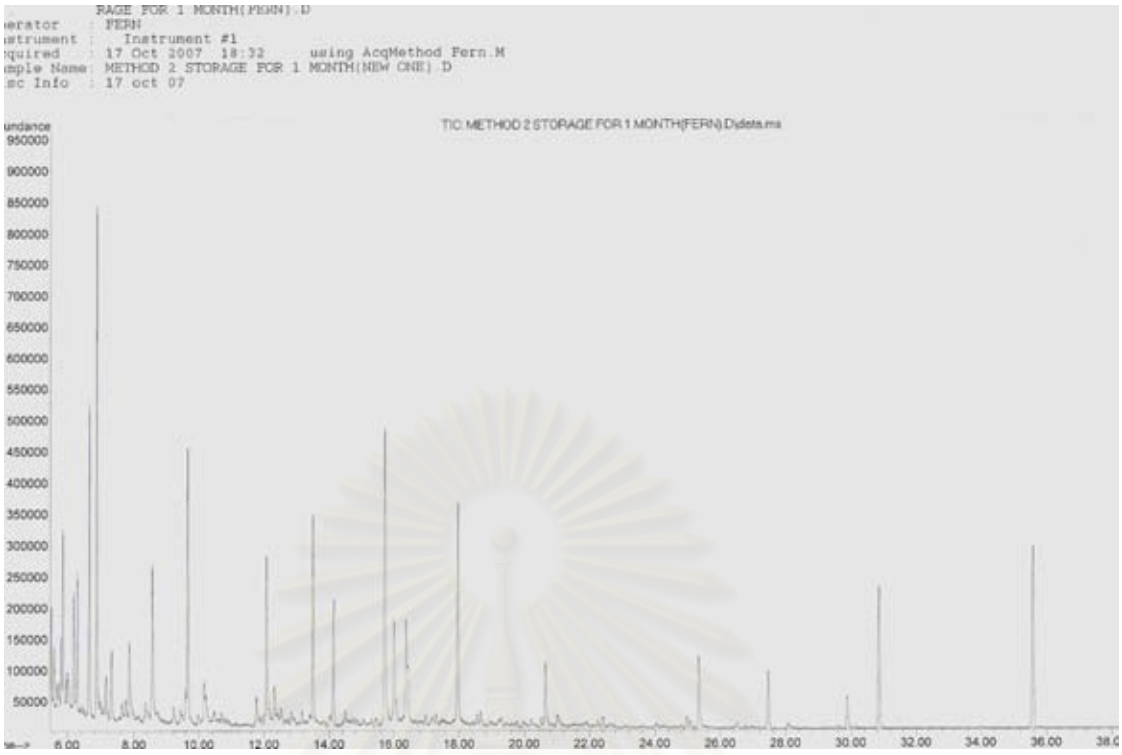


Figure B.10 The chromatogram of volatile compounds in macadamia nuts after 1 month storage

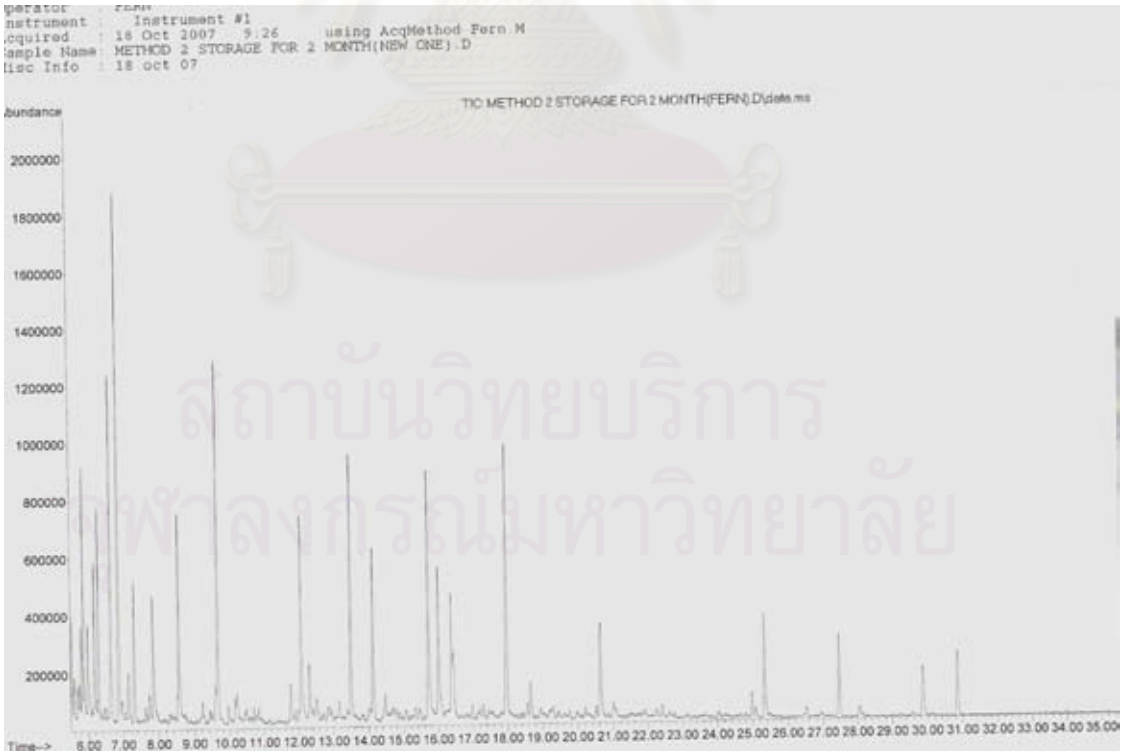


Figure B.11 The chromatogram of volatile compounds in macadamia nuts after 2 months storage

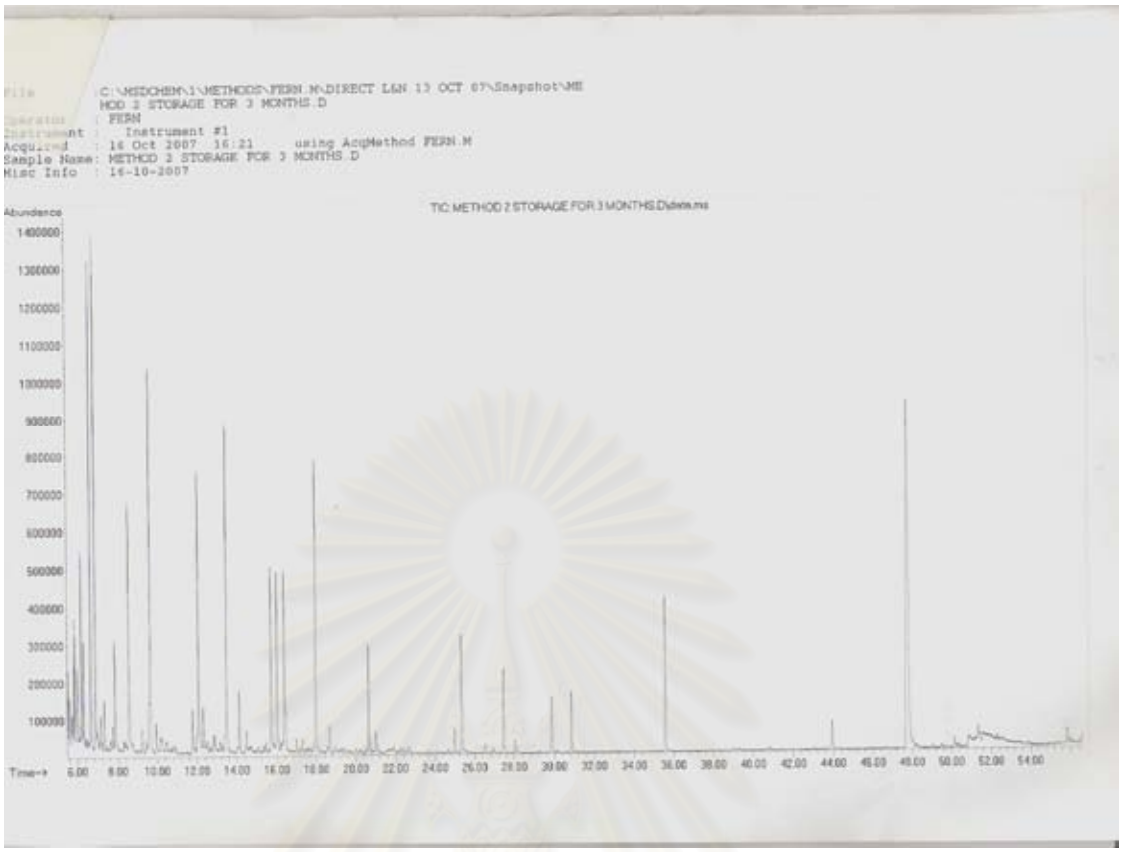


Figure B.12 The chromatogram of volatile compounds in macadamia nuts after 3 months storage

APPENDIX C

Mass spectra of volatile compounds identified in the study

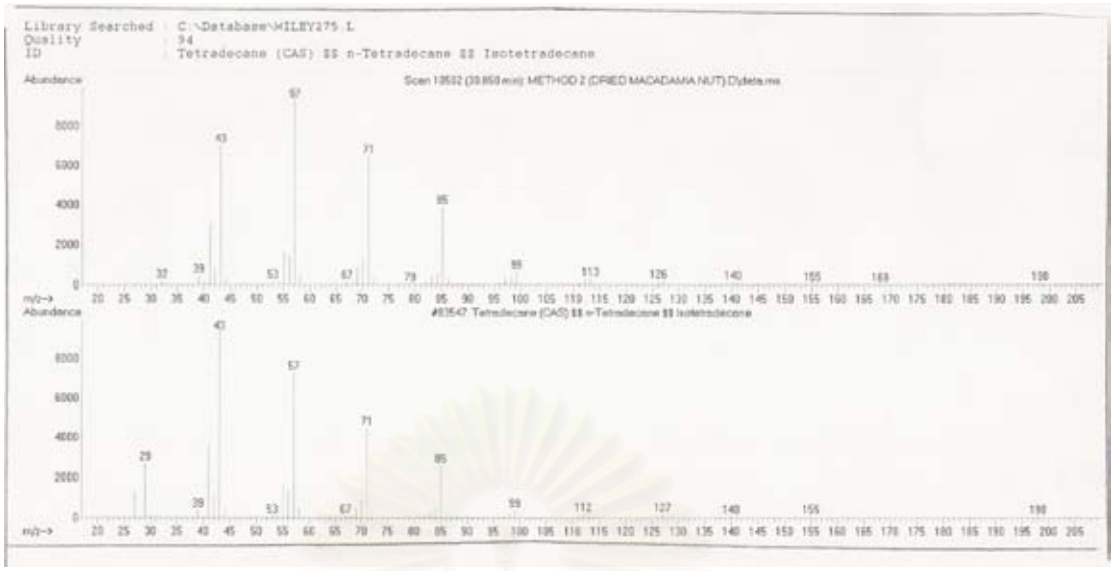


Figure C.1 Mass spectrum of Tetradecane

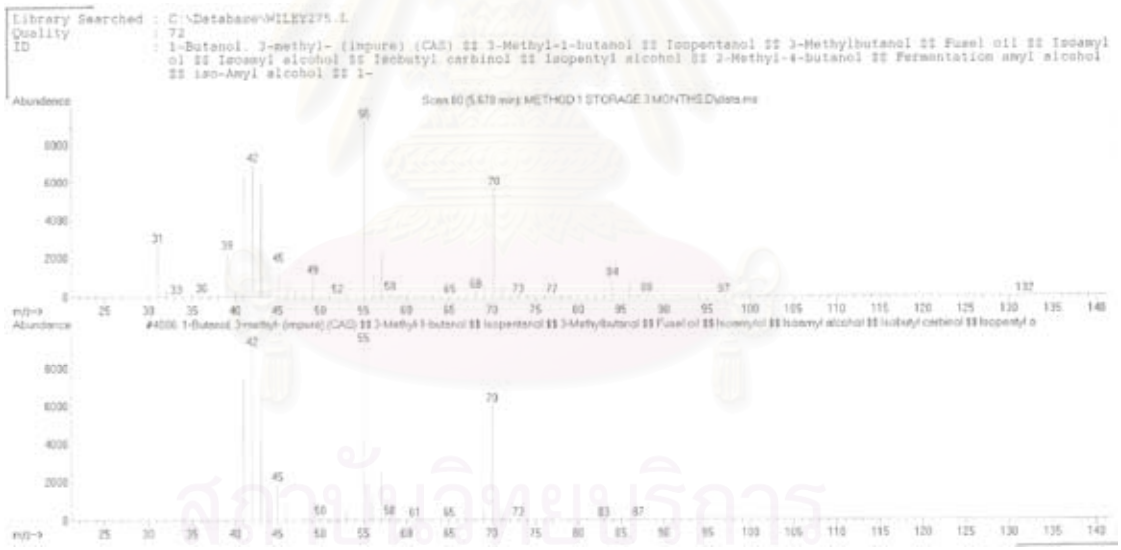


Figure C.2 Mass spectrum of Isopentanol

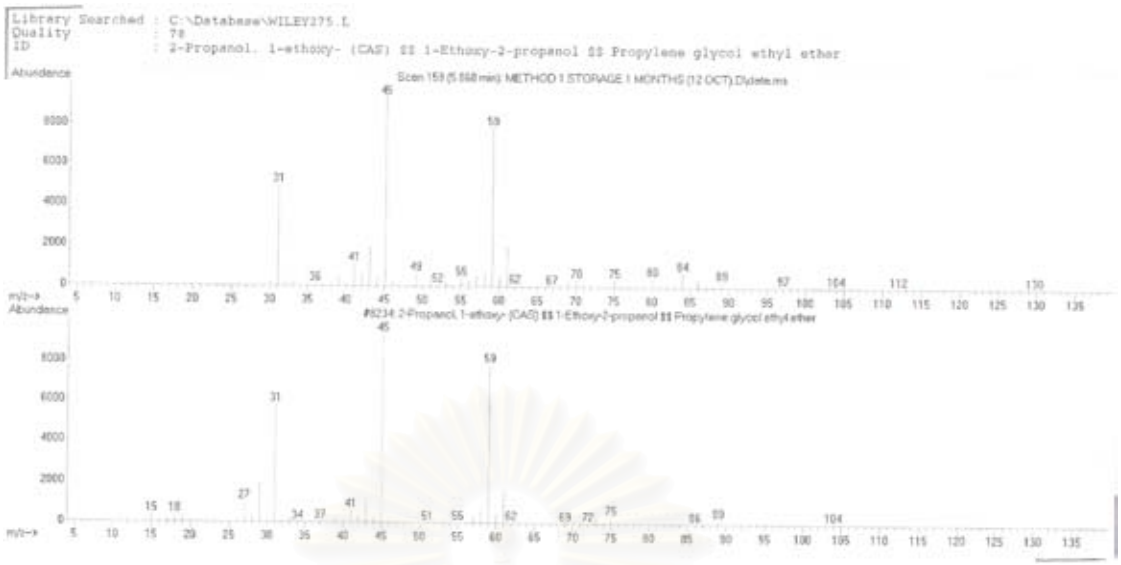


Figure C.3 Mass spectrum of 1-Ethoxy-2-Propanol

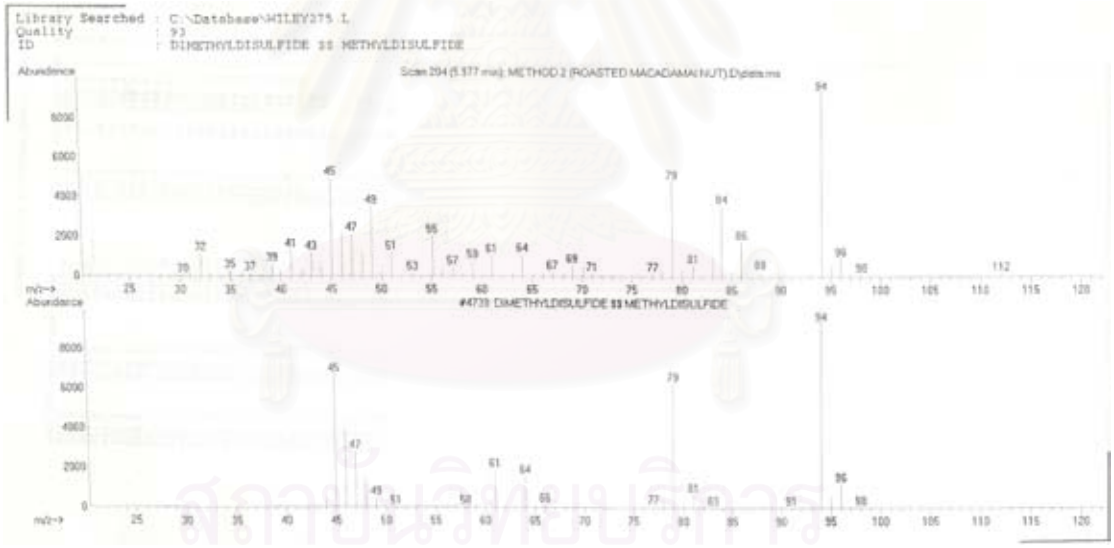


Figure C.4 Mass spectrum of Dimethyl disulfide

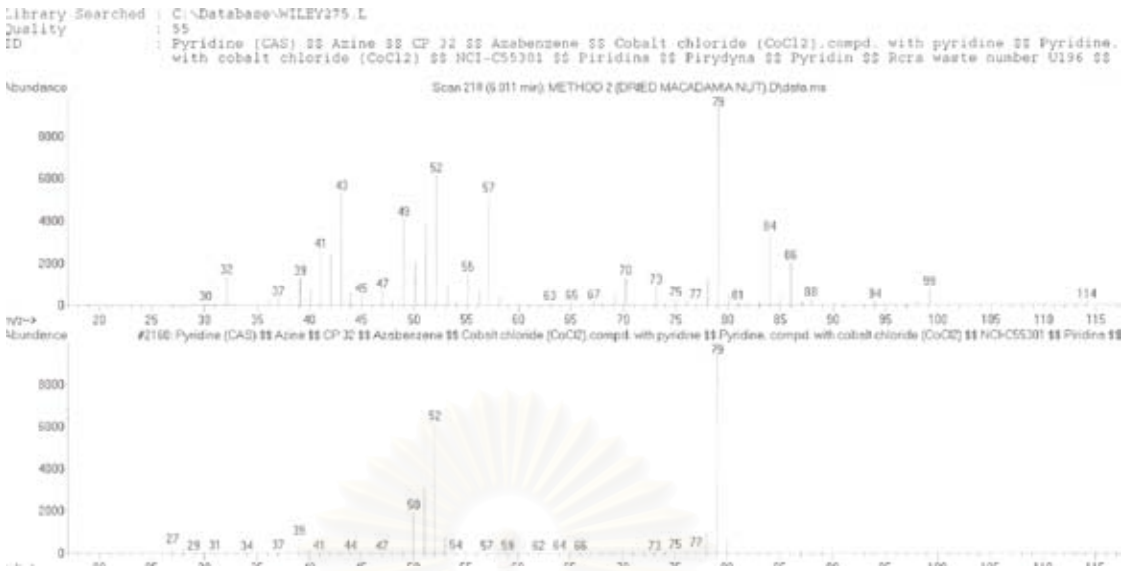


Figure C.5 Mass spectrum of Pyridine

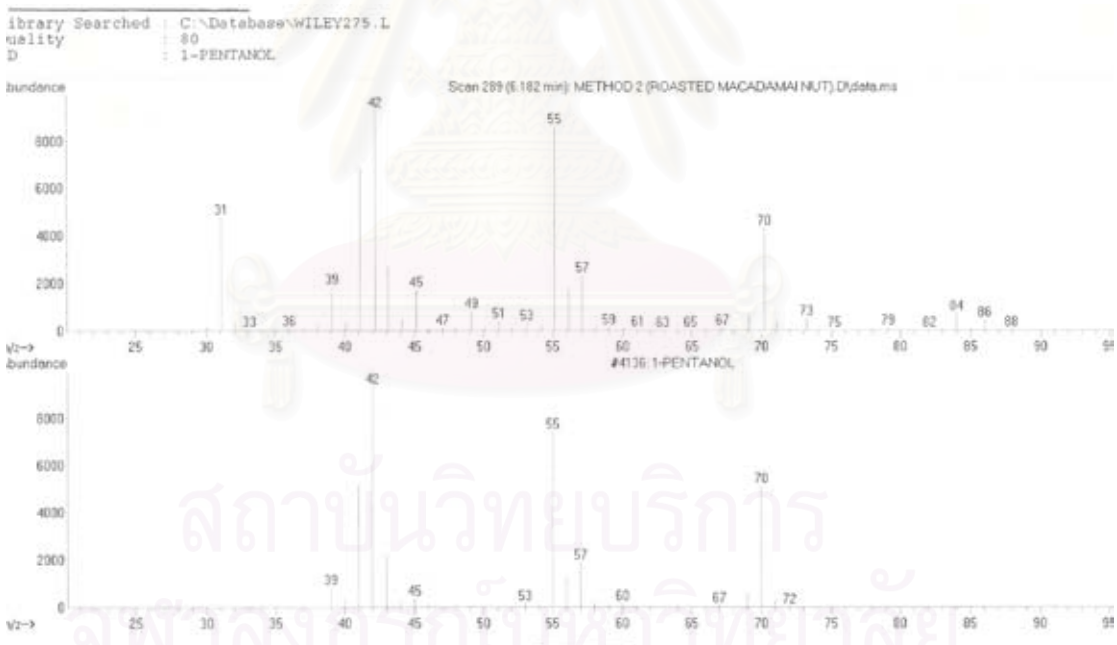


Figure C.6 Mass spectrum of 1-Pentano

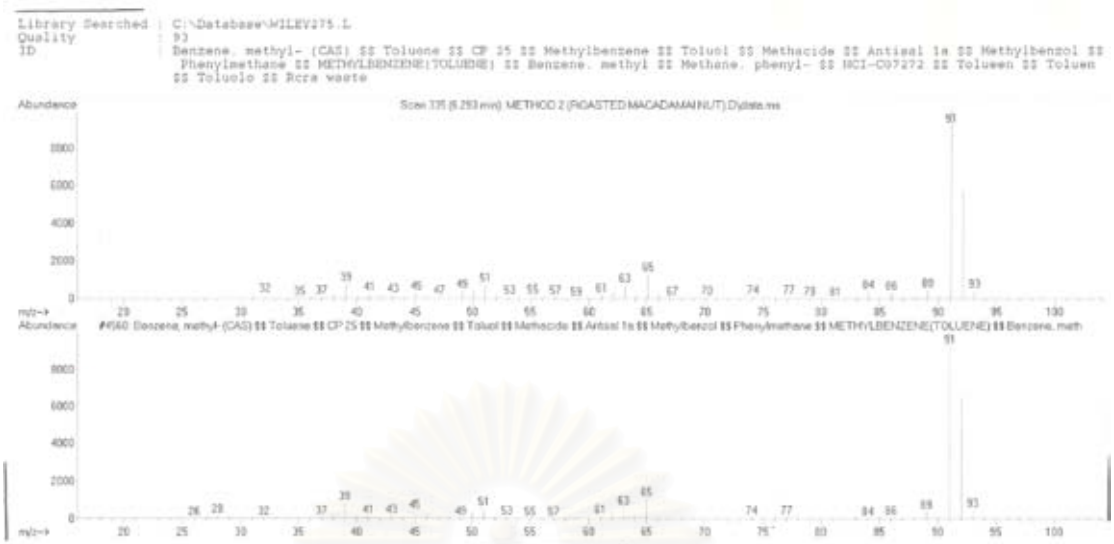


Figure C.7 Mass spectrum of Toluene

Figure C.8 Mass spectrum of Octane

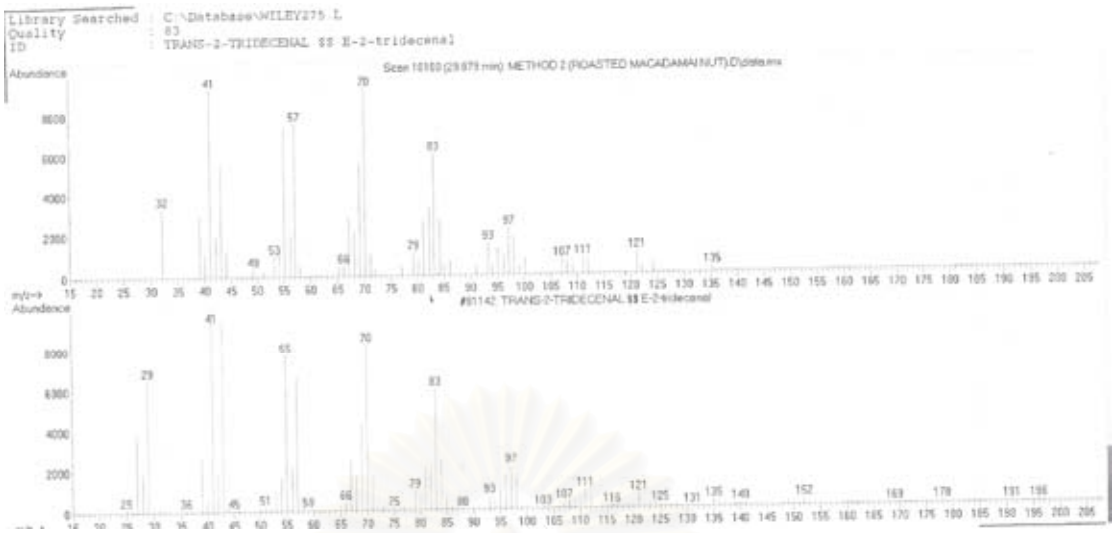


Figure C.9 Mass spectrum of Tran-2-tridecenal

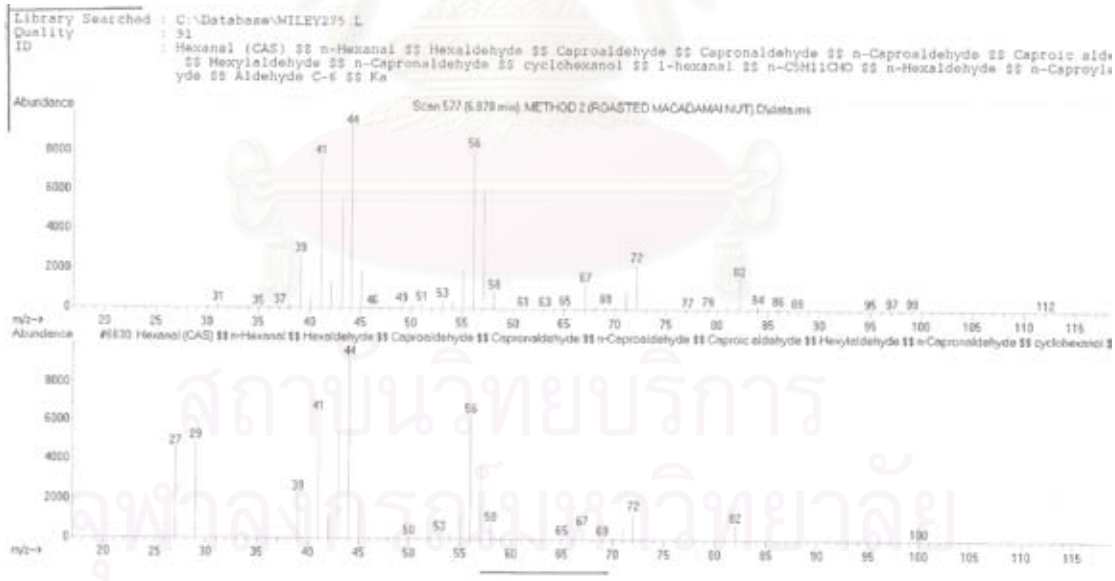


Figure C.10 Mass spectrum of Hexanal

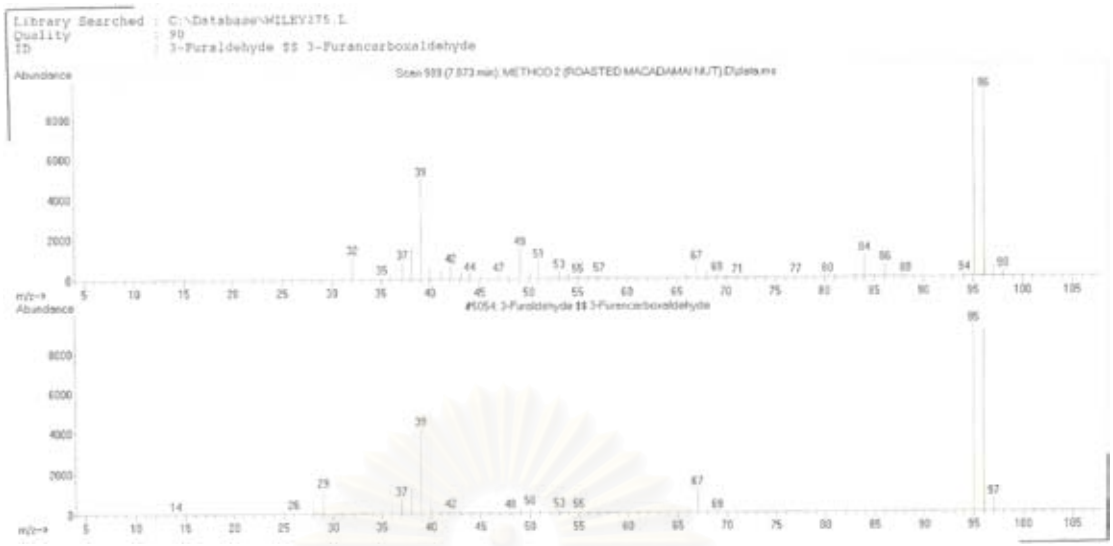


Figure C.11 Mass spectrum of 3-Furaldehyde

Figure C.12 Mass spectrum of 1-Hexano

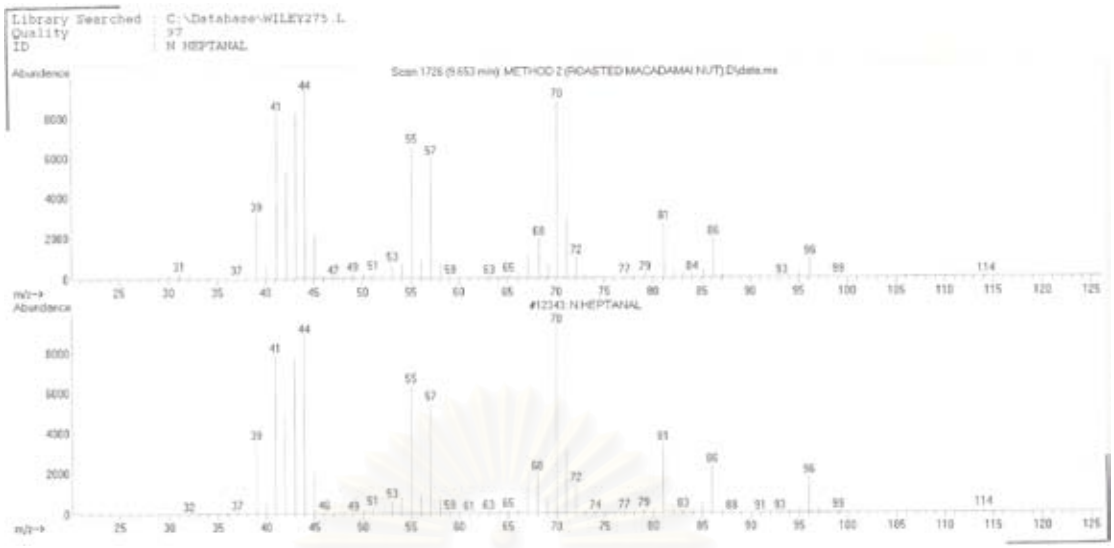


Figure C.13 Mass spectrum of Heptanal

Figure C.14 Mass spectrum of 2-Ethyl-1-hexano

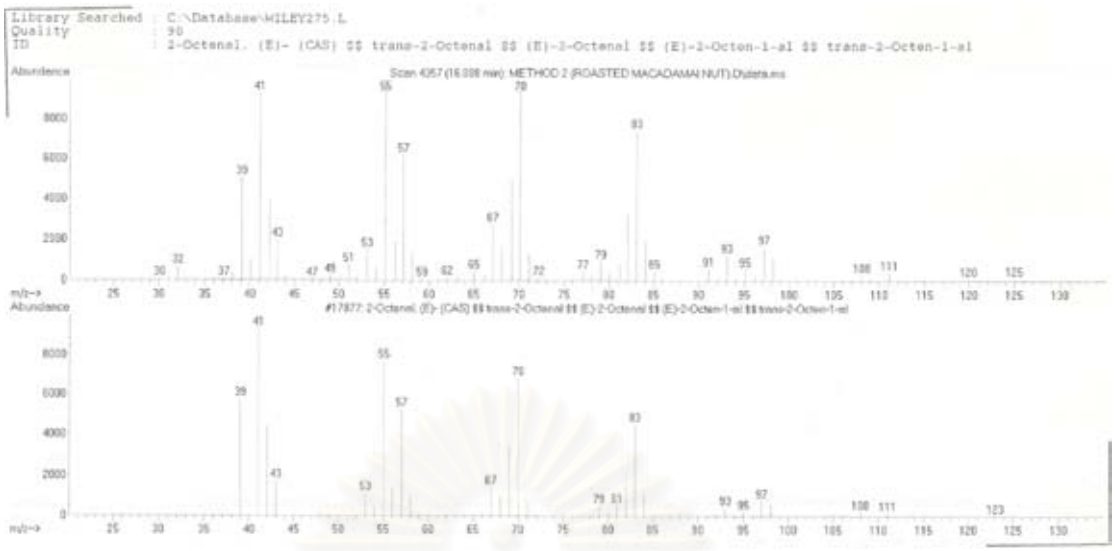


Figure C.15 Mass spectrum of 2-Octenal, Trans-2-Octenal

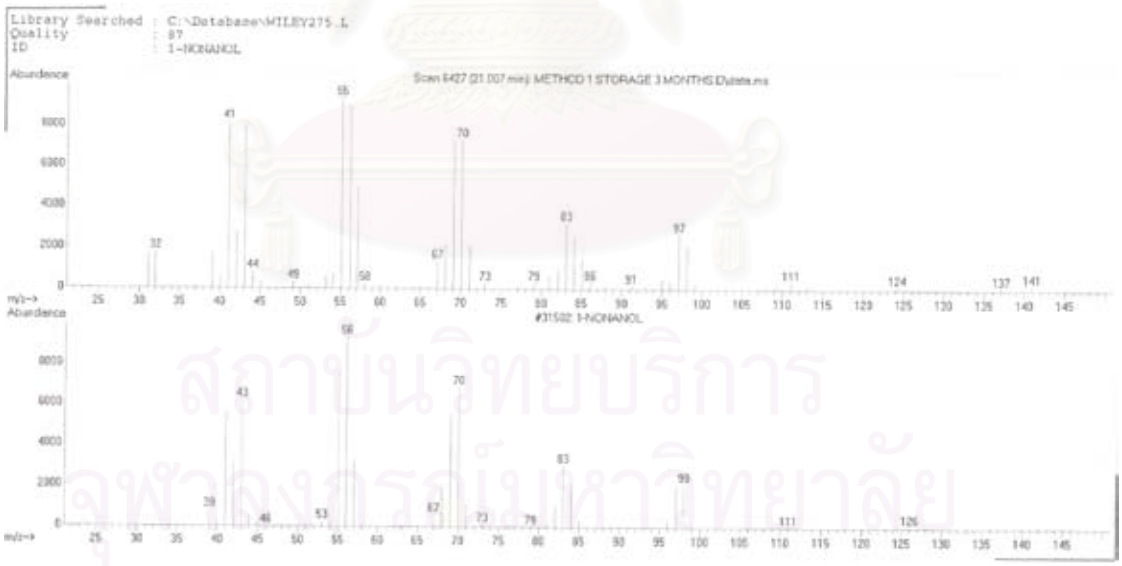


Figure C.16 Mass spectrum of 1-Nonanol

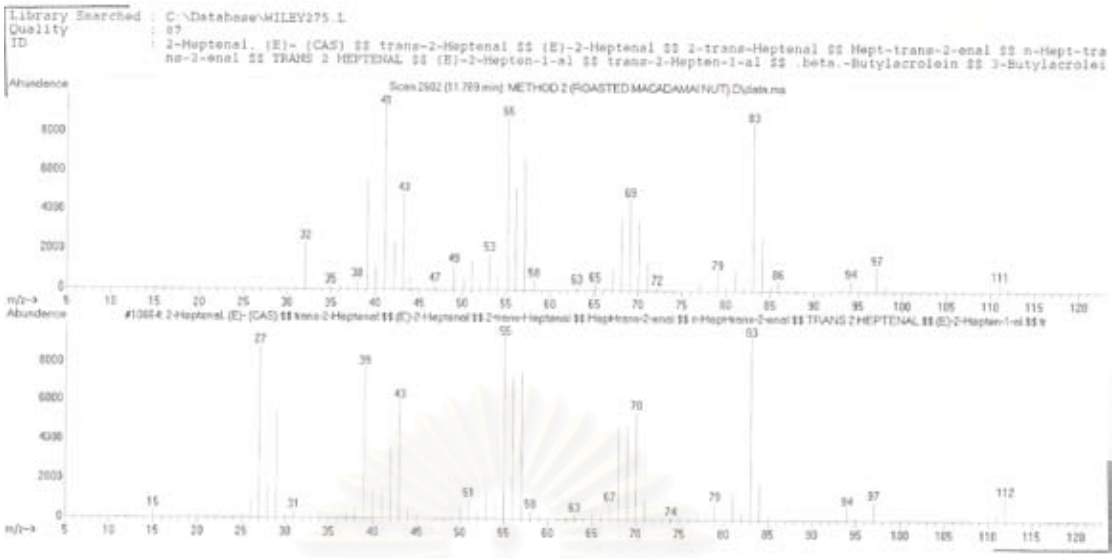


Figure C.17 Mass spectrum of 2-Heptenal

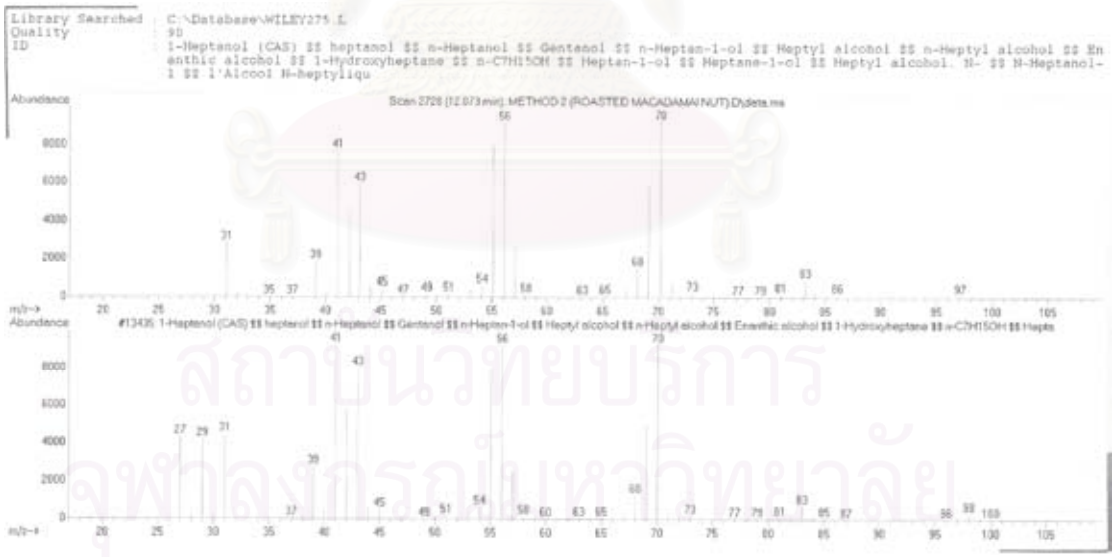


Figure C.18 Mass spectrum of 1-Heptanol

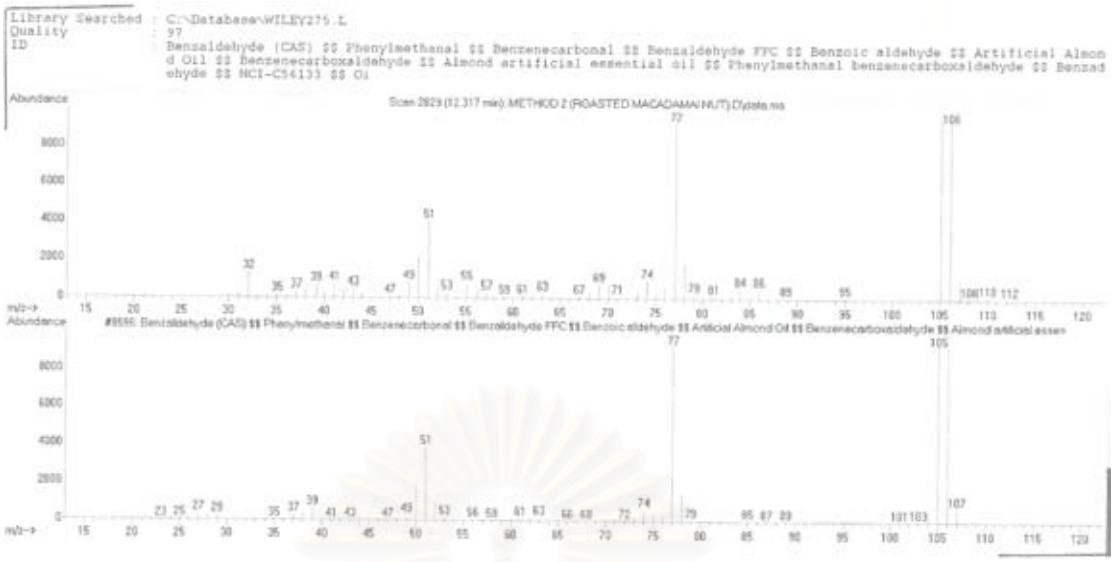


Figure C.19 Mass spectrum of Benzaldehyde

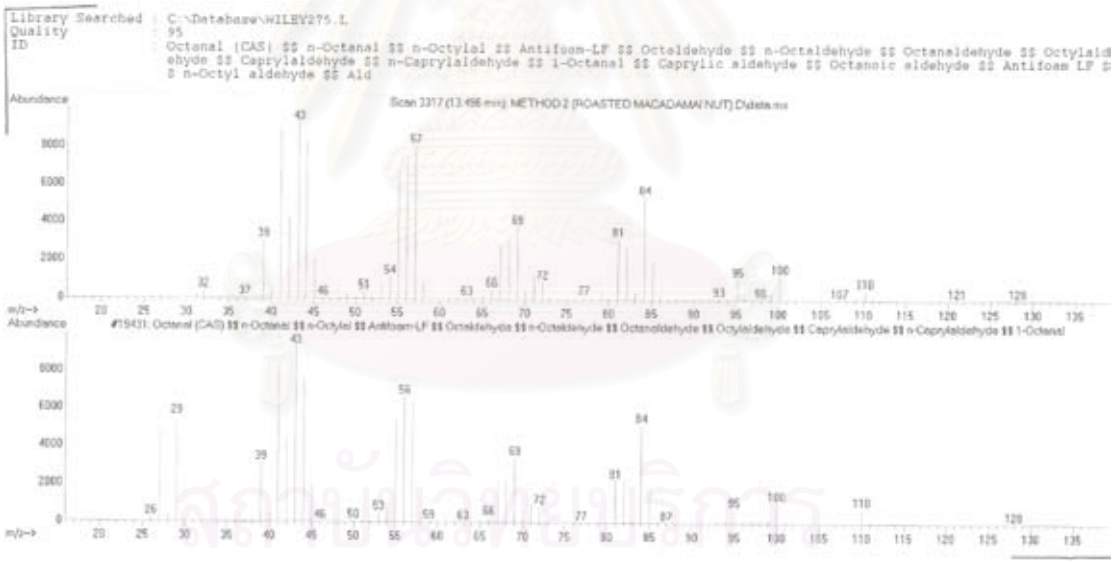


Figure C.20 Mass spectrum of Octanal

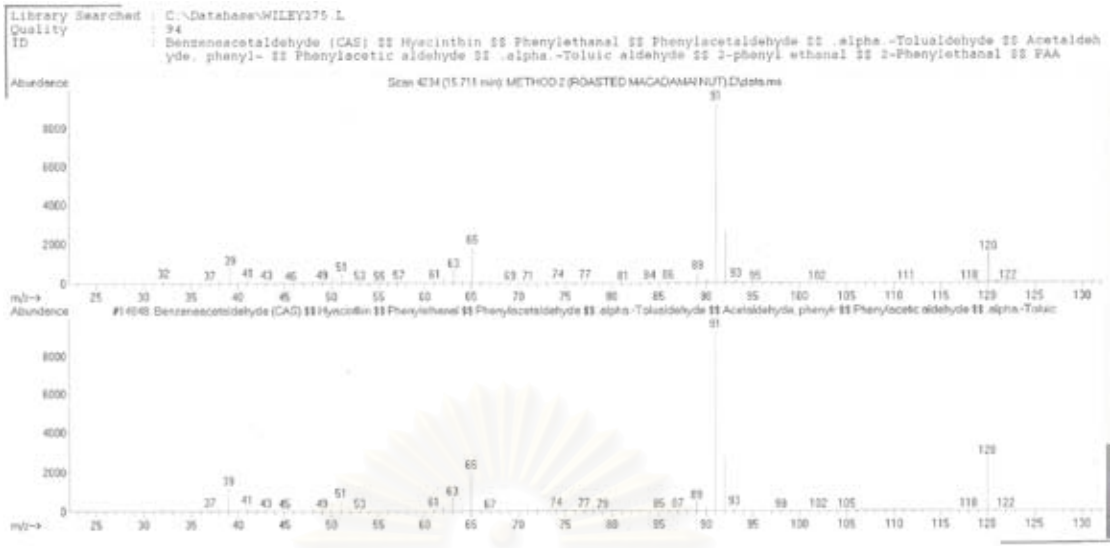


Figure C.21 Mass spectrum of Benzeneacetaldehyde

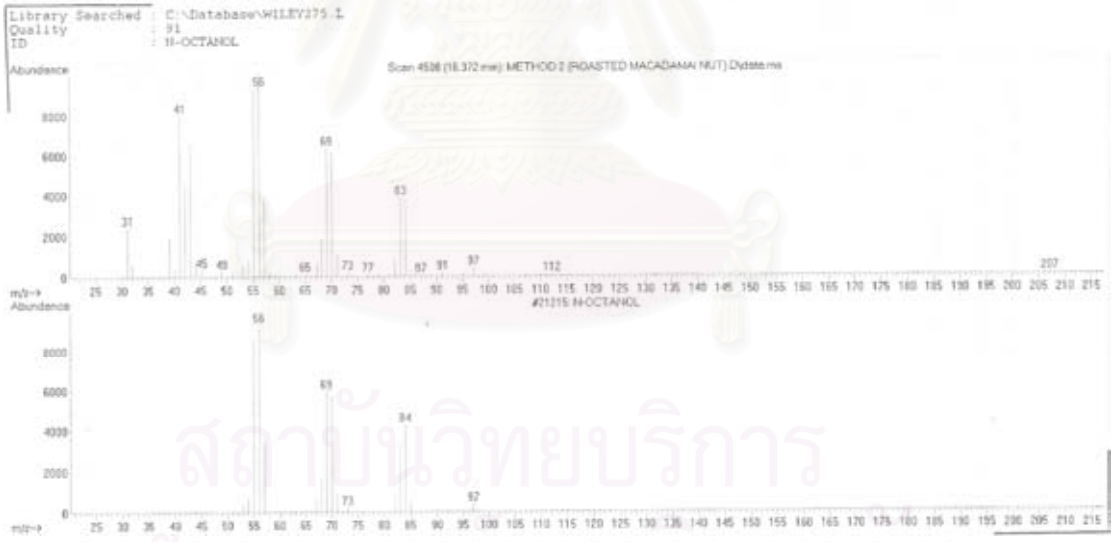


Figure C.22 Mass spectrum of N-Octanol

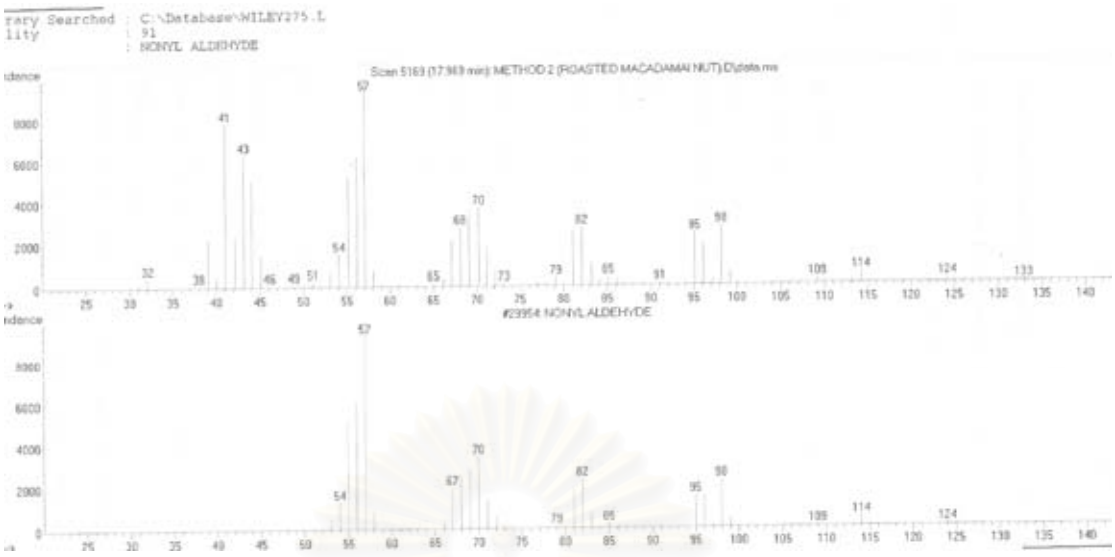


Figure C.23 Mass spectrum of Nonanal

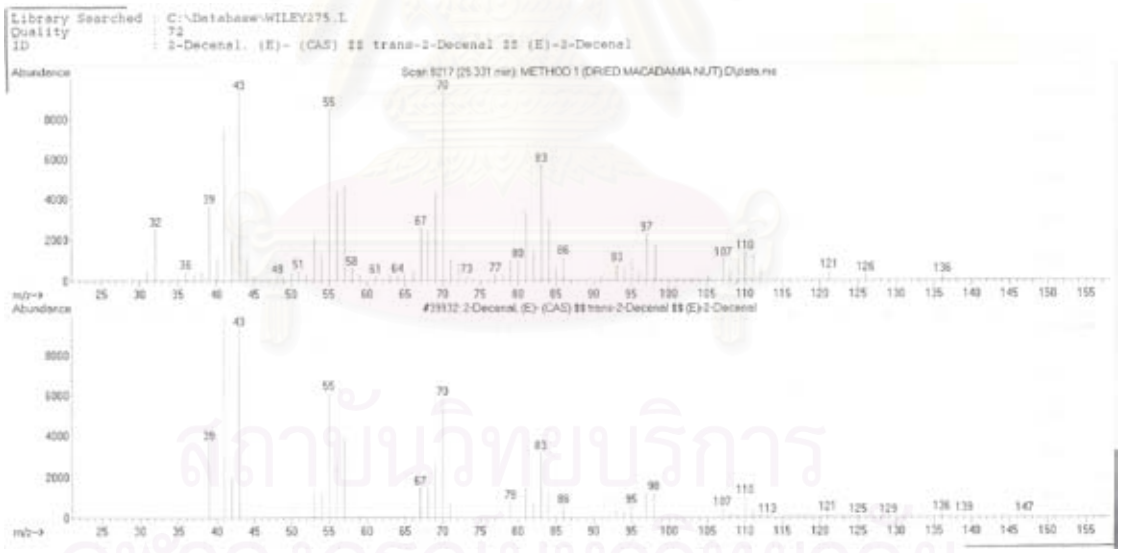


Figure C.24 Mass spectrum of 2-Decenal

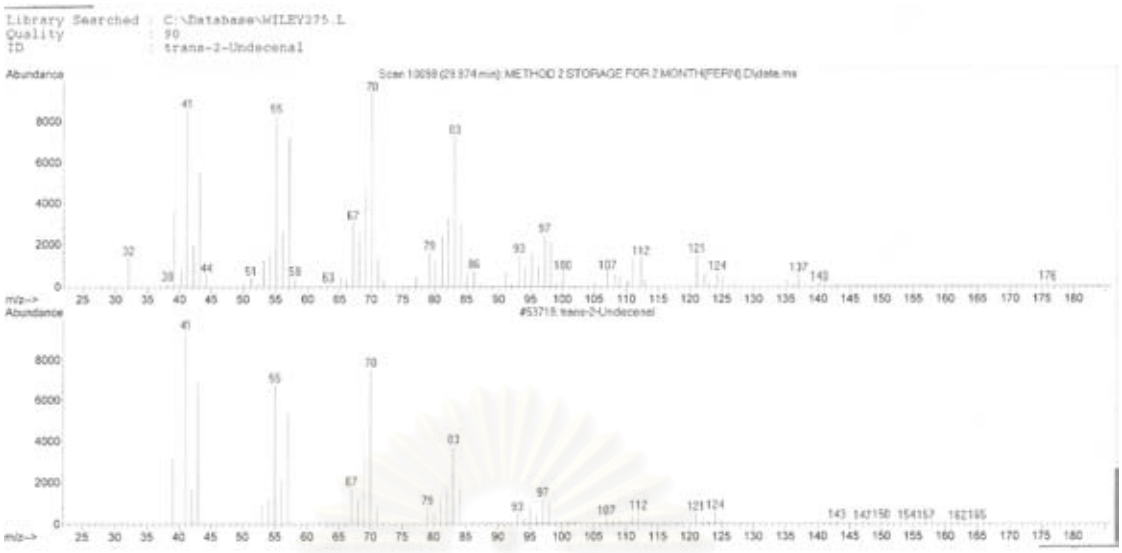


Figure C.25 Mass spectrum of Trans-2-Undecenal

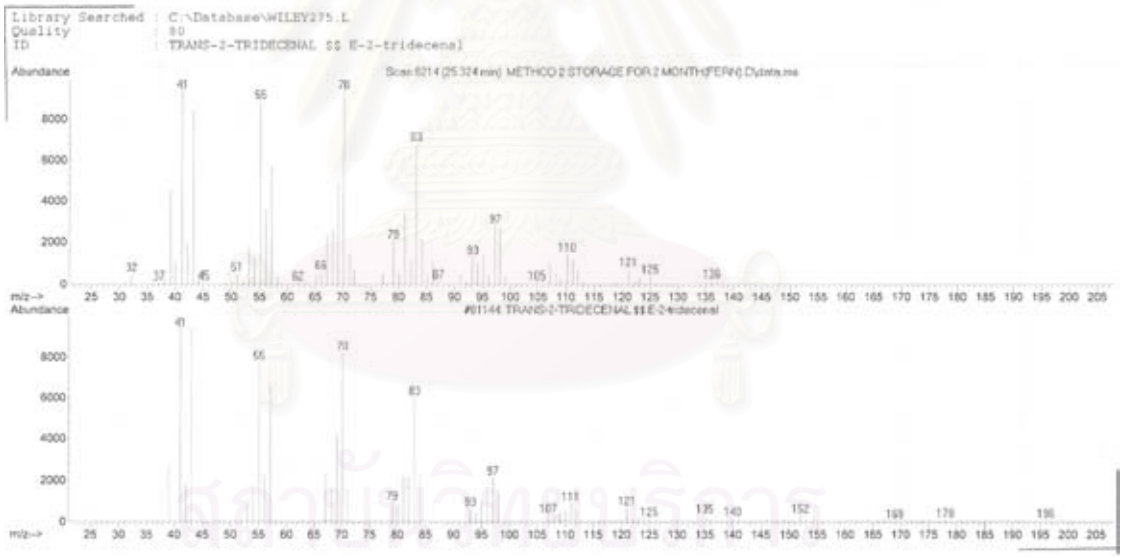


Figure C.26 Mass spectrum of Trans-2-Tridecenal

APPENDIX D

Standard of Fatty Acid Methyl Ester

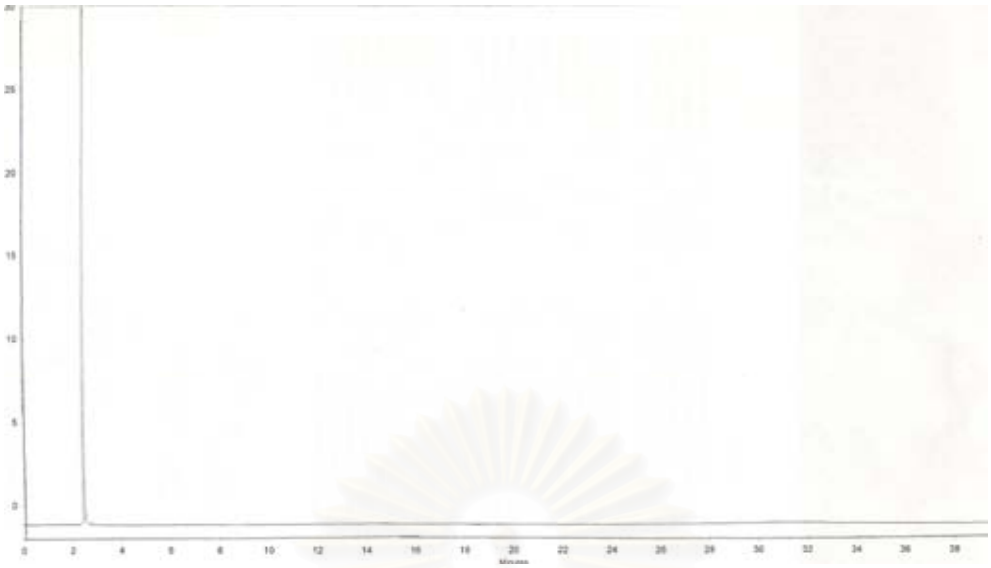


Figure D.1The chromatogram of purifed Isooctane (solvent) from GC-FID

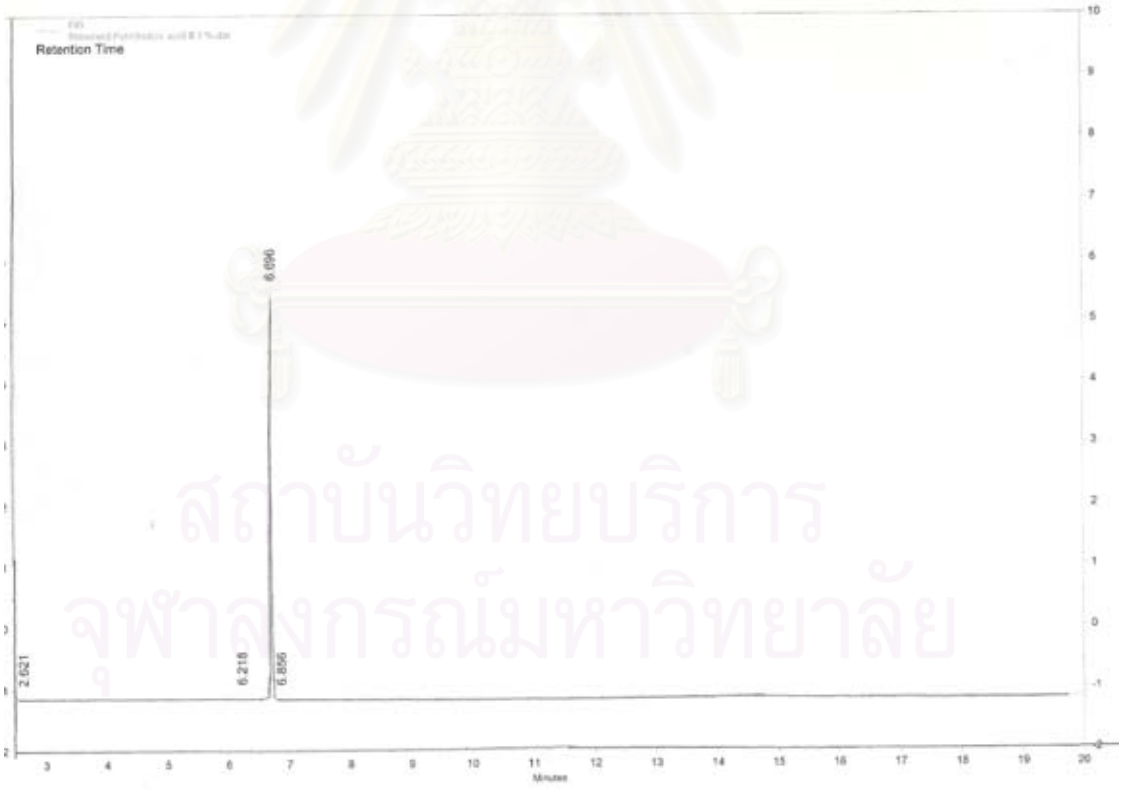


Figure D.2 The chromatogram of palmitoleic acid methyl ester RT= 6.696 min

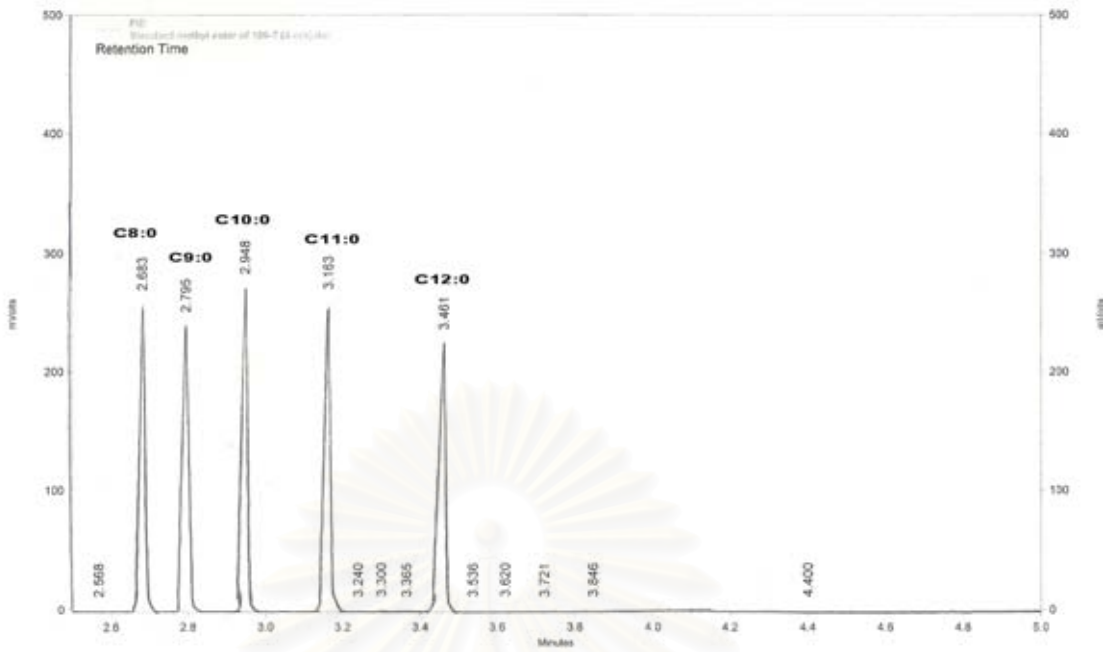


Figure D.3 The chromatogram of standard mixture methyl ester of octanoic acid C8:0, nonanoic acid, C9:0, decanoic acid C10:0, undecanoic acid C11:0 and lauric acid C12:0 with GC-FID

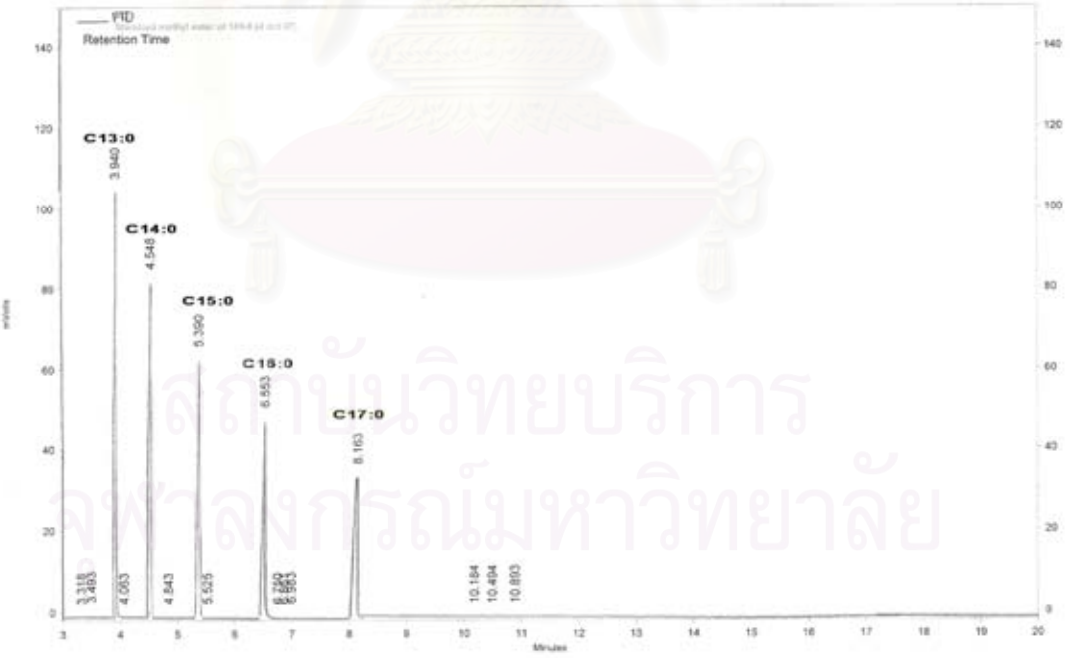


Figure D.4 The chromatogram of standard mixture methyl ester of tridecanoic acid C 13:0, myristic C14:0, pentadecanoic acidC15:0, palmitic acid C16:0 and heptadecanoic acid C17:0 with GC-FID

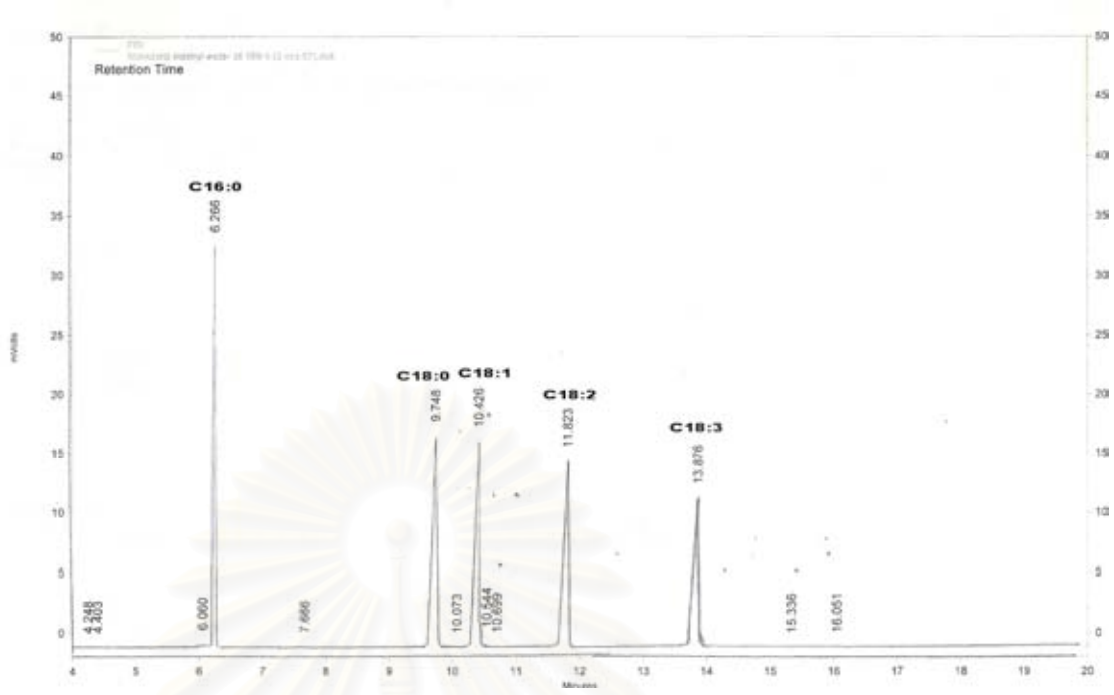


Figure D.5 The chromatogram of standard mixture methyl ester of palmitic acid C16:0, stearic acid, C18:0, oleic acid C18:1, linoleic acid C18:2 and linolenic acid C18:3 with GC-FID

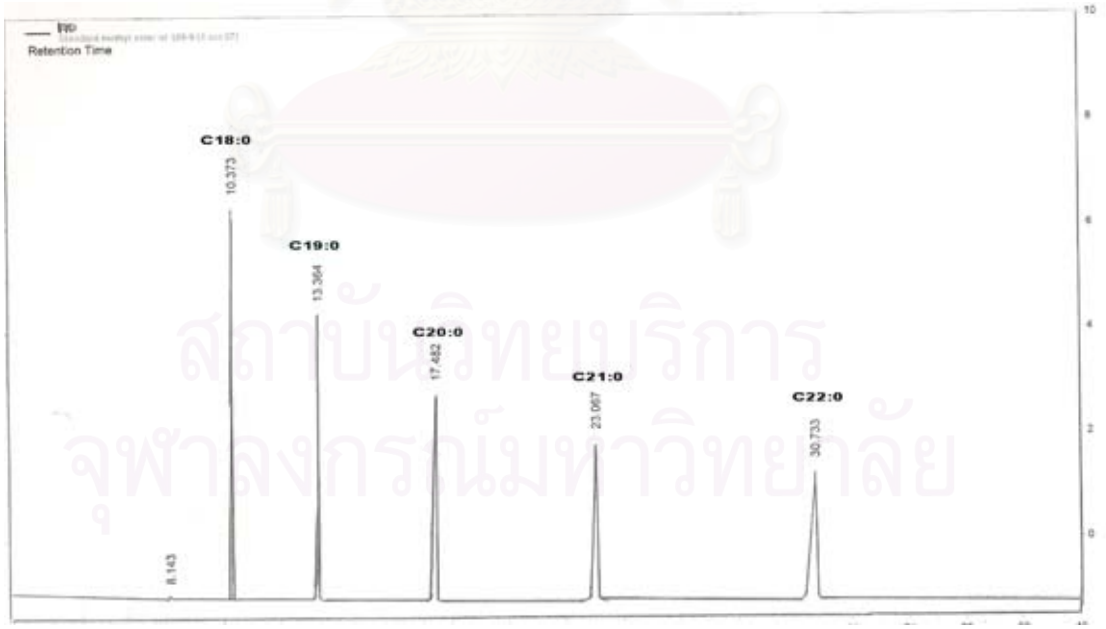


Figure D.6 The chromatogram of standard mixture methyl ester of stearic acid C18:0, nonadecanoic acid, C 19:0, arachidic acid C20:0, heneicosanoic acid C21:0 and behenic acid C22:0 with GC-FID

Plot log graph between corrected retention time (min) which was calculated by equation (D.1) and carbon number from the standard mixture of methyl ester. The log graph can indicate the exactly corrected retention time of each FAME. Moreover, the linearity of log graph can be used to predict the corrected retention time of other FAME. However, the log graph will be accurate when all saturated fatty acid is group as well as unsaturated fatty acid (Figures D.7 and D.8)

Corrected retention time (min) = RT of FAME – RT of solvent ….(D.1)

Where:

RT = Retention time (min)

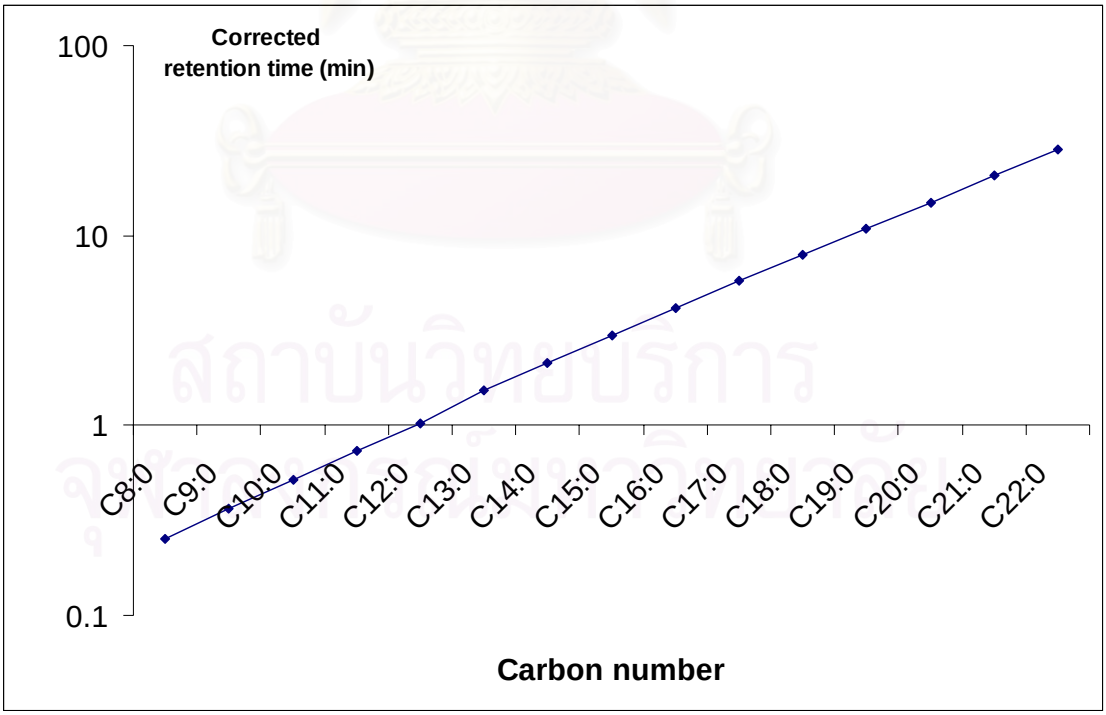


Figure D.7 The log graph between corrected retention time (min) of saturated fatty acid (C8:0 – C22:0) and carbon number

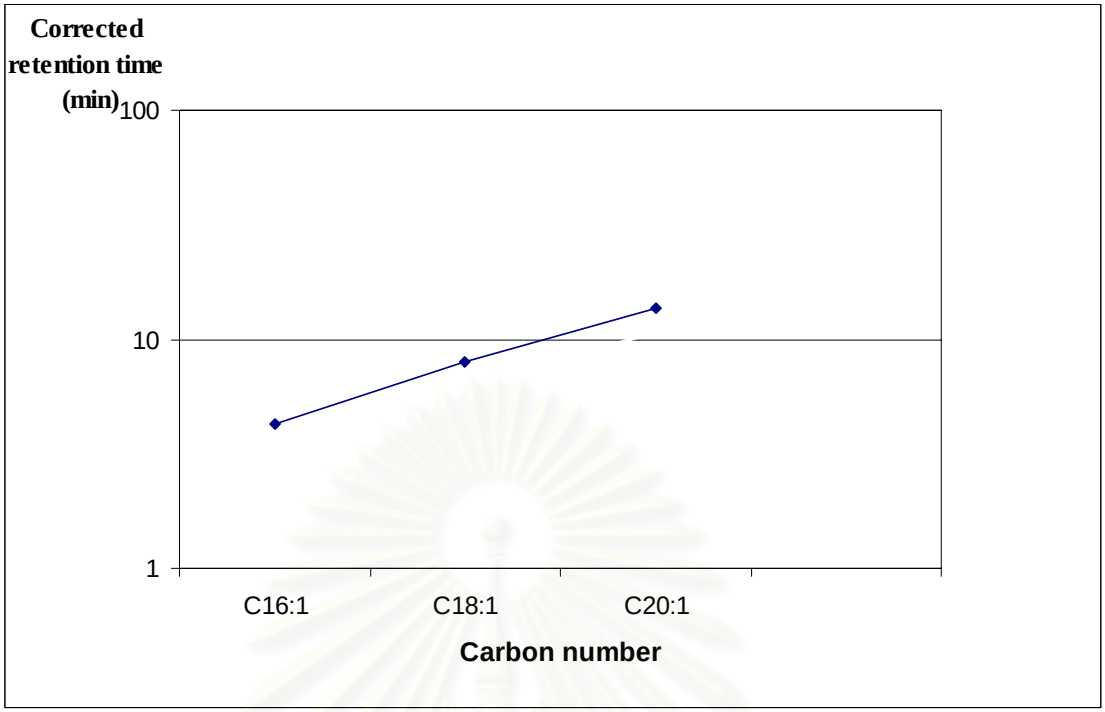


Figure D.8 The log graph between corrected retention time (min) of monounsaturated fatty acid (C16:1, C18:1 and C20:1)and carbon number

Standard curve of palmitoleic acid was constructed by plotting correct palmitoleic acid concentration correspond to its corrected peak area is shown in Table D.1. The chromatogram of purify palmitoleic acid (C16:1) was shown in Figure D.2.

Table D.1 Corrected area of palmitoleic acid at the different concentration

Concentration (mg) of palmitoleic acid methyl ester	Area	TRF	Corrected Area (min)
0.2	13067		13219.8839
2	97352		98491.0184
10	493876	1.0117	499654.3492
20	939542		950534.6414
100	5028648		5087483.182

The standard curve of corrected area of palmitoleic acid and concentration (mg) is shown in figure D.9.

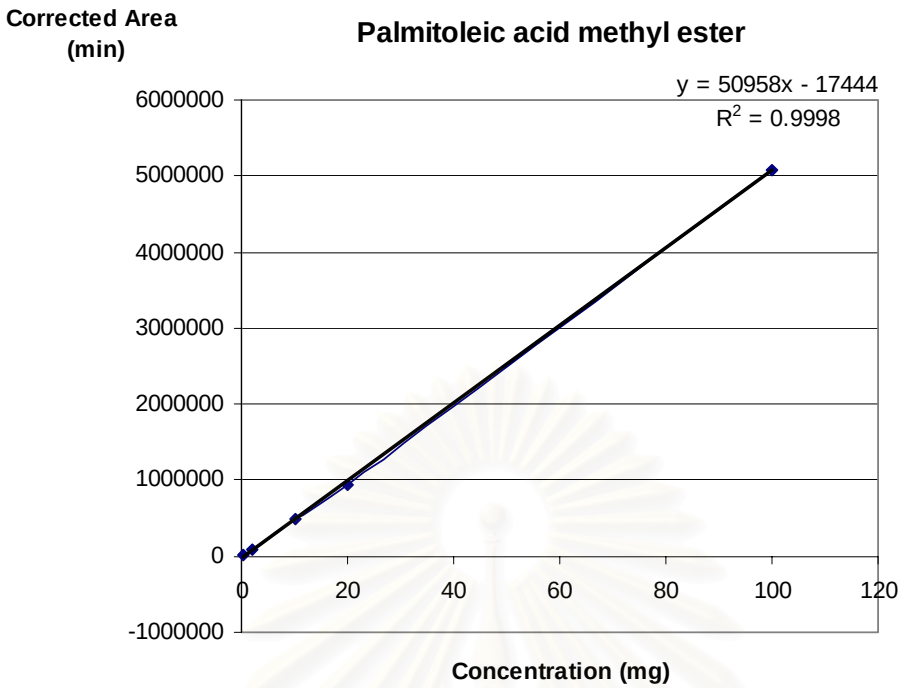


Figure D.9 Standard curve of corrected area of palmitoleic acid and concentration (mg)

The theoretical relative response factor (TRF) was used for conversion of raw peak area to corrected areas when analysing fatty acid methyl esters (FAME) by gas chromatography. The theoretical relative response factors (TRF) of each FAME are shown in Table D.2.

Table D.2 Theoretical relative response factor (TRF) for fatty acid methyl ester

Common Name	FAME	TRF
Lauric acid	C12:0	1.0771
Myristic acid	C14:0	1.0440
Palmitic acid	C16:0	1.0193
Palmitoleic acid	C16:1	1.0117
Stearic acid	C18:0	1.0000
Oleic acid	C18:1	0.9932
Linoleic acid	C18:2	0.9865
Linolenic acid	C18:3	0.9797
Arachidic acid	C20:0	0.9846
Eicosenic acid	C20:1	0.9785
Behenic acid	C22:0	0.9720

Peak area of sample fatty acid was then compared to peak area of palmitoleic acid in the sample. Then, the concentration of sample fatty acid can be calculated. In other words, palmitoleic acid is used as internal standard. The calculation of fatty acid methyl ester is shown in Table D.3.

Table D.3 Fatty acids composition of dried macadamia nut with hot air dryer

Common Name	FAME	Area peak	TRF	Corrected Area
Lauric acid	C12:0	1081	1.0771	164.7963
Myristic acid	C14:0	11234	1.044	1128.5640
Palmitic acid	C16:0	20468	1.0193	11450.8162
Palmitoleic acid	**C16:1**	**2722**	**1.0117**	**20707.4756**
Stearic acid	C18:0	88441	1	2722.0000
Oleic acid	C18:1	3764	0.9932	87839.6012
Linoleic acid	C18:2	184	0.9865	3713.1860
Linolenic acid	C18:3	3894	0.9797	180.2648
Arachidic acid	C20:0	2706	0.9846	3834.0324
Eicosenic acid	C20:1	375	0.9785	2647.8210
Behenic acid	C22:0	153	0.972	364.5000

Example : From dried macadamia nut

From the equation of palmitoleic acid

$$Corrected \quad area\,(y) = 50958x - 17444 \qquad \text{....(D.2)}$$

Where:

Y = Corrected area of palmitoleic acid methyl ester

$X(mg)$ = Concentration of palmitoleic acid methyl ester

The concentration of the other fatty acid methyl ester of interest was determined by using the formula below:

$$Conc\ FAME\ (mg) = \frac{Conc(mg)\ \ C16:1}{Corrected\ area\ C16:1} \times Corrected\ area\ FAME \qquad \text{....(D.3)}$$

The concentration of FAME was reported in mg/100 g d

APPENDIX E

Chromatogram of fatty acid methyl ester of macadamia nuts from GC-FID

The chromatograms of fatty acid methyl ester in macadamia nut subjected to different drying methods and storage duration with GC-FID.

E.1 The chromatograms of fatty acid methyl ester in macadamia nut subjected to hot air drying treatment are shown below.

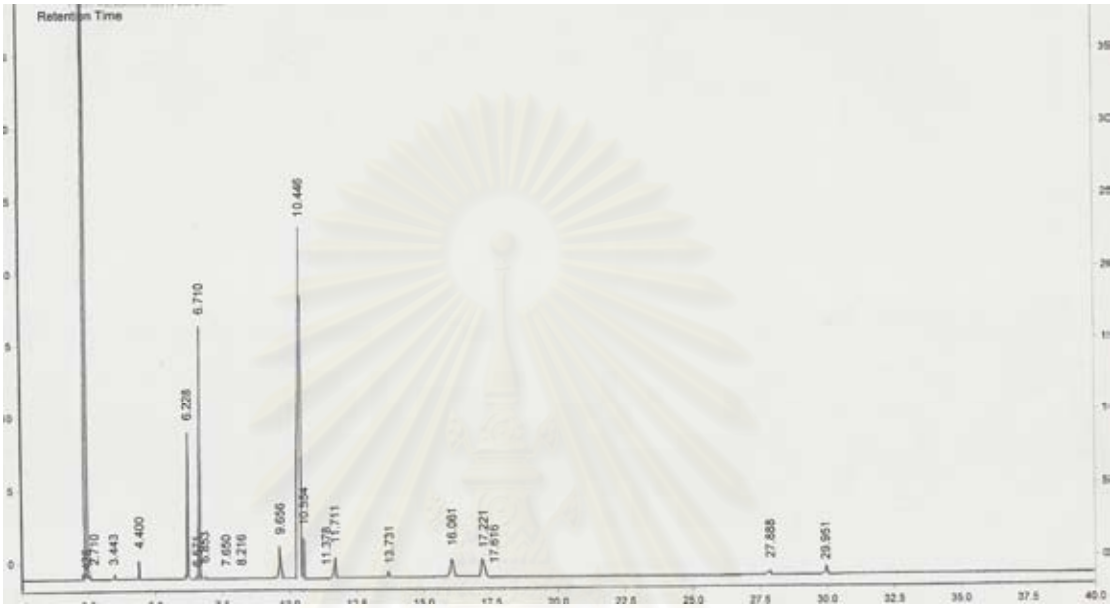


Figure E.1 The chromatogram of fatty acid methyl ester in fresh macadamia nuts

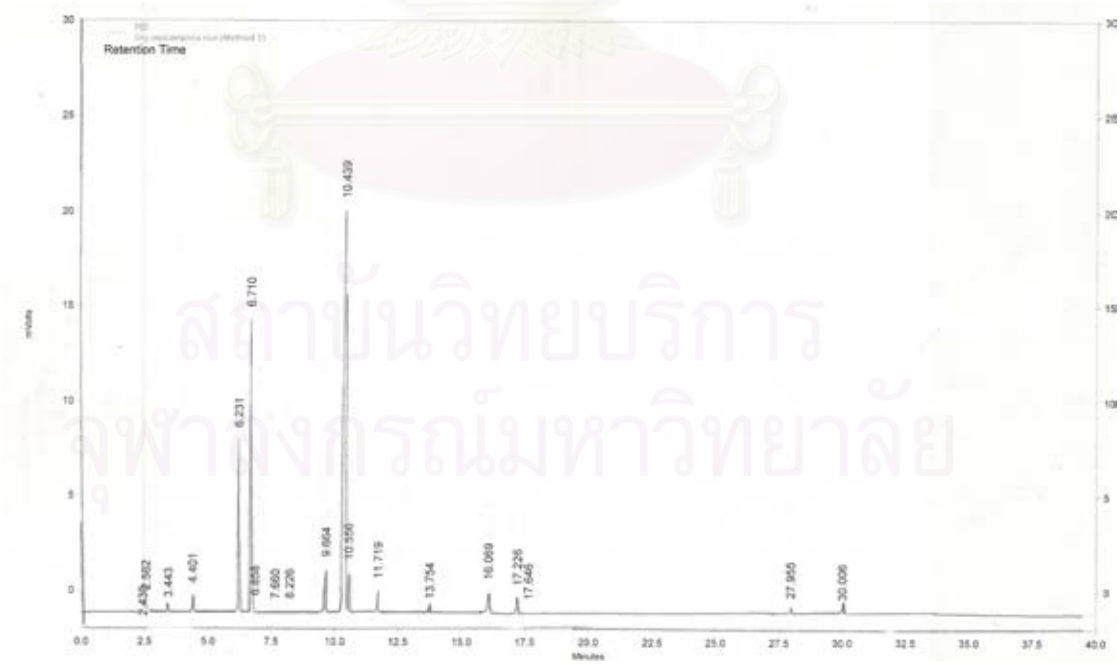


Figure E.2 The chromatogram of fatty acid methyl ester in dried macadamia nuts

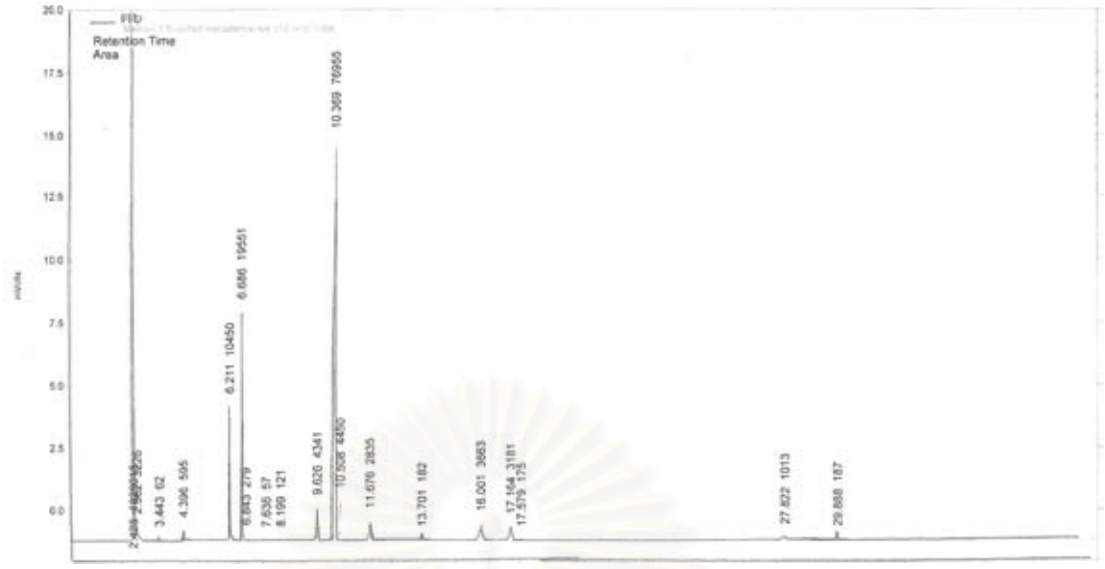


Figure E.3 The chromatogram of fatty acid methyl ester in roasted macadamia nuts

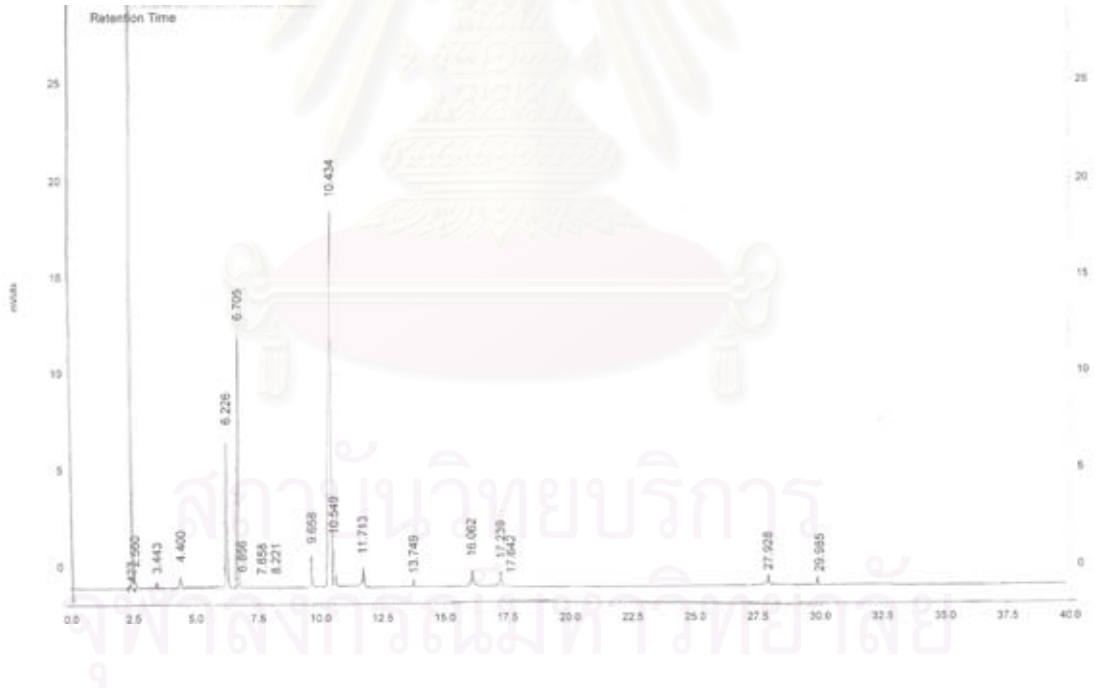


Figure E.4 The chromatogram of fatty acid methyl ester in macadamia nuts after 1 month storage

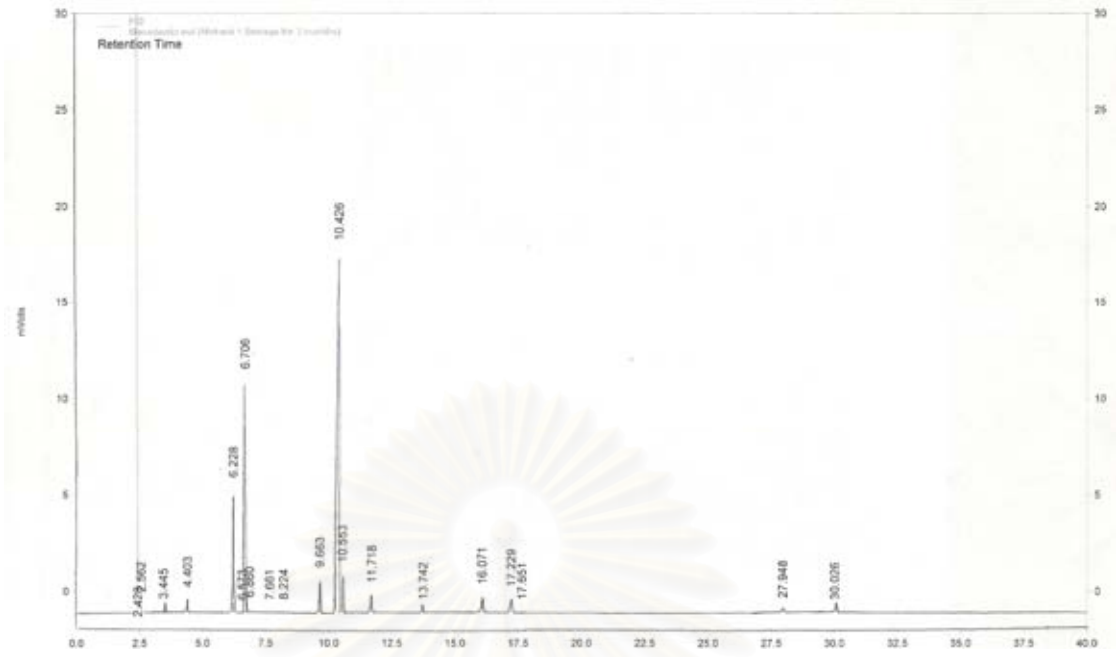


Figure E.5 The chromatogram of fatty acid methyl ester in macadamia nuts after 2 months storage

Figure E.6 The chromatogram of fatty acid methyl ester in macadamia nuts after 3 months storage

E.2 The chromatograms of fatty acid methyl ester in macadamia nut subjected to heat pump dryer combined with tunnel drying treatment are shown below.

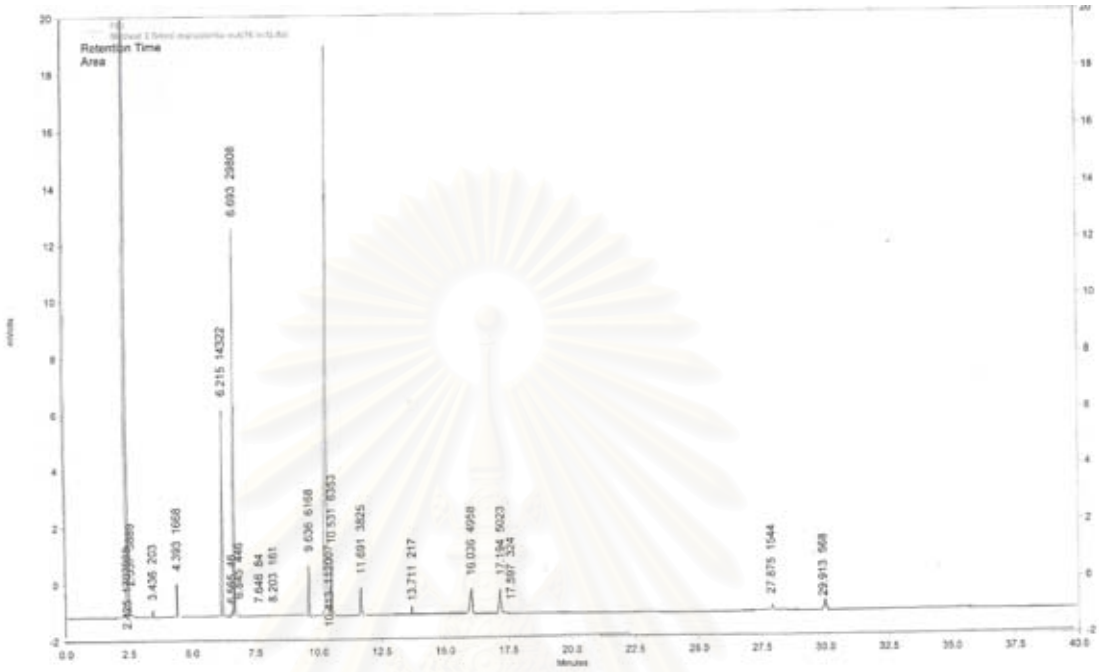


Figure E.7 The chromatogram of fatty acid methyl ester in dried macadamia nuts

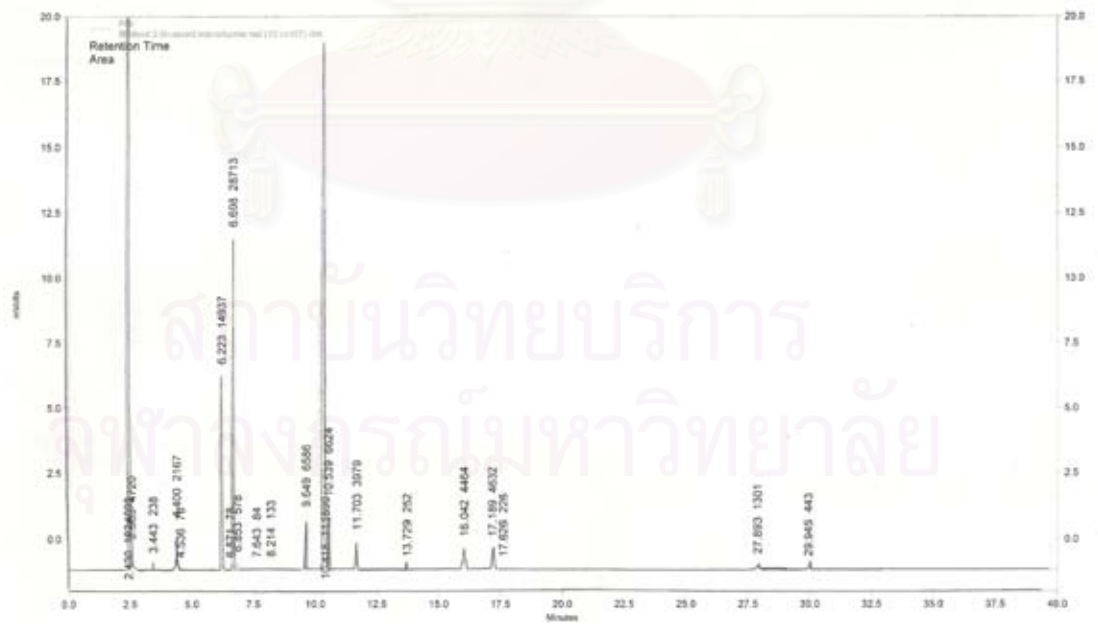


Figure E.8 The chromatogram of fatty acid methyl ester in roasted macadamia nuts

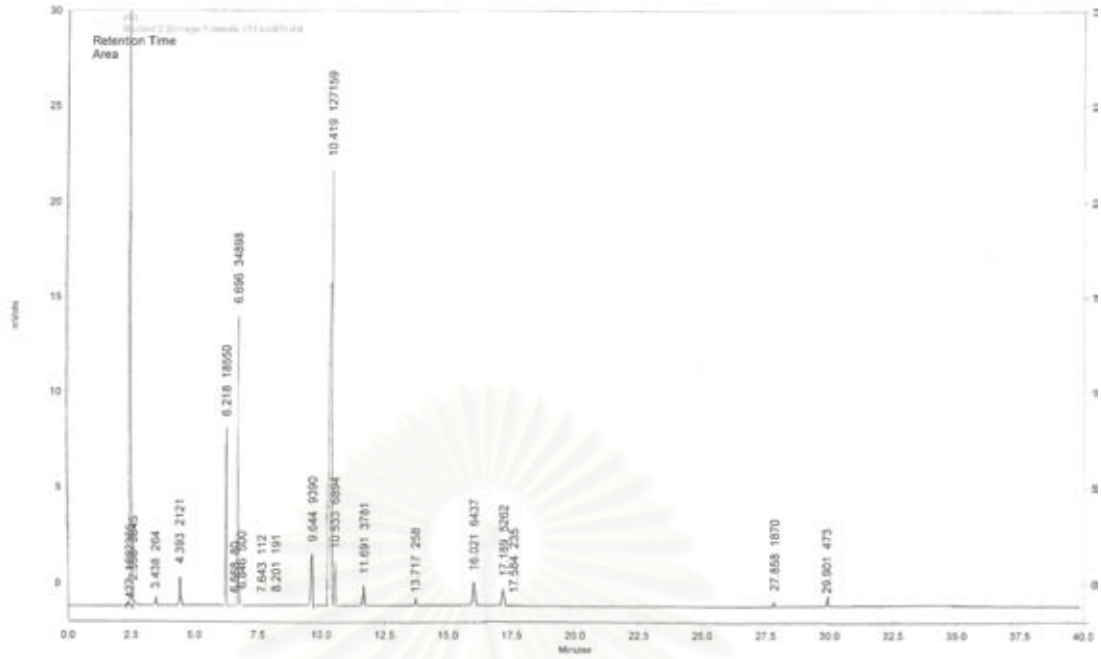


Figure E.9 The chromatogram of fatty acid methyl ester in macadamia nuts after 1 month storage

Figure E.10 The chromatogram of fatty acid methyl ester in macadamia nuts after 2 months storage

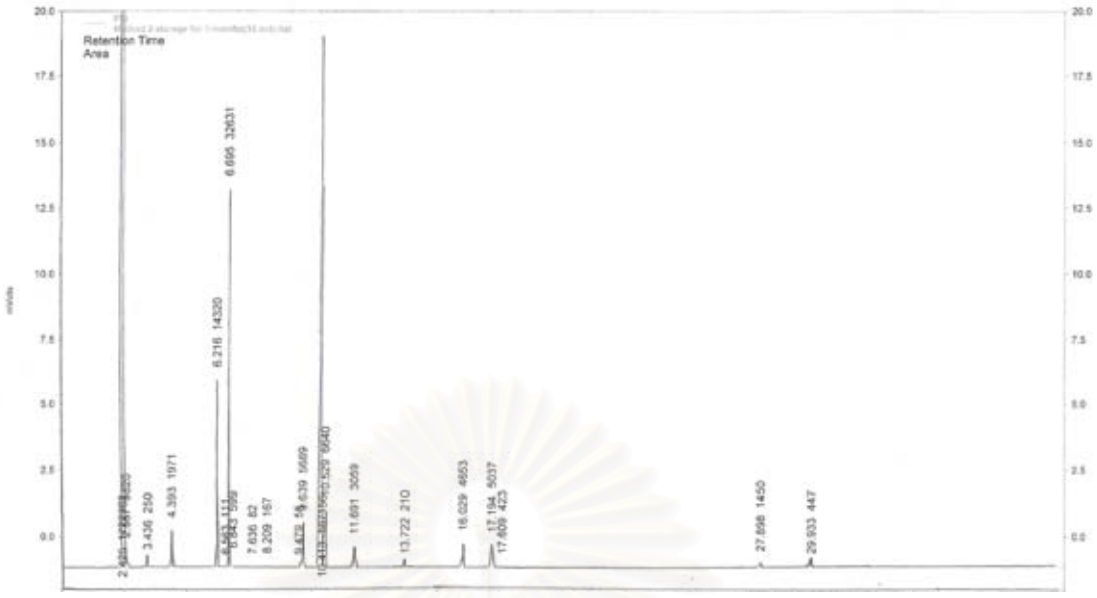


Figure E.11 The chromatogram of fatty acid methyl ester in macadamia nuts after 3 months storage

APPENDIX F

Publications derived from this project

Phattanayindee, S., Borompichaichartkul, C. and Srzednicki, G. 2008. The effect of storage temperature and storage time on quality of macadamia nuts. Proceedings of the 9th National Graduate Research Conference. Burapha University, Bangsaen, Chonburi, Thailand. (March 14-15, 2008).

VITAE

Miss Suporntip Phattanayindee was born on January 8, 1983 in Chachoengsao, Thailand. She obtained a Bachelor of Food science and Technology, Faculty of Science Thammasat University in academic year 2005.