## **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	
	1.4	Sterol Composition in Vegetable Oils Saponification	
	1.5		
	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	EXP	ERIMENTAL		
	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	RES	RESULTS AND DISCUSSION		
CHAPTER 4	CO	CONCLUSIONS		

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

microgram (s)

MS mass spectrometry

μl microlitre (s)

Me methyl

μg

mg miligram (s)

ml mililitre (s)

mm milimetre (s)

m.p. melting point

MW molecular weight

m/z mass/ion charge

NMR nuclear magnetic resonace

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 chromatogarm

TLC thin layer chromatography

TMS trimethylsilyl ethers

TMSCI 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 tr 20.73

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

Appx. Fig. 4A: Mass Spectra of Peak t<sub>r</sub> 11.38

Appx. Fig. 4B: Mass Spectra of Peak t<sub>r</sub> 12.66

Appx. Fig. 4C: Mass Spectra of Peak tr 13.99

Appx. Fig. 4D: Mass Spectra of Peak t<sub>r</sub> 14.60

Appx. Fig. 4E: Mass Spectra of Peak t<sub>r</sub> 15.76

Appx. Fig. 4F: Mass Spectra of Peak tr 16.70

Appx. Fig. 4G: Mass Spectra of Peak t<sub>r</sub> 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 tr 20.41

Appx. Fig. 4L: Mass Spectra of Peak t<sub>r</sub> 23.90

Appx. Fig. 4M: Mass Spectra of Peak tr 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