# CHAPTER 1

# INTRODUCTION

## 1.1 Introduction to Palm Oil

Malaysia is becoming the largest producer and exporter of palm oil in the world. Today palm oil is an important source of oils and fats in the world and is one of the world's most consumed edible oil. The current planting material in Malaysia is a cross of the *dura* and *pisifera* varieties known as *tenera*, all belonging to the *Elaeis guineensis* species originated in West Africa[1].

The oil palm tree is a sturdy perennial producing fruits throughout the year, with the production levels depending on weather conditions. It is a tropical plant which flourishes in areas with abundant rainfall. The tree starts to bear fruit within three years after planting and has a fruit yielding life span of about 25 years[2].

Palm oil and palm kernel oil are obtained from the oil palm fruit. Crude palm oil (CPO) is derived from the mesocarp (the fleshly portion of the fruit wall), whilst extraction from the hard nut inside the fruit (endosperm) yields palm kernel oil (PKO). Fibre is obtained from the pressed cake, or residue, which is expelled after oil extraction. Solvent extraction of the palm fibre produces fibre oil. Variations in chemical composition determine the chemical and physical properties of the three oils.

Palm oil consists mainly of glycerides where triglycerides made up the major component, with diglycerides and monoglycerides in small proportions[1]. Palm oil also contains about 1-8% of minor constituents such as carotenoids, vitamin E (tocophenols and tocotrienols), sterols, glycolipids, phospholipids, terpenic, phenolics and paraffin hydrocarbons. Among these the carotenoids, α-tocophenols, tocotrienols and sterols show valuable physiological and medical properties.

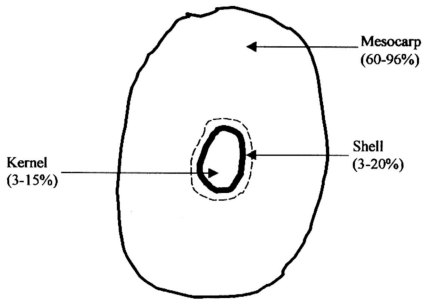



Fig. 1: Oil Palm Fruit (*tenera*)[3]

## 1.2 Non-Glyceride Components (Unsaponifiable Matter) and Their Nutritional Significance

The non-glyceride components content varies from oil to oil. It may range between 2-8% in some oils, but the constitution in most oils is 1% or below[4].

Edible oils with high non-glyceride components appear to contain a number of chemical factors which have hypocholesterolemic potential. Among the compounds useful in the prevention of hypercholesterolemia and the resulting coronary heart diseases are sterols and terpene alcohols (Table I)[4]. A number of research information suggested that the hypolipidemic effect is attributed mainly to cyclic terpene alcohols (dimethylsterols) and sterols present in the unsaponifiable fraction. Sterols and cycloartenol (a terpene alcohol) has been reported to be hypolipidemic in rats (Rukimini C and Raghuram TC, 1991; Kiribuchi M, Miura K, Oshima S, Kuga T and Mitani M, 1984)[4]. Sterols are considered to act by competitively inhibiting cholesterol synthesis in the lever and cholesterol absorption from the gut. Triterpene alcohols inhibit cholesterol esterase activity non-competitively when present with 2-phenylethanol, resulting in a slow hydrolysis of cholesterol esters and subsequently a decrease in cholesterol absorption[4].

Table 1: Hypocholesterolemic Effect of Non-Glyceride Components of Vegetable Oils (Phytosterols and Terpene Alcohols)[4]

| Component | Oils Rich in | Mechanism of Action |
|---|---|---|
| ***Phytosterols*** | | |
| • Stigmasterol | Rice bran | Cholesterol absorption |
| • Sitosterol | Rapeseed | Cholesterol excretion |
| • Campesterol | Corn oil | |
| • Brassicasterol | Sesame oil | |
| ***Terpene alcohols*** | | |
| • Oryzanol | Rice bran | Cholesterol excretion |
| • Cycloartenol | Olive, soybean | Increased through bile |
| • 24-Methylene cycloartenol | Linseed | cholesterol absorption |

Besides sterols and terpene alcohols, other non-glyceride components such as vitamin E and carotenoids have long been known to possess antioxidant property.

### 1.3 Sterols

Sterol is a group of steroid which contains hydroxyl group at C3 and side chain with 8 or more carbon atoms at C17[5]. Sterols are widely distributed in animal and particularly the plant kingdoms[5], with bacteria being the only forms of life not possessing them[6]. They are highly crystalline, and may be classified on the basis of occurrence as zoosterols (animals), phytosterols (plants), mycosterols (yeast and fungi), and marine sterols (sponges)[7]. Plant sterols differ from animal sterols in having either one or two extra carbons at C24[8]. The $\Delta^5$-unsaturatation form of sterols are referred to as stenols whilst their saturated derivatives are called stanols[7].

Cholesterol is the most common sterol present in nearly all animal tissues, but is rare in plants. It is an integral part of cell membrane, and as the precursor of steroid hormones and bile acids[6]. Human adults consist about 250 g cholesterol in average[9]. Free sterol forms 17% of the solid matter of human brain and is the chief constituent of gall-stones[7].

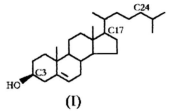
In 1879, sitosterol, the first sterol to be isolated in a state of purity was obtained from cereal grains. 27 years later, Windause and Hauth successfully obtained stigmasterol from Calabar beans (*Phytostigma venenonsum*)[10]. Sterols occur in plants as free alcohols, as esters of long-chain fatty acids, or as glycosides. They are present in seeds, fruits, roots or green tissues[10]. Sterol pattern is not the same in all parts of the plant and its seeds and it changes during plant development[11].

The principal sterols in most plants are the $\Delta^5$-sterols, β-sitosterol and stigmasterol. The next most abundant sterol is campesterol. The abundance of campesterol ranges from 23-80% in the Bryophyta, from 20-90% in seedless vascular plants, and from 30-80% in seed-bearing vascular plants[11]. Cholesterol, 24-methylenecholesterol, and isofucosterol are widely distributed as minor components.

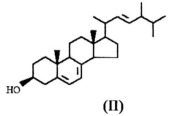
Fig. 2: Structures of Selected Sterols.

(I) Cholesterol, (II) Ergosterol, (III) Brassicasterol, (IV) Campesterol,

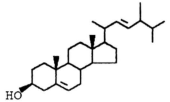
(V) $\Delta^5$-Avenasterol, (VI) Stigmasterol, (VII) β-Sitosterol, (VIII) Sitostanol



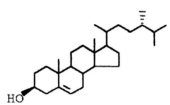

(I)



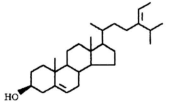

(II)



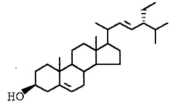

(III)



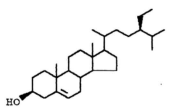

(IV)



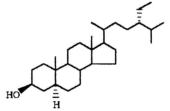

(V)




(VI)



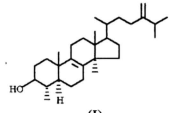

(VII)



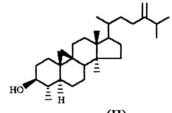

(VIII)

Fig. 3: Structures of Selected 4-Methylsterols.

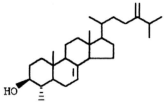
(I) Obtusifoliol, (II) Cycloeucalenol, (III) Gramisterol, (IV) Citrostadienol



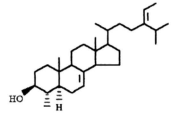

(I)




(II)



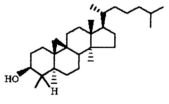

(III)



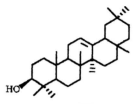

(IV)

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

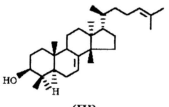
(I) Cycloartanol, (II) β-Amyrin, (III) Butyrospermol, (IV) Cycloartenol, (V) α-Amyrin, (VI) Lupeol, (VII) 24-Methylenecycloartanol



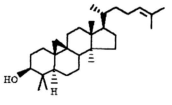

(I)



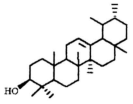

(II)



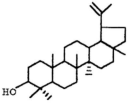

(III)



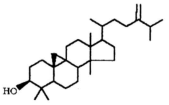

(IV)




(V)




(VI)




(VII)

## 1.4 Sterol Composition in Vegetable Oils

Sterols are among the lipids of nutritional interest that are frequently determined in fats and oils and other foods. Much research work has been carried out during the past three decades to identify the sterol composition in commonly used edible oils.

Slover *et al.*[12] analysed the sterol fraction from thirteen vegetable oils and identified β-sitosterol, stigmasterol and campesterol in every oil studied. T. M. Jeong *et al.*[13] reported the sterol composition of twenty vegetable oils. All oils except pumpkin seed oil consist mainly of $\Delta^5$-sterols while $\Delta^7$-sterols are present only in small proportion at the most. Much effort has been done on sterol determination by Itoh *et al.*[13-16] in the 70's. Other than the major sterols, the wide spread occurrence of $\Delta^7$- stigmastenol, $\Delta^5$-avenasterol and $\Delta^7$-avenasterol was demonstrated in other vegetable oils. Shea butter is particularly rich in these minor sterols.

Table 2[17] (adapted from Itoh *et al.*, 1973a, b, 1974) lists the sterol content of some commonly occurring vegetable oils. It is evident that a majority of the oils contain β-sitosterol, stigmasterol and campesterol. Cholesterol was reported to be present in small amounts in some of these oils at levels less than 40 mg/kg, except cocoa butter oil and linseed oil with a slightly higher amount. Brassicasterol, which is characteristic of the Cruciferae family, is present in rapeseed oil at level of 612 ppm. Coffee seed, rice bran, and wheat germ oils have relatively high amounts of $\Delta^5$-avenasterol (>900 mg per 1 kg oil). Rice bran, safflower, sunflower, shea butters, and wheat germ oils are all good

sources of $\Delta^7$-stigmastenol (>500 ppm). The level of sterols in palm oil is lower than for most vegetable oils.

The content and composition of sterols in crude palm oil and palm kernel oil have been reported by several authors such as Itoh *et al.* (1973)[14,15,16], J. L. Weihrauch and J. M. Gardner (1978)[31], Downes (1982, 1985)[18,20] and T. S. Tang (1996)[21], but in many of these papers only the dominant sterols have been studied, and none has been reported for the sterol constituents of oil extracted from palm fibre. For comparison, the literature data on the percent distribution of individual sterols in the sterol fractions of crude palm oil and crude palm kernel oil are presented in Table 3 to 5.

From the tables, the following sterols (including members of methylsterols and terpene alcohols) are found to be present in crude palm oil and crude palm kernel oil:

CPO – cholesterol, brassicasterol, campesterol, stigmasterol, β-sitosterol, $\Delta^5$-avenasterol, $\Delta^7$-stigmastenol, $\Delta^7$-avenasterol, obtusifoliol, cycloeucalenol, gramisterol, citrostadienol, cycloartanol, cycloartenol, 24-methylenecycloartanol.

CPKO - cholesterol, brassicasterol, campesterol, stigmasterol, β-sitosterol, $\Delta^5$-avenasterol, $\Delta^7$-stigmastenol, $\Delta^7$-avenasterol, obtusifoliol, cycloeucalenol, gramisterol, citrostadienol, β-amyrin, butyrospermol, cycloartenol, α-amyrin, lupeol, 24-methylenecycloartanol.

The presence of sitostanol, a sterol which is harvested from pine tree[22], has not yet been reported by any previous studies.

Table 2: Sterol Composition (mg/kg) of Some Commonly Occurring Vegetable Oils (Adapted from Itoh *et al*., 1973a, b, 1974)[17]

| Vegetable oil | Cholesterol | Brassicasterol | Campesterol | Stigmasterol | β-Sitosterol | $\Delta^5$ -Avenasterol | $\Delta^7$ -Stigmastenol | $\Delta^7$ -Avenasterol | Unknown |
|---|---|---|---|---|---|---|---|---|---|
| Castor oil | - | - | 285 | 627 | 1254 | 599 | 57 | 28 | - |
| Cocoa butter | 59 | - | 266 | 769 | 1746 | 88 | 29 | - | - |
| Coconut | 23 | - | 18 | 296 | 1322 | 319 | 136 | - | - |
| Coffee seed | - | - | 3488 | 3672 | 9914 | 1101 | 183 | - | - |
| Corn oil | - | - | 2691 | 702 | 7722 | 468 | 117 | - | - |
| Cottonseed | - | - | 170 | 42 | 3961 | 85 | - | - | - |
| Illipe butter | - | - | 739 | 323 | 3234 | 227 | 46 | - | - |
| Kapok | - | - | 256 | 57 | 2451 | 57 | 28 | - | - |
| Linseed | 42 | - | 1218 | 378 | 1932 | 546 | 84 | - | - |
| Olive (France) | - | - | 28 | 14 | 1310 | 29 | 58 | - | - |
| **Palm** | **26** | - | **358** | **204** | **1894** | **51** | **25** | - | - |
| **Palm kernel** | **40** | - | **118** | **145** | **924** | **79** | **13** | - | - |
| Peanut | - | - | 360 | 2160 | 1536 | 192 | 72 | 24 | - |
| Poppy | - | - | 605 | 83 | 1870 | 55 | 55 | - | 83 |
| Rapeseed | - | 612 | 1530 | - | 3549 | 122 | 306 | - | - |
| Rice bran | - | - | 5056 | 2709 | 8849 | 903 | 180 | 361 | - |
| Safflower | - | - | 452 | 313 | 1809 | 35 | 696 | 104 | 69 |
| Safflower (oleic-rich) | - | - | 576 | 384 | 1996 | 38 | 576 | 192 | 77 |
| Sal | - | - | 1469 | 918 | 3427 | 306 | - | - | - |
| Sesame | - | - | 1170 | 616 | 3819 | 431 | 123 | - | - |
| Shea butters | - | - | - | - | - | - | 944 | 281 | 132 |
| Soybean | - | - | 720 | 720 | 1908 | 108 | 108 | 36 | - |
| Sunflower | - | - | 313 | 313 | 2352 | 156 | 588 | 156 | 39 |
| Wheat germ | - | - | 5702 | - | 17336 | 1555 | 777 | 518 | - |

Table 3: Comparison with Literature Data for Sterol Composition in Crude Palm Oil[17-19]

| | REFERENCE | a | b | c | d | e | f | g | h | i |
|---|---|---|---|---|---|---|---|---|---|---|
| STEROLS % | • Cholesterol $C_{27}H_{46}O$ MW 386 | 2.2-6.7 | 1.1-8.8 | 4.3 | 1 | 2.7 | 2.2 | 8 | 3.5 | 1.9 |
| | • Brassicasterol $C_{28}H_{46}O$ MW 398 | nd | 0.0-tr | tr | nd | nd | nd | nd | nd | - |
| | • Campesterol $C_{28}H_{48}O$ MW 400 | 18.7-29.1 | 14.0-23.4 | 19.8 | 14 | 23.5 | 18 | 20.3 | 23 | 23.9 |
| | • Stigmasterol $C_{29}H_{48}O$ MW 412 | 8.9-13.9 | 8.0-13.3 | 12.1 | 8 | 11.3 | 11 | 10.4 | 11.7 | 11.4 |
| | • β-Sitosterol $C_{29}H_{50}O$ MW 414 | 50.2-62.1 | 58.1-70.4 | 62.1 | 74 | 60.7 | 58.1 | 61.3 | 57.7 | 59.7 |
| | • $\Delta^5$-Avenasterol $C_{29}H_{48}O$ MW 412 | 0.0-2.8 | < 2.0 | 1.7 | 2 | 1.7 | 0.3 | tr | 2.5 | - |
| | • $\Delta^7$-Stigmastenol $C_{29}H_{50}O$ MW 414 | 0.2-2.4 | <1.0 | - | 1 | tr | tr | tr | 0.7 | - |
| | • $\Delta^7$-Avenasterol $C_{29}H_{48}O$ MW 412 | 0.0-5.1 | <1.9 | - | nd | nd | 1.9 | nd | 1 | - |
| | Others | - | - | - | - | - | - | - | - | 3.1 |
| | Total sterols (mg/kg) | 362-672 | - | 1170 | - | - | - | - | 389-481 | 210-620 |

Note:
nd = not detected
(-) = no figures given
tr = trace

a = Downes (1982)
b = Codex (1978)
c = Weihrauch & Gardner (1978)
d = Itoh *et al.* (1973)
e = Mannino & Amelotti (1975)
f = Prevot & Mordret (1976)
g = Castang *et al.* (1976)
h = Rossel & Pritchard (1990)
i = Siew (1990)

Table 4: Comparison with Literature Data for Sterol Composition in Crude Palm Kernel Oil[17,20,21]

| | REFERENCE | a | b | c | d | e | f | g | h | i |
|---|---|---|---|---|---|---|---|---|---|---|
| STEROLS % | • Cholesterol $C_{27}H_{46}O$ MW 386 | 1.0-3.7 | 0.5-2.2 | 0.5-3.0 | 3 | 2.1 | 0.5 | 1.2 | 1.7 | 1.7 |
| | • Brassicasterol $C_{28}H_{46}O$ MW 398 | nd-0.3 | - | 0-tr | tr | tr | - | - | 0.1 | 0.1 |
| | • Campesterol $C_{28}H_{48}O$ MW 400 | 8.4-12.7 | 9.2-10.8 | 8.7-12.0 | 9 | 8.6 | 11.4 | 10.7 | 10 | 9.7 |
| | • Stigmasterol $C_{29}H_{48}O$ MW 412 | 12.3-16.1 | 12.4-16.6 | 11.0-15.9 | 11 | 13.6 | 15.9 | 13.7 | 13.7 | 14.4 |
| | • β-Sitosterol $C_{29}H_{50}O$ MW 414 | 62.6-70.4 | 64.7-73.4 | 68.0-70.4 | 70 | 69.3 | 70 | 67.1 | 67 | 67.3 |
| | • $\Delta^5$-Avenasterol $C_{29}H_{48}O$ MW 412 | 4.0-9.0 | 2.0-6.8 | 2.7-6.0 | 6 | 5 | 2.7 | 5.5 | 6.2 | 4.5 |
| | • $\Delta^7$-Stigmastenol $C_{29}H_{50}O$ MW 414 | nd-2.1 | 0-0.3 | <1.0 | 1.0 | 1.4 | - | 0.3 | 0.6 | 0.3 |
| | • $\Delta^7$-Avenasterol $C_{29}H_{48}O$ MW 412 | nd-1.4 | 0-tr | - | tr | tr | - | - | 0.1 | 0.2 |
| | Others | nd-2.7 | - | - | - | - | - | - | 0.7 | 1.9 |
| | Total sterols (mg/kg) | 792-1187 | - | - | - | 1400 | 900 | 810 | 792-1187 | 985-1228 |

Note:
nd = not detected
(-) = no figures given
tr = trace

a = Downes (1985)
b = Castang (1981)
c = Codex (1978)
d = Itoh *et al.* (1973)
e = Weihrauch & Gardner (1978)
f = Seher & Vogel (1976)
g = Homberg & Bielefeld (1976)
h = Rossel & Pritchard (1990)
i = Tang Thin Sue (1996)

Table 5: Comparison of 4-Methylsterol Composition in Crude Palm Oil and Crude Palm Kernel Oil (Adapted from Itoh *et al*., 1974)[15,16]

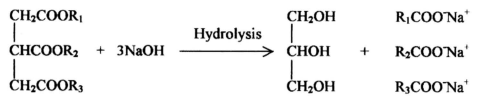
| 4-Methylsterols (%) | CPO | CPKO |
|---|---|---|
| • Obtusifoliol $C_{30}H_{50}O$ MW 426 | 17 | 13 |
| • Cycloeucalenol $C_{30}H_{50}O$ MW 426 & Gramisterol $C_{29}H_{48}O$ MW 412 | 67 | 20 |
| • Citrostadienol $C_{30}H_{50}O$ MW 426 | 9 | 30 |
| Unknown | 7 | 37 |

Table 6: Comparison of Terpene Alcohol (Dimethylsterol) Composition in Crude Palm Kernel Oil and Crude Palm Kernel Oil (Adapted from Itoh *et al*., 1974)[15,16]

| Dimethylsterols (%) | CPO | CPKO |
|---|---|---|
| • Cycloartanol $C_{30}H_{52}O$ MW 428 | 2 | - |
| • β-Amyrin $C_{30}H_{50}O$ MW 426 | - | 4 |
| • Butyrospermol $C_{30}H_{50}O$ MW 426 | - | 9 |
| • Cycloartenol $C_{30}H_{50}O$ MW 426 | 60 | 41 |
| • α-Amyrin $C_{30}H_{50}O$ MW 426 | - | 29 |
| • Lupeol $C_{30}H_{50}O$ MW 426 | - | 13 |
| • 24-Methylenecycloartanol $C_{31}H_{52}O$ MW 440 | 34 | 4 |
| Unknown | 4 | - |

## 1.5 Saponification

Saponification is an alkaline process in which glycerides are hydrolysed to produce glycerol and salts of carboxylic acids. The chemistry involved in saponification is as below.



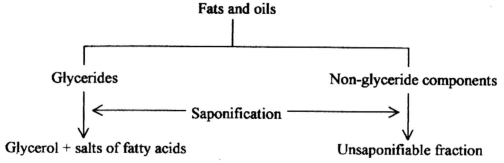

where $R_1$, $R_2$ and $R_3$ are hydrocarbon chains.

Glycerol and salts of fatty acids formed in the process are soluble in water, whereas other compounds in the unsaponifiable fraction (e.g. sterols, hydrocarbons, terpene alcohols) are water insoluble. Thus, the unsaponified matters can be separated from the saponified fraction by doing liquid-liquid extraction. The reasons of doing unsaponification are:

i) High percentage of triglycerides may be interfered with chromatographic procedures

ii) A lot of the polar multifunctional components are not stable in gas chromatograph

iii) To remove triglycerides and other water soluble components (e.g. phospholipids, phenolics, nitrogen and phosphorus containing compounds)




## 1.6 Separation Methods

The physicochemical properties of many of the sterols are very similar, since they are only differed structurally from one another only at epimeric centres, in the length of the side chain, and in the degree of unsaturation of the ring system and side chain. The differences in relation to the entire molecule are small, thus separation of the different components of the mixtures is difficult. Column chromatography, thin layer chromatography, high-performance liquid chromatography and gas chromatography have been used for separation.

### 1.6.1 Column Chromatography

Column chromatography is useful in separation of sterols especially when sterol fraction is high in mass (>10 mg)[23]. Adsorption or ordinary phase systems (alumina or silica gel) eluted with gradients of moderate polarity separate sterols in order of increasing polarity. This chromatographic system is good for separating sterols according to the kind and number of their oxygen functions (W. R. Nes *et al.*, 1985)[23]. Reverse phase chromatography system employs lipophyllic hydrophobic sephadex derivatives as the stationary phase, elution with polar solvents separate sterols in order of decreasing

polarity and increasing size. Such system has been employed by W. R. Nes *et al.* (1976)[23] and Chiu *et al.* (1985)[23] in the isolation of sterols with C24 methyl and ethyl groups. These systems are more sensitive to structural changes than the normal systems. Argentation column chromatography, in which silica is impregnated with a solution of $AgNO_3$ and dried, is most often used in the separation of sterols as their acetates. The systems have been developed that enhance the separation of sterol differing in the number and position of double bonds from a variety of sources (Lutsky *et al.*, 1971, 1975)[23]. Polar solvents are usually used.

### 1.6.2 Thin Layer Chromatography (TLC)

TLC can be used for two different purposes:

(a) Separation of sterols fraction from unsaponifiable material

Preparative TLC is usually performed for the purpose of preliminary fractionation. Development of the plate can be performed by using several types of solvent mixture, for example hexane:diethyl ether (3:1), benzene:acetone (95:5)[24], or many others. Unsaponifiable matter is then separated into three or four zones. This method was widely used by the early investigators, and it is still a chief method used to separate the sterols fraction from unsaponifiable material at present.

(b) Separation of the individual sterols from the sterol fraction

TLC systems (adsorption, argenetic, and reversed-phase) have been developed which can be used in the separation of component sterols from sterols fraction

that are less than 10 mg[23]. Reversed-phase paper chromatography is frequently used, and paraffin oil being the most common stationary phase. De Zatti, Campella and Jacini separated ergosterol and fucosterol from β-sitosterol by using liquid parrafin as the stationary phase, and mobile phase made of 85% pyridine and 15% water[10]. Only limited success has been achieved by this method. The technique is not regarded as a satisfactory one for the separation of sterols, because the $R_F$ values of several sterols (e.g. stigmasterol and β-sitosterol) are identical, and they are commonly occur together.

### 1.6.3 Reverse Phase High-Performance Liquid Chromatography

Reverse phase HPLC, typically with the aid of a column containing C18 or C8 residues chemically bonded to silica and methanol-based solvent systems, is a powerful chromatographic technique which has great utility for sterol analysis as well as for the isolation of individual sterols[11]. Diode-array multiple wavelength detectors can be used for obtaining UV spectra of the components as they elute from the column. Simple and complex reverse phase HPLC systems have been used by a number of workers to isolate individual sterols from a variety of sources (W. D. Nes *et al.*, 1985; Poll *et al.* 1985)[23].

### 1.6.4 Gas Chromatography (GC)

Gas chromatography is the technique of choice for the separation of thermally stable and volatile organic compounds. It accomplishes the separation by partitioning the components of a chemical mixture between a moving (mobile) gas phase and a stationary

phase. It is an excellent tool for the separation, detection, and quantitation of the components of a complex mixture, but is not a good tool for qualitative identification. For good qualitative analysis, gas chromatography should be combined with some other analytical system, and often, confirmation by two or more methods is desirable. Mass spectrometry is frequently a preferred choice because of its high sensitivity and relatively specific spectral information. The basic components of a gas chromatography are shown as follow:

Gas carrier → Flow regulator → Column →Detector →Recorder

↑

Injector

Flame ionisation detector (FID) is the most commonly employed for sterol analysis in GC because of (a) its sensitivity (b) its universality (c) it is relatively insensitive to temperature changes during analysis (d) it has a large linear mass range of response.

## 1.7 Gas Chromatography-Mass Spectrometry (GC-MS)

Mass spectrometer is an analytical technique which is able to detect and characterise organic compounds, giving definite and structural information from minute amounts of material. The combination of GC and MS is particularly useful for lipid studies because complex mixture may be separated and identified. In general, any compounds that are volatile enough for GC separation are sufficient volatile for MS analysis. Compounds which are non-volatile can usually be studied after derivatisation.

In electron ionisation, mass spectra are obtained by bombarding the molecules of a compound in the vapour phase at low pressure with electrons. Interaction of molecules with electrons causes ionisation and produces a series of parent ions and fragment ions. The bombarding voltage commonly used is 70 eV. At this electron energy the total probability of ionisation is high, and there is good sensitivity to detection.

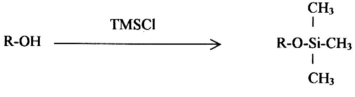
The nature of the ions and their relative abundances depend upon the structure of the molecule being studied. Fragmentations arise from simple cleavage of bonds in the parent ions, or from atomic arrangement at the moment of fragmentation. Such rearrangement is known to occur to a certain extent in molecules containing two or more carbon atoms, and it is very common with unsaturated hydrocarbons or where migration of hydrogen atoms is involved.

### 1.8 Derivatives of Sterols for Gas Chromatography

Compounds of high molecular weight or containing polar functional groups may be not sufficiently volatile, or too strongly attracted to the stationary phase and tail badly if to be analysed by GC. Derivatisation is aimed to increase the compound volatility, to reduce adsorption on GC column, and to stabilise molecules which are thermally labile at GC condition. The formation of derivatives is essential to protect the sterols from decomposing during gas chromatography.

Derivatives were used for gas-chromatographic studies of sterols, chiefly with the object of reducing the polarity associated with hydroxylic group. The commonly employed derivatives are the trimethylsilyl ethers (TMS), acetates, trifluoroacetates and methyl ethers. TMS and acetates are the easiest ones to prepare.

Trimethylsilylation was introduced for steroid work by Lukkainen, VandenHeuvel, Haahti and Horning in 1961[25]. It is the most widely employed derivatisation technique in combined GC-MS. The silylating agents commonly used are bistrimethylsilylacetamide (BSA) and N,O-bis(trimethylsilyl)-trifluoroacetamide (BSTFA). Pyridine is used as a solvent during derivatisation, it also acts an acid scavenger and basic catalyst if required.




When sterols possess similar situated hydroxyl groups and differ in positions remote from the hydroxyl groups are analysed, the elution pattern for the derivatives is virtually identical to that observed for the free sterols. However, greater detector response will be observed for the TMS ether compared to free sterols, because irreversible adsorption during chromatography is reduced. If the TMS derivative formation is from related hydroxy sterols differing in the stereochemistry or position of the hydroxyl group, changes in separation factors are often observed[26].

## 1.9 Significant of Study

Of recent years the number of coronary heart disease cases in this country has been increasing. Apart from the genetic factor, problem of the heart is often associated with abnormal depositing of cholesterol in the arteries. Cholesterol that response for hardening of artery walls is the bad cholesterol or also called low-density lipoprotein (LDL). High levels of LDL have been linked to heart diseases while high levels of high-density lipoprotein (HDL) may act as a protection against heart diseases. This is because the LDL carries cholesterol into the blood stream but the HDL takes it out[3].

Generally, blood cholesterol alone does not give a complete picture to one's susceptibility to coronary heart disease. The determining factor is the proportion of the LDL against the HDL present in the blood. The liver and other tissues control the serum cholesterol levels. A high intake of saturated fats in the diet may increase serum cholesterol levels due to an increase in LDL. On the other hand, polysaturated and monosaturated fats reduce serum cholesterol levels to some degree[3].

Today the general public is becoming increasingly aware of maintaining their good health and wellbeing. LDL can be decreased by both drugs, diet and lifestyle changes. Few years ago, a 12% of sitostanol ester has been incorporated into a Finnish margarine[22]. Sitostanol is the 5α-saturated derivative of sitosterol. Experiments conducted have shown that the new margarine can cause a 10-14% reduction of LDL cholesterol[22]. Similarly, diet provided with phytosterols at levels of 1.7 g to 3.2 g per day has been reported to reduce the LDL cholesterol in blood by from 4.0% to 6.9%[27].

Although the content and intake should be quite high to show the hypocholesterolemic effect, but "natural product" is usually more preferred by consumers compared to synthetic cholesterol-lowering drugs.

## 1.10 Aims and Objectives

The role of sterols in living organisms is being increasingly studied. The objective of this project is to study the sterol composition in crude palm oil, crude palm kernel oil and crude fibre oil. It is aimed to search for sitostanol in the palm oil, which is known to be able to reduce blood cholesterol level, as well as to investigate the possibility of the existence of other related sterols which have not been detected in palm oil.