

	Page
ACKNOWLEDGEMENTS	x
ABSTRACT	xi
OBJECTIVES	xvi
ABBREVIATIONS	xviii
LIST OF FIGURES	xxi
LIST OF TABLES	xxiv
 <b>CHAPTER 1 INTRODUCTION</b>	 1
1.1 THE PALM OIL INDUSTRY	1
1.1.1 General	1
1.1.2 Characteristics of Palm Oil	2
1.1.3 Minor Components in Crude Palm Oil	3
1.1.4 Carotenoids in Palm Oil	4
1.1.5 Extraction of Palm Oil	6
(a) Sterilisation	6
(b) Stripping	6
(c) Digestion	6
(d) Oil Extraction	7
(e) Clarification and Purification of Crude Palm Oil	7
1.1.6 Refining of Palm Oil	9
1.2 CAROTENOIDS	13
1.2.1 Introduction	13
1.2.2 Structure	13

1.2.3	Nomenclature	14
1.2.4	Biosynthesis	16
1.2.5	Physical Properties	20
	(a) Absorption Spectroscopy	20
	(b) Other Spectroscopic Methods	21
1.2.6	Chemical Properties of Carotenoids	22
1.2.7	Applications of Carotenoids	22
	(a) Colourant	22
	(b) Medical Applications	23
1.3	ANALYTICAL TECHNIQUES	24
1.3.1	Introduction	24
1.3.2	Extraction	25
1.3.3	Saponification	26
1.3.4	Separation of Carotenoids	26
	(a) Phase Separation	27
	(b) Open Column Chromatography	27
	(c) Thin Layer Chromatography	27
	(d) Gas Liquid Chromatography	28
	(e) High-Performance Liquid Chromatography	28
1.3.5	Detection	30
1.4	RECOVERY OF CAROTENOIDS	31
1.4.1	Introduction	31
1.4.2	Natural Carotenoids	31
1.4.3	Recovery of Carotenoids from Palm Oil	32
1.5	PROPERTIES OF CAROTENOIDS	34
1.5.1	Antioxidant and Singlet-Oxygen Quenching Properties	34
	(a) A Singlet-Oxygen Quencher	34
	(b) Antioxidant Activity of Carotenoids	35

1.5.2 Photofunctions in Plants	35
1.5.3 Physiological Functions of Carotenoids	36
(a) Pro-vitamin A Activity	36
(b) Photoprotection	39
(c) Immunoenhancement	39
(d) Lung Cancer	39
(e) Other Cancers	40
(f) Cataract	41
1.5.4 Safety	41
 <b>CHAPTER 2 RESULTS AND DISCUSSION</b>	 42
2.1 DETERMINATION OF CAROTENES IN VARIOUS OIL PALM SPECIES	42
2.1.1 Analysis of Palm Oil Carotenoids by Non-Aqueous Reversed-Phase HPLC	42
2.1.2 Carotenoids in Various Oil Palm Species	54
2.2 OTHER MINOR CONSTITUENTS IN VARIOUS OIL PALM SPECIES	60
2.2.1 Vitamin E Compositions	60
2.2.2 Sterol Compositions	65
2.3 EXTRACTION OF CAROTENE-ENRICHED OIL	69
2.3.1 Residual Oil from Pressed Palm-Fruit Fibres	69
(a) Organic Solvent Extraction	70
(b) Liquid CO <sub>2</sub> Extraction	71
(c) Chemical Composition of Residual Fibre Oil	72
2.3.2 Oil from Second Pressings of Mesocarp Fibres	74
2.3.3 Oil Extracted from Exocarp and Mesocarp	77

2.4	RELATIVE DISTRIBUTION OF MINOR CONSTITUENTS IN FIBRE, "SECOND-PRESSED" AND EXOCARP OILS	79
2.4.1	Carotene Profiles of Oil Extracts	79
2.4.2	Other Minor Constituents	81
	(a) Vitamin E	81
	(b) Sterols	82
2.4.3	Fatty Acid Compositions of Fibre and "Second-Pressed" Oils	84
2.4.4	Other Quality Parameters of Fibre and "Second-Pressed" Oils	85
2.5	RECOVERY OF CAROTENES FROM PALM OIL	88
2.5.1	Introduction	88
	(a) Physical Recovery Methods	88
	(b) Chemical Recovery Methods	89
2.5.2	Molecular Distillation of Esters	90
2.5.3	Carotene Profile by HPLC	94
2.5.4	Other Constituents	95
2.5.5	Applications of Carotene Concentrate	96
2.5.6	Toxicology Study	99
2.6	PRODUCTION OF RED PALM OIL	100
2.6.1	Introduction	100
2.6.2	Pretreatment of Crude Palm Oil	101
2.6.3	Deacidification and Deodorisation	105
2.6.4	Pilot Plant Production of Red Palm Oil	111
2.6.5	Carotene Composition of Deacidified and Deodorised Red Palm Oil	114
2.6.6	Oxidative Stability of Red Palm Oil	116
2.6.7	Applications of Red Palm Oil	119

2.7	PHOTOPROTECTIVE AND ANTIOXIDANT ACTIVITIES OF PALM CAROTENES	121
2.7.1	Effect of Palm Carotenes on the Photooxidation of Fatty Acids	122
2.7.2	Effect of Palm Carotenes on LDL Oxidation	133
	(a) $\text{Cu}^{2+}$ -Catalysed Autoxidation of LDL	135
	(b) Photooxidation of LDL	137
2.8	EFFECT OF VARIOUS ANTIOXIDANTS ON THE APPH-INDUCED OXIDATIONS OF LDL AND PLASMA	141
2.8.1	Introduction	141
2.8.2	AAPH Oxidation of LDL Isolated from Human plasma	142
2.8.3	AAPH oxidation on Human Plasma	149
2.9	CAROTENE DISTRIBUTION AND OXIDATIVE STABILITY OF LDL	162
2.9.1	Introduction	162
2.9.2	Carotene Distribution	163
2.9.3	Distribution of Retinyl Esters	171
2.9.4	Retinol Distribution	178
2.9.5	Susceptibility of LDL to Oxidation	181
	(a) Fatty Acid Composition of Plasma and LDL	182
	(b) Oxidative Stability of LDL	185
	<b>CHAPTER 3 EXPERIMENTAL</b>	194
3.1.	EXTRACTION OF PALM OILS	194
3.1.1	Materials	194
3.1.2	Oil Palm Species and Varieties	194
3.1.3	Residual Oil from Screw-Pressed Fibres	195
	(a) Solvent Extraction	195
	(b) High Pressure Liquid $\text{CO}_2$ Extraction	195

3.1.4	Double-Screw Pressing of Fibres	197
3.1.5	Exocarp and Mesocarp of the Oil Palm Fruits	197
3.2.	ANALYSIS OF CAROTENES BY NON AQUEOUS REVERSED-PHASE HPLC	198
3.2.1	Materials	198
3.2.2	Saponification Procedure	198
3.2.3	HPLC Analysis	198
3.2.4	Spectrophotometric Determination of Carotenoids	199
3.2.5	Iodine-Catalysed Photoisomerisation of Carotenes	200
3.3.	HPLC ANALYSIS OF TOCOPHEROLS AND TOCOTRIENOLS	200
3.4.	DETERMINATION OF STEROLS	201
3.4.1	Materials	201
3.4.2	Saponification	201
3.4.3	Isolation of Sterols by TLC	201
3.4.4	Quantification of Sterols	202
3.5.	FATTY ACID COMPOSITIONS OF VARIOUS PALM OIL AND PALM OIL PRODUCTS	202
3.5.1	Preparation of Fatty Acid Methyl Esters	202
3.5.2	Analysis of Fatty Acid Composition by Gas Chromatography	203
3.6.	PREPARATION OF CAROTENE CONCENTRATE	203
3.6.1	Materials	203
3.6.2	Determination of Free Fatty Acid Content (VOTC Method)	204
3.6.3	Laboratory Scale Transesterification	205
3.6.4	Molecular Distillation of Methyl Esters	205
3.6.5	Pilot Plant Production of Esters	206
3.6.6	Pilot Plant Production of Carotene Concentrate	206

3.7. CAROTENE CONCENTRATE IN POWDER FORM	207
3.7.1 Materials	207
3.7.2 Preparation of Palm Carotene Powder	207
3.7.3 Determination of Carotene Content	208
3.7.4 Stability of Carotene Powder	208
3.8. PREPARATION OF DEACIDIFIED AND DEODORISED RED PALM OIL	209
3.8.1 Materials	209
3.8.2 Pretreatment of Crude Palm Oil	209
3.8.3 Deodorisation and Deacidification	209
3.8.4 Pilot Plant Production of Deodorised and Deacidified Red Palm Oil	210
3.8.5 Determination of Induction Period	210
3.8.6 Analytical Methods	212
3.9. PHOTOPROTECTIVE AND ANTIOXIDANT ACTIVITIES OF PALM CAROTENES	212
3.9.1 Materials	212
3.9.2 Preparation of Methyl Esters of Soybean Oil	212
3.9.3 Photosensitised Oxidation of Fatty Acid Methyl Esters	213
3.9.4 Analysis of the FAC of Photosensitized Oxidation Products	213
3.9.5 Determination of Total Carbonyl Compounds with 2,4-Dinitrophenylhydrazine	215
(a) Preparation of Carbonyl Derivatives	215
(b) Separation of Carbonyl Derivatives by Preparative TLC	215
(c) Quantitation of Carbonyl Compounds	216
3.10 EFFECT OF PALM CAROTENES ON LDL OXIDATION	216
3.10.1 Materials	216
3.10.2 Isolation of Low Density Lipoprotein	217

3.10.3	Dialysis of Isolated LDL Solution	218
3.10.4	Protein Determination (Lowry Method)	218
3.10.5	Oxidation of LDL Supplemented with Palm Carotenes	220
	(a) Photooxidation	220
	(b) Autooxidation	221
3.10.6	Autooxidation of LDL Supplemented with Vitamin E	221
3.11.	EFFECT OF CAROTENES, VITAMIN E AND VITAMIN C ON THE OXIDATION OF PLASMA AND LDL	222
3.11.1	Materials	222
3.11.2	Palm Carotene Supplementation	222
3.11.3	Azo-Initiated Oxidation	223
3.11.4	Determination of Conjugated Dienes in LDL	223
3.11.5	Determination of $\alpha$ -Tocopherol in Plasma and LDL	223
	(a) Extraction of Lipid Components from Plasma/LDL	223
	(b) HPLC Analysis of $\alpha$ -Tocopherol	224
3.11.6	Determination of $\beta$ -Carotene in Plasma	224
	(a) Extraction of Carotenes from plasma	224
	(b) HPLC Determination of $\beta$ -Carotene	225
3.11.7	Quantification of Lipid Hydroperoxides	225
	(a) Extraction of Lipid Hydroperoxides	225
	(b) Preparation of Isoluminol and Microperoxidase Solutions	225
	(b) HPLC Analysis of Lipid Hydroperoxides	225
3.11.8	Quantification of Ascorbic Acid	226
	(a) Extraction of Ascorbic Acid	226
	(b) HPLC Analysis	226
3.11.9	Carbonyl Assays	227



3.12 CAROTENE DISTRIBUTION AND OXIDATIVE STABILITY OF LDL	228
3.12.1 Materials	228
3.12.2 Animals and Diets	228
3.12.3 Oxidation of LDL	230
3.12.4 Total Fatty Acid Composition	230
(a) Extraction of Lipids	230
(b) Transesterification/Esterification of Esters/Fatty acids	230
(c) Gas Chromatographic Analysis	231
3.12.5 Carotene, Retinol and Retinyl Esters in Rabbit's Plasma and Organs	231
(a) Extraction of Carotenes, Retinol and Retinyl Esters	231
(b) HPLC Analysis	232
3.12.6 Analysis of Plasma/LDL Vitamin E	232
REFERENCES	233
LIST OF PUBLICATIONS	251