

PERPUSTAKAAN UNIVERSITI MALAYA

ACM- 8693

INVC. 18/1/01

**ISOLATION AND IDENTIFICATION
OF STEROLS IN PALM OIL AND
THEIR ANALYSIS BY GC AND GC-MS**

HAN AI CHEN

UNIVERSITY OF MALAYA

2000

Perpustakaan Universiti Malaya



A510030405

**ISOLATION AND IDENTIFICATION
OF STEROLS IN PALM OIL AND
THEIR ANALYSIS BY GC AND GC-MS**

A PROJECT REPORT SUBMITTED TO THE
FACULTY OF SCIENCE
UNIVERSITY OF MALAYA
IN THE PART-FULFILMENT OF SCGS 5189
FOR THE DEGREE OF MASTER OF SCIENCE
IN ANALYTICAL CHEMISTRY AND INSTRUMENTAL ANALYSIS

BY
HAN AI CHEN

SUPERVISED BY
DR. KAMAL AZIZ KETULY

DEPARTMENT OF CHEMISTRY
UNIVERSITY OF MALAYA
KUALA LUMPUR
MAY 2000

ACKNOWLEDGMENT

I wish to express my deepest gratitude to Dr. Kamal Aziz Ketuly for supervising and giving guidance throughout this project. Also not forget to thank Dr. Choo Yuen May (PORIM) for her collaborations on this project and supply of palm oil samples and some of the standard sterols. Their advice and comments are always in my appreciation. My special thanks to Professor C. J. W. Brooks (Chemistry Dept., Glasgow University, Scotland, United Kingdom) for donating the rest of the sterol standards.

Thanks also to Mr. Siew Yau Foo for his guidance and assistance in the use of GC-MS. I am indebted to many friends, especially Mr. Wong Chee Kong and Mr. Nasr Yousef M. J. Omar (MSc research student at Chemistry Dept., UM), for their assistance in various ways.

Finally, appreciation to Mr. Low Tek Sing and my beloved parents for their support and encouragement until completion of my study.

ABSTRACT

The unsaponifiable matters were separated from crude palm oil, crude palm kernel oil, and crude palm fibre oil. The sterol fractions were isolated by preparative thin layer chromatography and were then analysed by gas chromatography and combined gas chromatography-mass spectrometry. Qualitative separations of sterols are illustrated. Tables are presented showing the sterol composition determined in the oils, values obtained for individual contents are in broad agreement with other published results. There were no major differences between crude palm oil, crude palm kernel oil and crude palm fibre oil with respect to their sterols composition. β -sitosterol was found as the most predominant component in the sterol fraction from all oils. Sitostanol and ergosterol, of which their existence was not reported in any previous studies on palm oil, were found in the crude palm fibre oil samples, but were not detected in the crude palm oil and crude palm kernel oil samples.

CONTENTS

Acknowledgement	ii
Abstract	iii
Contents	iv
Abbreviations	1
List of Figures	3
List of Tables	8

CHAPTER 1 INTRODUCTION

1.1	Introduction to Palm Oil	9
1.2	Non-Glyceride Components (Unsaponifiable Matter) and Their Nutritional Significance	11
1.3	Sterols	12
1.4	Sterol Composition in Vegetable Oils	17
1.5	Saponification	23
1.6	Separation Methods	24
1.6.1	Column Chromatography	24
1.6.2	Thin Layer Chromatography (TLC)	25
1.6.3	Reverse Phase High-Performance Liquid Chromatography	26
1.6.4	Gas Chromatography (GC)	26
1.7	Gas Chromatography-Mass Spectrometry (GC-MS)	27

1.8	Derivatives of Sterols for Gas Chromatography	28
1.9	Significant of Study	30
1.10	Aims and Objectives	31
CHAPTER 2	EXPERIMENTAL	
2.1	General	32
2.2	Sources of Samples	32
2.3	Chemicals and Reagents	34
2.4	Sterol Reference Standards	34
2.5	Apparatus	34
2.6	Procedure	35
	2.6.1 Saponification	35
	2.6.2 Extraction	35
	2.6.3 Isolation of the Sterols by Thin Layer Chromatography	36
2.7	Derivatisation	38
2.8	Analysis of Sterol Fraction by GC	40
2.9	Identification and Confirmation by using GC-MS	41
	2.9.1 Scan Mode Identification	42
	2.9.2 Selected Ion Monitoring (SIM)	42
CHAPTER 3	RESULTS AND DISCUSSION	44
CHAPTER 4	CONCLUSIONS	67

REFERENCES	68
APPENDIX I	72
APPENDIX II	81
APPENDIX III	117
APPENDIX IV	154

ABBREVIATIONS

Appx.	appendix
BSTFA	N,O-bis(trimethylsilyl)-trifluoroacetamide
cm	centimetre
CPO	crude palm oil
CPKO	crude palm kernel oil
CPFO	crude palm fibre oil
FID	flame ionisation detector
Fig.	Figure
g	gram (s)
GC	gas chromatography
GC-MS	gas chromatography-mass spectrometry
HDL	high-density lipoprotein
HPLC	high-performance liquid chromatography
kg	kilogram (s)
i.d.	internal diameter
LDL	low-density lipoprotein
MS	mass spectrometry
µg	microgram (s)
µl	microlitre (s)
Me	methyl
mg	miligram (s)
ml	mililitre (s)

mm	milimetre (s)
m.p.	melting point
MW	molecular weight
m/z	mass/ion charge
NMR	nuclear magnetic resonance
PORIM	Palm Oil Research Institute of Malaysia
ppm	part per million
v/v	volume over volume
w/v	weight over volume
SIM	selected ion monitoring
TIC	total ion chromatogram
TLC	thin layer chromatography
TMS	trimethylsilyl ethers
TMSCl	trimethylchlorosilane

LIST OF FIGURES

Fig. 1: Oil Palm Fruit (*tenera*)

Fig. 2: Structures of Selected Sterols

Fig. 3: Structures of Selected 4-Methylsterols

Fig. 4: Structures of Selected Terpene Alcohols (Dimethylsterols)

Fig. 5: Illustration of a Soxhlet Extraction in Progress

Fig. 6: TLC Chromatogram of Unsaponifiable Matters on Silica Plate

Fig. 7: Flow Chart of Sample Preparation

Fig. 8: GC Chromatogram of Standard Mixture (Cholesterol, Brassicasterol, Ergosterol, Campesterol, Stigmasterol, β -Sitosterol, Sitostanol)

Fig. 9: GC Chromatogram of Derivatised Standard Mixture (Cholesterol TMS, Brassicasterol TMS, Ergosterol TMS, Campesterol TMS, Stigmasterol TMS, β -Sitosterol TMS, Sitostanol TMS)

Fig. 10: Fragment A

Fig. 11: Fragmentation of $\Delta^{24(28)}$ -Sterols

Appx. Fig. 1: GC-MS Chromatogram of Derivatised Standard Mixture (Cholesterol TMS, Brassicasterol TMS, Ergosterol TMS, Campesterol TMS, Stigmasterol TMS, β -Sitosterol TMS, Sitostanol TMS)

Appx. Fig. 1A: Mass Spectra of Cholesterol TMS

Appx. Fig. 1B: Mass Spectra of Brassicasterol TMS

Appx. Fig. 1C: Mass Spectra of Ergosterol TMS

Appx. Fig. 1D: Mass Spectra of Campesterol TMS

Appx. Fig. 3H: Mass Spectra of Peak t, 20.73

Appx. Fig. 4: GC-MS Chromatogram of CPFO Sample No.9 (TMS)

Appx. Fig. 4A: Mass Spectra of Peak t, 11.38

Appx. Fig. 4B: Mass Spectra of Peak t, 12.66

Appx. Fig. 4C: Mass Spectra of Peak t, 13.99

Appx. Fig. 4D: Mass Spectra of Peak t, 14.60

Appx. Fig. 4E: Mass Spectra of Peak t, 15.76

Appx. Fig. 4F: Mass Spectra of Peak t, 16.70

Appx. Fig. 4G: Mass Spectra of Peak t, 17.33

Appx. Fig. 4H: Mass Spectra of Peak t, 18.30

Appx. Fig. 4I: Mass Spectra of Peak t, 18.74

Appx. Fig. 4J: Mass Spectra of Peak t, 19.92

Appx. Fig. 4K: Mass Spectra of Peak t, 20.41

Appx. Fig. 4L: Mass Spectra of Peak t, 23.90

Appx. Fig. 4M: Mass Spectra of Peak t, 26.01

Appx. Fig. 5: GC-MS Chromatogram of CPO Sample No.6 (TMS)

Appx. Fig. 5A: TIC of SIM Mode Analysis, CPO Sample No.6 (TMS)

Appx. Fig. 5B: TIC of SIM Mode Analysis, CPO Sample No.6 (TMS), Spiked

Appx. Fig. 6: GC-MS Chromatogram of CPO Sample No.11 (TMS)

Appx. Fig. 6A: TIC of SIM Mode Analysis, CPO Sample No.11 (TMS)

Appx. Fig. 6B: TIC of SIM Mode Analysis, CPO Sample No.11 (TMS), Spiked

Appx. Fig. 7: GC-MS Chromatogram of CPO Sample No.18 (TMS)

Appx. Fig. 7A: TIC of SIM Mode Analysis, CPO Sample No.18 (TMS)

Appx. Fig. 7B: TIC of SIM Mode Analysis, CPO Sample No.18 (TMS), Spiked

Appx. Fig. 8: GC-MS Chromatogram of CPO Sample No.20 (TMS)

Appx. Fig. 8A: TIC of SIM Mode Analysis, CPO Sample No.20 (TMS)

Appx. Fig. 8B: TIC of SIM Mode Analysis, CPO Sample No.20 (TMS), Spiked

Appx. Fig. 9: GC-MS Chromatogram of CPKO Sample No.3 (TMS)

Appx. Fig. 9A: TIC of SIM Mode Analysis, CPKO Sample No.3 (TMS)

Appx. Fig. 9B: TIC of SIM Mode Analysis, CPKO Sample No.3 (TMS), Spiked

Appx. Fig. 10: GC-MS Chromatogram of CPKO Sample No.5 (TMS)

Appx. Fig. 10A: TIC of SIM Mode Analysis, CPKO Sample No.5 (TMS)

Appx. Fig. 10B: TIC of SIM Mode Analysis, CPKO Sample No.5 (TMS), Spiked

Appx. Fig. 11: GC-MS Chromatogram of CPKO Sample No.10 (TMS)

Appx. Fig. 11A: TIC of SIM Mode Analysis, CPKO Sample No.10 (TMS)

Appx. Fig. 11B: TIC of SIM Mode Analysis, CPKO Sample No.10 (TMS), Spiked

Appx. Fig. 12: GC-MS Chromatogram of CPKO Sample No.15 (TMS)

Appx. Fig. 12A: TIC of SIM Mode Analysis, CPKO Sample No.15 (TMS)

Appx. Fig. 12B: TIC of SIM Mode Analysis, CPKO Sample No.15 (TMS), Spiked

Appx. Fig. 13: GC-MS Chromatogram of CPFO Sample No.7 (TMS)

Appx. Fig. 13A: TIC of SIM Mode Analysis, CPFO Sample No.7 (TMS)

Appx. Fig. 13B: TIC of SIM Mode Analysis, CPFO Sample No.7 (TMS), Spiked

Appx. Fig. 14: GC-MS Chromatogram of CPFO Sample No.8 (TMS)

Appx. Fig. 14A: TIC of SIM Mode Analysis, CPFO Sample No.8 (TMS)

Appx. Fig. 14B: TIC of SIM Mode Analysis, CPFO Sample No.8 (TMS), Spiked

Appx. Fig. 15: GC-MS Chromatogram of CPFO Sample No.9 (TMS)

Appx. Fig. 15A: TIC of SIM Mode Analysis, CPFO Sample No.9 (TMS)

Appx. Fig. 15B: TIC of SIM Mode Analysis, CPFO Sample No.9 (TMS), Spiked

Appx. Fig. 16: GC-MS Chromatogram of CPFO Sample No.13 (TMS)

Appx. Fig. 16A: TIC of SIM Mode Analysis, CPFO Sample No.13 (TMS)

Appx. Fig. 16B: TIC of SIM Mode Analysis, CPFO Sample No.13 (TMS), Spiked