STUDIES ON THE COMPOSITION AND STRUCTURE OF PALM OIL GLYCERIDES

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CERTIFICATE

This is to certify that the thesis entitled **"Studies on the Composition and Structure of Palm Oil Glycerides"** is an authentic record of the research work carried out by Mrs. Susanna George under my supervision in partial fulfilment of the requirements for the Degree of Doctor of Philosophy of the Cochin University of Science and Technology, Cochin and further that no part there of has been presented before for any degree.

31/12/93

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DECLARATION

I, **Susanna George**, hereby declare that this thesis entitled **"Studies on the Composition and Structure of Palm Oil Glycerides"** is a bonafide record of research work done by me and that no part of this thesis has been presented earlier for any degree, diploma title or recognition.

Susanna George

Trivandrum,

December, 1993.

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ABSTRACT

During the last couple of decades, the oil palm has emerged as the second largest source of edible oil in the world. Recently oil palm has been introduced commercially in India to augment edible oil supply in the country. Currently, about 10,000 hectares are under oil palm cultivation in India, and it is envisaged to cover about 6 lakh hectares in the coming years. Though oil palm is a major commercial oil crop, not much basic information on the lipids of the fruit (the source of palm oil) is available even where oil palm is cultivated in a very large scale. Being a new crop to India, it is of paramount importance to understand the basic chemistry/biochemistry of the lipids, which in turn, may find practical applications in the area of processing and product development.

The present investigation entitled **"Studies on the Composition and Structure of Palm Oil Glycerides"** was designed with a view to elucidate the lipid composition and structure under conditions such as fruit development and processing.

The fresh mesocarp of mature oil palm fruits contained 45.2% lipid. 95.27% of the total lipid consisted of triacylglycerols while diacylglycerols, monoacylglycerols and free fatty acids contributed 3.05%. Phospholipids and glycolipids comprised 0.83% of the total lipids. Phosphatidylcholine, phosphatidylethanolamine and phosphatidylinositol were the major phospholipid classes in mature oil palm fruit mesocarp.

The major fatty acids of the total lipids and also of the various lipid classes of mature oil palm fruit mesocarp were 16:0, 18:1 and 18:2 (contributing more than 90%) with minor amounts of 12:0, 14:0, 18:0 and 18:3. The fatty acid composition of the triacylglycerols was similar to that of the total lipids with a saturated-unsaturated fatty acid ratio of 49/51. Diacylglycerols, phospholipids and glycolipids contained higher levels of unsaturated fatty acid while monoacylglycerols and free fatty acids were characteristically higher in saturated fatty acids. The association of fatty acid with lipid class was evident; while 16:0 tended to concentrate in the neutral lipids, particularly triacylglycerols, 18:2 and 18:3 preferred phospholipids and glycolipids respectively.

Distribution of fatty acids at the sn-2-position and at the 1,3-positions of triacylglycerols revealed that 16:0 was predominantly esterified to the sn-1,3-positions. 18:1 on the other hand, showed a preference for the sn-2-position. 18:2 was more evenly distributed among the three positions than 18:1.

The triacylglycerol composition of oil palm fruit mesocarp calculated from Ag^{+}TLC data compared well with the values calculated from lipase hydrolysis data. Monoene (32.3%) and diene (30.84%) triacylglycerols were the major triacylglycerol classes. POP (19.26%) and POO (19.79%) were the major triacylglycerol species present. The triacylglycerol composition followed the 1,3-random-2-random distribution theory of Vander Wal.

A differential distribution profile of the phospholipids and glycolipids in the anatomically distinct regions of the mature oil palm fruit was indicated. Extremely high content of phospholipids and glycolipids were noticed in the exocarp of the fruit as compared to the fleshy mesocarp. Significant differences were also noticed in the composition of the lipid classes of mesocarp of three varieties of the oil palm fruit. Fatty acid composition of the total lipids from the three varieties studied did not show appreciable differences. However, high concentration of unsaturated fatty acids, 18:2 and 18:3 in the polar lipids and correspondingly lower proportions in the neutral fraction were noticed.

Lipid accumulation in the oil palm fruit mesocarp during fruit development occurred between 16 and 20 weeks after anthesis (WAA). Upto 16 WAA, oil content was low, followed by an extremely rapid phase of oil accumulation between 16 and 20 WAA and a final phase when only minor amounts of lipid was deposited. During the early stages of fruit development, the lipids were predominantly composed of polar lipids, partial glycerides and free fatty acids. By 24 WAA, triacylglycerols accounted for 95% of the lipid present. In the early states of fruit development, there was a higher content of unsaturated fatty acids with correspondingly lower level of saturated fatty acids in all the lipid classes. This trend was reversed towards the end of maturation.

There was an exponential rate of increase of triacylglycerols and their fatty acids (particularly 16:0 and 18:1) towards the end of fruit development. The fatty acid composition of the triacylglycerols in

the early stages of development, prior to active accumulation of lipid, was more or less similar, but differed appreciably from the later stages, and the transition of fatty acid composition towards that of normal palm oil occurred at around 16 WAA stabilizing by 20 WAA. All fatty acids increased in terms of absolute quantity. There was an overall consistency in fatty acid positional distribution irrespective of development stage. More saturated fatty acids were found to be esterified at the 1,3-positions and more unsaturated fatty acids at the sn-2-position of the triacylglycerols. Higher rate of incorporation of 16:0 at the 1,3-positions during the active phase of triacylglycerol synthesis was observed while 18:1 exhibited a reverse trend.

The monoene and diene triacylglycerols were the major triacylglycerols that were accumulated during development of oil palm fruit. The triacylglycerol composition revealed a pattern of association of fatty acid with triacylglycerol class. For any given stage of development, 16:0 was associated with the monoene triacylglycerols whereas 18:1 was associated with the diene triacylglycerols. After 16 WAA, the rate of incorporation of 16:0 to the monoene and of 18:1 to the diene triacylglycerols, was found to be higher with the increasing synthesis of these acids. 16:0 and 18:1 contributed to the formation of SOS and SOO respectively.

During palm oil extraction, oil loss occurs mainly at three stages of processing, namely, sterilization, pressing and clarification. Data relating to the lipid classes showed that sterilizer condensate had the highest levels of free fatty acids (24%) followed by press fibre

(12.5%) and sludge effluent (10.9%) as compared to raw palm oil (1.5%). Diacylglycerol and monoacylglycerol contents were markedly higher for these streams. Press fibre was characterized by extremely high proportions of phospholipids and glycolipids. Greater proportion of saturated fatty acids and correspondingly lower unsaturated fatty acids were present in sterilizer condensate. Phospholipids were characterized by high levels of 18:2 and glycolipids by 18:3 in all the process streams. The results are discussed in detail in Chapter 3.

CHAPTER 1

INTRODUCTION

1.1. SCOPE OF OIL PALM

1.1.1. World scenario

Although the history of the oil palm can be traced to antiquity, its commercial importance as a source of edible oil was recognized only in the twentieth century. The latter half of the present century witnessed a phenomenal growth of oil palm cultivation to meet the increasing demand for edible oil and oleochemicals (Mielke, 1987). Incredible productivity coupled with the perennial oil source favored the adoption of oil palm in the large scale estates of South East Asia particularly Malaysia and Indonesia, which account for around 77 percent of the palm oil supply of the world (Anon, 1992a). Palm oil has thus emerged from obscurity to become the second largest edible oil in the world during the last couple of decades (Anon, 1992b). The present annual production of palm oil is about 11.4 million tonnes (Anon, 1992b)

and at the present rate of growth it is expected that palm oil may surpass soybean oil production to qualify as the largest edible oil by the turn of the century. The commercial importance of palm oil therefore needs no further emphasis.

1.1.2 The Indian Edible Oil Scene

It is a paradox that with the largest area (23 million hectares, 1989-91) under oilseed cultivation, India has been the largest importer of edible oil in the world till recently (Holla and Rajan, 1992). According to the available estimates, the country requires 26 million tonnes of oilseeds (equivalent to 7.1 million tonnes of vegetable oil) by the turn of the century (Rao, 1991). The contributing factors to this situation primarily are the low productivity of traditional oilseeds and poor agro-techniques and management practices (Achaya, 1991). Unless urgent measures are taken to augment the edible oil supply in the country, the shortage of this essential commodity would further increase resulting in massive outflow of foreign exchange (Holla and Rajan, 1992). It may be mentioned here that per capita availability of fats and oils in India (6.71 kg/year) is among the lowest in the world and is equivalent to less than 10 percent of the total calory intake (Achaya, 1991; Iyer, 1991; Rao, 1992). To attain the normal intake of fats and oils to around 20 percent of the total calories, the present availability of edible fats and oils in the country will have to be doubled, meaning massive efforts to improve the

productivity of traditional seeds and to find alternative high potential oil crops.

1.1.3 Potential of Oil Palm Cultivation in India

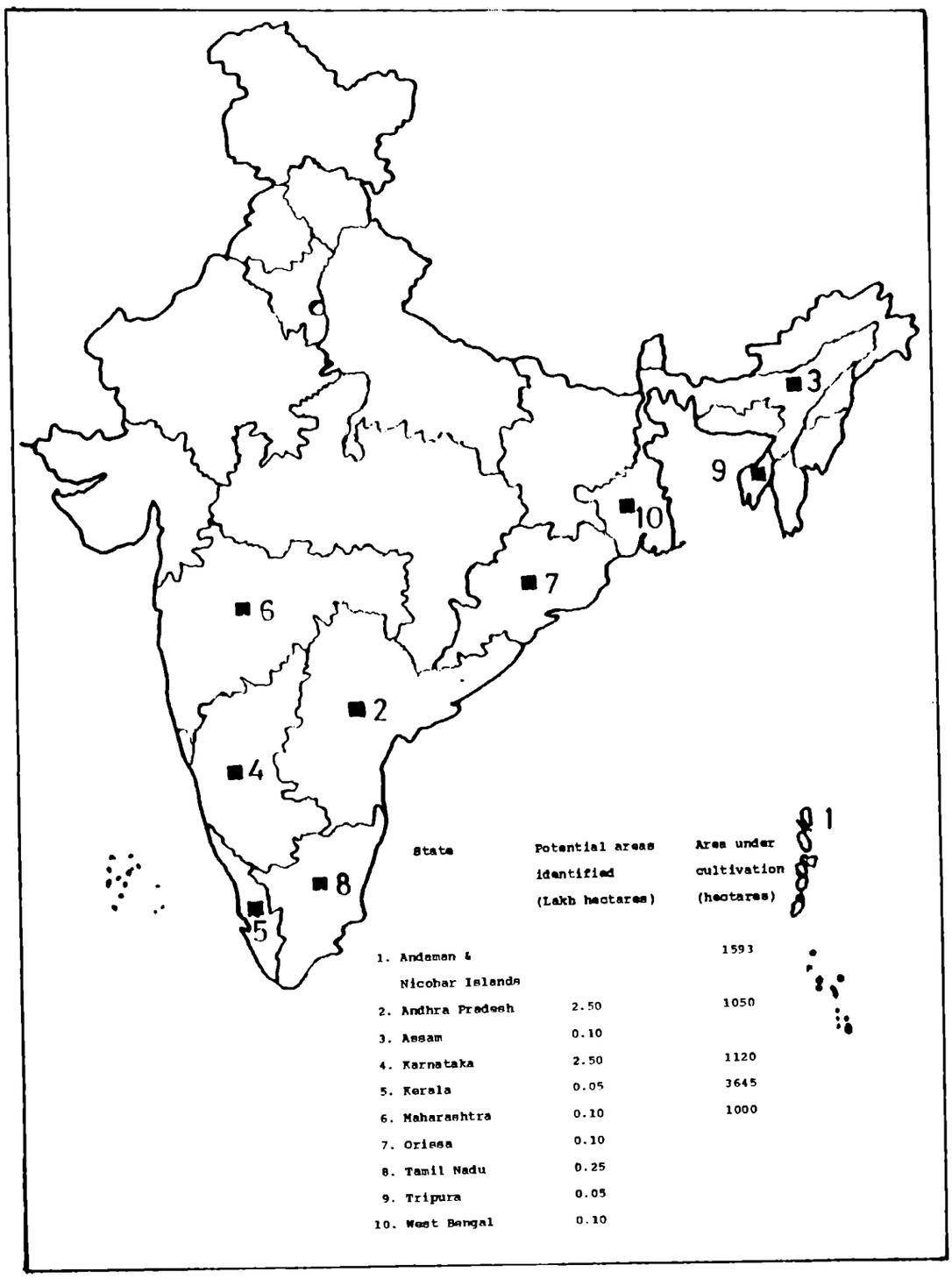
It is in this context that Oil Palm assumes great significance for India as an alternative oil crop though its potential as a source of edible oil for the country has been recognized only very recently. It is well known that the oil palm has the highest genetic potential among the oil crops and has achieved average productivity of 4-5 tonnes per hectare per year (Rosenquist, 1991). It has also been demonstrated that by tissue culture techniques, the productivity can further be enhanced to 8 tonnes per hectare per year (Paranjothy et al, 1990). Thus under identical agro-climatic conditions, oil palm can yield 6-7 times more than the other traditional oilseeds cultivated in India. Attempts made towards commercial cultivation of oil palm in Andaman and Nicobar Islands and Kerala State have met with reasonable success with yield levels of 2.8 to 3 tonnes per hectare per year having been achieved even under average management conditions (Chadha and Rethinam, 1991a; Holla and Rajan, 1992). The availability of indigenous palm oil during 1989-90 was approximately 3000 tonnes; all of which was non-edible and used in the soap industry (Holla and Rajan, 1992).

The Chadha Committee constituted by the Government of India (1986-1988) has identified 5.75 lakh hectares in nine states in India to bring under oil palm cultivation in the coming years (Chadha and

Rethinam, 1991b) (Figure 1). The Government of India has approved a massive oil palm plantation programme aimed at achieving an oil output level of 0.76 million tonnes by 2010 AD and above 2 million tonnes by 2020 AD (Anon, 1992c), to eradicate the edible oil deficiency in the country.

1.1.4 Present Status of Oil Palm in India

Currently, oil palm is commercially cultivated in Kerala State (3645 hectares) and in Andaman and Nicobar Islands (1593 hectares) (Abraham, 1991). Further expansion is being focussed in Andhra Pradesh and Karnataka as the bulk of the area (87 percent) identified for oil palm fall in these two states (Figure 1). In order to support the oil palm plantation development, scientific research related to plant breeding, crop protection and management practices, and seedling production are actively pursued at Central Plantation Crops Research Institute (C.P.C.R.I), Palode, Trivandrum, a constituent of Indian Council of Agricultural Research (I.C.A.R) (Chadha, 1990). Owing to the unique processing requirements for oil palm fruits, palm oil extraction is integral to plantation development. Oil palm fruits after harvest is highly perishable and therefore have to be processed on the same day. The presence of an active lipase in the fruit mesocarp and its activation under favorable conditions such as over-ripening, mechanical injury, storage, etc., result in extremely high free fatty acid in the oil and consequent problems associated with down stream processing and



State	Potential areas identified (Lakh hectares)	Area under cultivation (hectares)
1. Andaman & Nicobar Islands		1593
2. Andhra Pradesh	2.50	1050
3. Assam	0.10	
4. Karnataka	2.50	1120
5. Kerala	0.05	3645
6. Maharashtra	0.10	1000
7. Orissa	0.10	
8. Tamil Nadu	0.25	
9. Tripura	0.05	
10. West Bengal	0.10	

Figure 1. States identified with areas for oil palm cultivation in India

storage of the oil (Arumughan et al., 1989,1991; Sundaresan et al., 1990; Arumughan, 1991). This necessitates optimization of processing capacity and integration of the plantation size to produce edible grade palm oil. The Regional Research Laboratory, Trivandrum (RRL-T), a constituent of the Council of Scientific and Industrial Research (C.S.I.R), has been actively engaged in the post harvest science and technology of oil palm to provide necessary technological input to plantation development programme envisaged in the country. As a result, RRL-T has optimized the process technology for edible oil extraction to the Indian agricultural situation (Sundaresan et al , 1990). After four years of field trials, scientists at RRL-T have now set up India's first commercial plant for edible palm oil at Pedavegi in West Godavari district of Andhra Pradesh (Arumughan, 1992). Scientific investigations relating to oil palm fruit biochemistry have also been pursued at RRL-T.

1.2 BOTANY OF OIL PALM

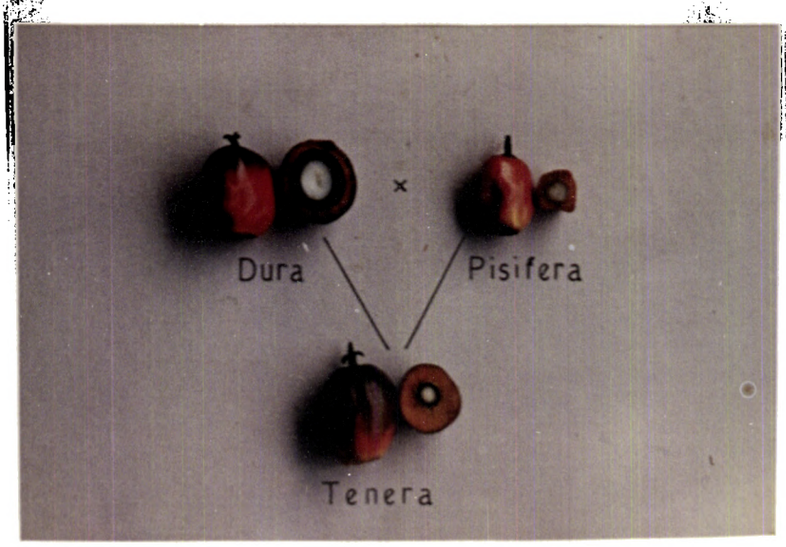
The oil palm belongs to the family 'Arecacae' and is botanically known as Elaeis guineensis Jacq. The generic name 'Elaeis' is derived from the Greek word 'elaion' meaning oil while the specific name 'guineensis' is indicative of its origin from the Guinea Coast of West Africa (Hartley, 1977). There are three species under the genus Elaeis, namely, E. guineensis, E. odora and E. oleifera. However, only E. guineensis is adopted for commercial cultivation primarily due to its high yielding potential.

Hot, humid, tropical climate and equitable distribution of rainfall ranging from 2000-3000 mm are ideal for oil palm cultivation. South East Asia (Malaysia and Indonesia), West Africa (mainly Nigeria and Ivory Coast) and Central America are the major oil palm growing areas is the world. (Mielke, 1987; Moll, 1987). The mature stem of the oil palm is an erect and fairly uniform column having persistent leaf bases (Photograph I). The crown of the adult palm has a continuous succession of pinnately compound leaves arranged in a spiral fashion.

The oil palm is monoecious and possess separate male and female inflorescences (Hartley, 1977). In the female inflorescence, the flowers are arranged spirally around the rachis of a number of spikelets. The female flower consists of six sepaloid segments in two whorls, tricarpellate ovary and rudimentary androecium. The sessile stigma has three prominent lobes which are retained in the mature fruit. The male inflorescence has a long peduncle having finger-like spikelets

I. A ma[illegible] oil palm [illegible]

III. Mature oil palm fruits



IV. Fruit forms of the oil palm

with numerous flowers. Each male flower has a perianth of six minute segments, a tubular androecium with six anthers and a rudimentary gynoecium.

Oil palm under favorable conditions starts flowering from 18 months onwards. Pollination is aided by wind or insects. Earlier, manual pollination was practised in plantations to obtain uniform and higher yield. This practice was later dispensed with in favor of the insect Elaeidobius kamerunicus, popularly known as 'weevil' in modern oil palm plantations (Taniputra et al, 1991).

A mature oil palm fruit bunch is ovoid and may reach 50 cm in length and 35 cm in breadth (Photograph II). It contains from a few hundred to a few thousand fruits depending on genetic and environmental factors and palm age. Bunch weights range from 5 kg in young palms to as much as 40kg in mature palms in good state (Wood, 1987). Ripening is usually from the tip downwards.

From the day of anthesis, it takes about 180 days for the development of fruit to full maturity (Hartley, 1977). The oil palm fruit is a sessile drupe, varying in shape from nearly spherical to ovoid or elongated and bulging at the top (Photograph III). In length it varies from 2-5 cm, in weight from 3g to over 30g. It is bright orange - red in color when ripe. The fresh mesocarp (fruit coat) of mature oil palm fruits contain around 40-45 percent oil and is the source of commercial palm oil. The endosperm or 'seed' also contains reserve energy as oil, palm kernel oil. There are three varieties or fruit forms of E. guineensis, viz., dura, pisifera and tenera (Photograph IV). Dura

(D) and pisifera (P) are the parental varieties while tenera is the hybrid (DXP). Whereas tenera is the commercial cultivar for plantations, the dura and pisifera are raised in the seed gardens for hybridization (Hartley, 1977; Maycock, 1985).

1.3 LIPIDS: OCCURRENCE AND STRUCTURE

According to Christie (1987), "Lipids are fatty acids and their derivatives, and substances related biosynthetically or functionally to these compounds". The term is used to include such diverse compounds as fatty acids, acylglycerols, wax esters, cholesterol, bile acids, terpenes and carotenoids (Christie, 1982).

1.3.1. Classification of Lipids

The lipids of plant and animal origin consist of fatty acids linked by an ester bond to the trihydric alcohol, glycerol, or to other alcohols such as cholesterol, or by amide bonds to long-chain bases or on occasion to other amines. In addition, they may contain alkyl moieties other than fatty acids, phosphoric acid, organic bases, carbohydrates or many other components which can be released by hydrolytic procedures (Sonntag, 1979a; Christie, 1982; Gunstone, 1986; Kates, 1986). Lipids may be classified into two broad classes; 'simple lipids' are those which on hydrolysis yield at most two types of primary hydrolysis products per mole; 'complex lipids' yield three or more primary hydrolysis products per mole. The term 'neutral lipid' and 'polar lipid' respectively are also used frequently to define these groups.

Simple Lipids: Triacylglycerols, partial glycerides and free fatty acids

Triacylglycerols (less accurately termed triglycerides) are by far the most abundant single lipid class and virtually all the common important fats and oils of animal and plant origin consists almost entirely of this lipid class. Hence fats and oils are generally defined as "mixtures of mixed triacylglycerols" (Sonntag, 1979a). In a triacylglycerol, each hydroxyl group of the trihydric alcohol, glycerol is esterified to a fatty acid (Litchfield, 1972; Sonntag,1979a; Christie, 1982, 1987; Harwood, 1986; Kates, 1986) (Figure 2). The types of fatty acids present, and their intermolecular and intramolecular distribution in the triacylglycerol molecules influence the physico-chemical properties of the fat.

Diacylglycerols (diglycerides) and the **monoacylglycerols** (monoglycerides) contain two moles and one mole of fatty acid per mole of glycerol respectively (Figure 2) and are rarely present at greater than trace levels in fresh animal and plant tissues. 1,2-Diacyl-sn-glycerols however, are important as intermediates in the biosynthesis of triacylglycerols and other lipids. 2-Monoacyl-sn-glycerols are formed as intermediates or end products of the enzymatic hydrolysis of triacylglycerols.

Fatty acids: Christie (1987) defines fatty acids as "compounds synthesised in nature via condensation of malonyl coenzyme A units by a fatty acid synthase complex". In general, they are long-chain aliphatic monocarboxylic acids containing even number of carbon atoms in straight

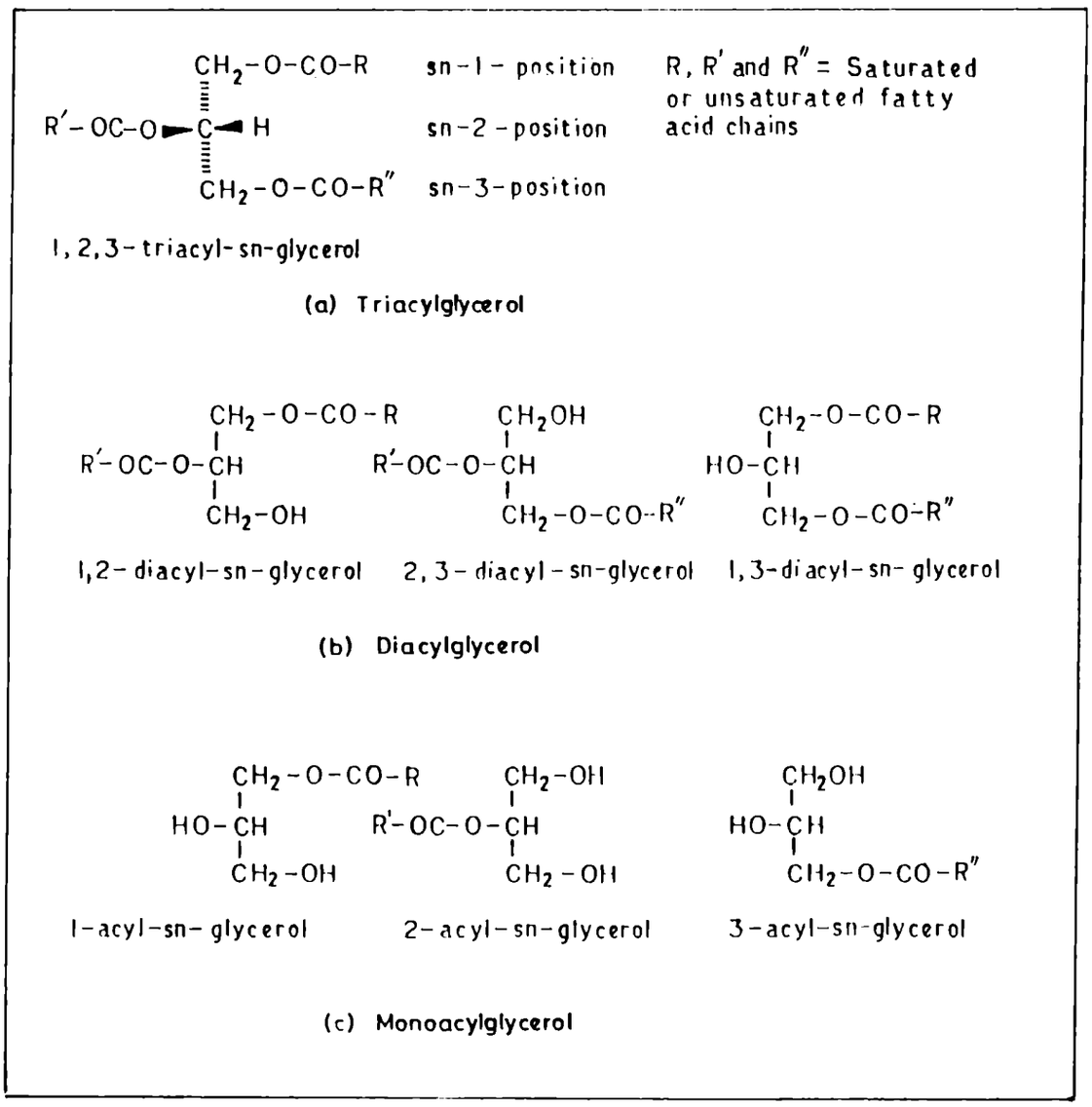


Figure 2. The structures of acylglycerols.

chains (C_4 to C_{24}) and are saturated or unsaturated. The common fatty acids of plant tissues are C_{16} and C_{18} straight chain compounds with zero to three double bonds of a cis configuration (Deuel, 1951; Sonntag, 1979a; Harwood, 1980; Christie, 1982; Gunstone, 1986; Kates, 1986). Table 1 gives the names of fatty acids commonly occurring in commercial fats and oils.

Other simple lipids present in minor quantities in nature are sterol esters, carotenoid esters, alkyl ethers and plasmalogens, wax esters and acylated steryl glycosides (Snyder, 1969; Mahadevan, 1971; Mudd and Garcia, 1975; Kolattukudy, 1976; Harwood, 1980, 1986; Christie, 1982; Kates, 1986).

Complex Lipids: glycerophospholipids and glycoglycerolipids

The complex or polar lipids consist of the glycerophospholipids (or phospholipids) which contain a polar phosphorus moiety and a glycerol backbone and the glyceroglycolipids (or glycolipids) which contain a polar carbohydrate moiety. Sphingolipids contain a long-chain base, generally sphingosine, fatty acid and inorganic phosphate, carbohydrate or other complex organic compounds (Harwood, 1980, 1986; Christie, 1982;Kates, 1986).

The structures of the common glycerophospholipid constituents of plant and animal tissues are shown in Figure 3. **Phosphatidic acid** or 1,2-diacyl-sn-glycerol-3-phosphate is found naturally only in trace amounts in tissues, but it is important metabolically as a precursor of most other glycerolipids.

Table 1 The Names and Designations of Fatty Acids Occurring in Commercial Fats and Oils (Christie, 1982)

Systematic name	Trivial name	Shorthand designation
Butanoic	Butyric	4:0
Hexanoic	Caproic	6:0
Octanoic	Caprylic	8:0
Decanoic	Capric	10:0
Dodecanoic	Lauric	12:0
Tetradecanoic	Myristic	14:0
Hexadecanoic	Palmitic	16:0
Octadecanoic	Stearic	18:0
Eicosanoic	Arachidic	20:0
Docosanoic	Behenic	22:0
Tetracosanoic	Lignoceric	24:0
Cis-9-Hexadecenoic	Palmitoleic	16:1(n-7)
Cis-9-Octadecenoic	Oleic	18:1(n-9)
Cis-13-Docosenoic	Erucic	22:1(n-9)
9,12-Octadecadienoic	Linoleic	18:2(n-6)
6,9,12,-Octadecatrienoic	γ-Linolenic	18:3(n-6)
5,8,11,14-Eicosatetraenoic	Arachidonic	20:4(n-6)
9,12,15-Octadecatrienoic	α-Linolenic	18:3(n-3)
5,8,11,14,17-Eicosapentaenoic		20:5(n-3)
4,7,10,13,16,19-Docosahexaenoic		22:6(n-3)

Phosphatidylglycerol or 1,2-diacyl-sn-glycero-3-phosphoryl-1'-sn-glycerol tends to be a trace constituent of tissues, although it does appear to have important functions in lung surfactant and in plant chloroplasts. **Diphosphatidylglycerol** or cardiolipin is related structurally to phosphatidylglycerol and is an important constituent of mitochondrial lipids, especially heart muscles and is common in bacteria. Phosphatidic acid, phosphatidylglycerol and diphosphatidylglycerol are acidic phospholipids.

Phosphatidylcholine or 1,2-diacyl-sn-glycero-3-phosphoryl-choline (commonly termed lecithin) is usually the most abundant lipid in the membranes of animals, plants and microorganisms. Together with sphingomyelin (the other choline containing phospholipid) it comprises much of the lipid in the external monolayer of the plasma membrane of animal cells.

Phosphatidylethanolamine or 1,2-diacyl-sn-glycero-3-phosphoryl-ethanolamine (cephalin) is frequently the second most abundant phospholipid class in animal and plant tissues and can be the major lipid class in microorganisms. The N-acyl derivative is found in certain seeds.

Phosphatidylinositol or 1,2-diacyl-sn-glycero-3-phosphoryl-1-sn-myoinositol is a common minor constituent of animal, plant and microbial lipids. Often in animal tissues and yeasts it is accompanied by small amounts of polyphosphoinositides. Other minor phospholipid components occurring in plant and animal tissues are lysophosphatidyl-

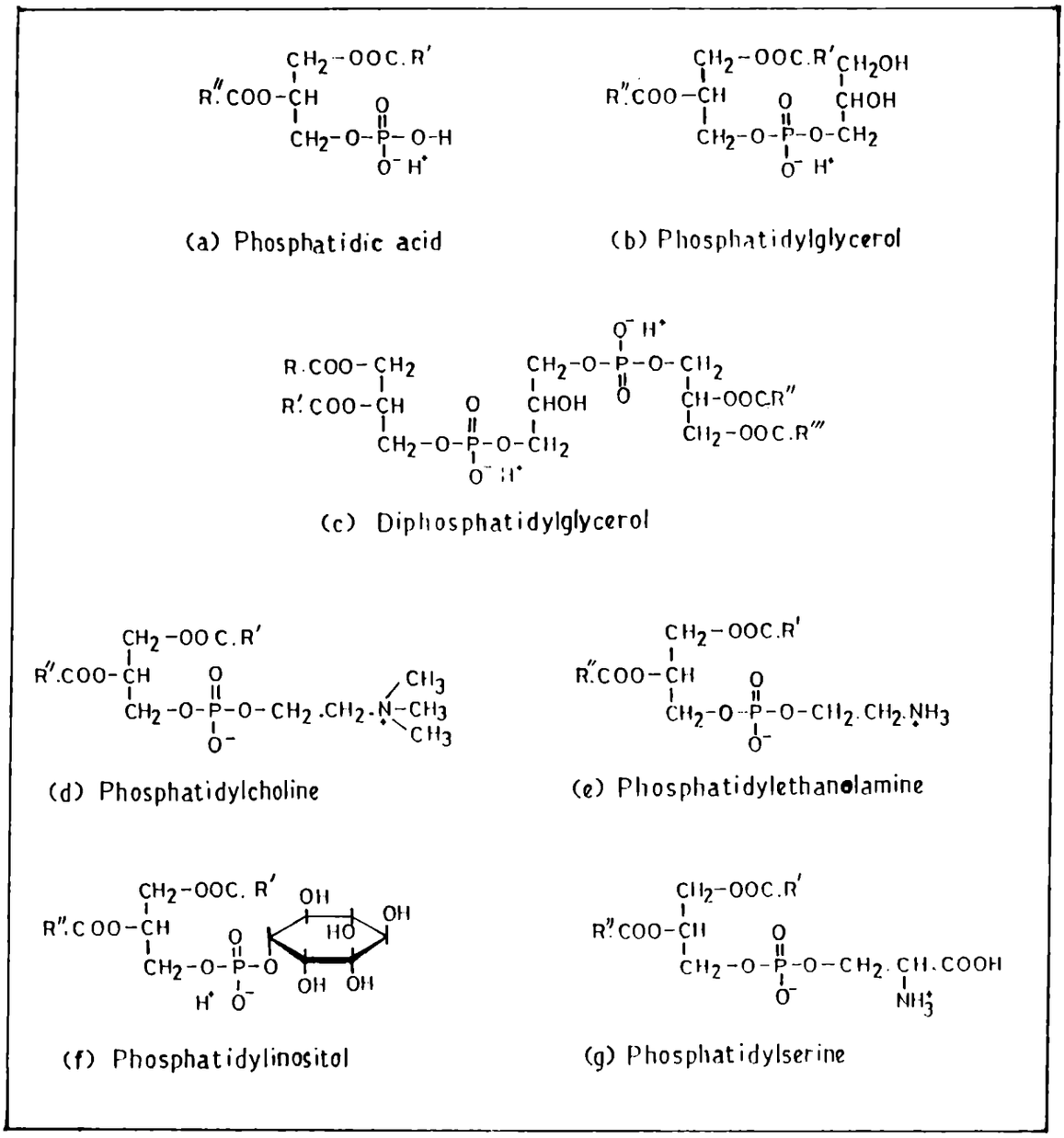


Figure 3. The structures of the principal plant glycerophospholipids.

choline, lysophosphatidylethnolamine and phosphonolipids. The presence of lysophosphatidylcholine and ethanolamine are usually indicative of lipid degradation before or during extraction. The sphingolipids (ceremides, sphingomyelin and gangliosides) although major lipids in animals (particularly nervous tissue) are of minor importance in plants.

Glycoglycerolipids (glycosyldiacylglycerols): Plant tissues especially tend to contain appreciable amounts of lipids in which 1,2-diacyl-sn-glycerol is joined by a glycosidic linkage at position sn-3 to a carbohydrate moiety (Figure 4). These compounds are especially important in the photosynthetic membranes of cyanobacteria, algae and higher plants. The main components are monogalactosyldiacylglycerol or 1,2-diacyl-3-O-β-D-galactopyranosyl-sn-glycerol and digalactosyldiacylglycerol or 1,2-diacyl-3-O-[α-D-galactopyranosyl-(1'->6')-O-β-D-galactopyranosyl]-sn-glycerol. The galactosylglycerides contain large amounts of polyunsaturated fatty acid, eg > 90% α-linolenic acid in chloroplastic diacylgalactosylglycerol (Carter et al. 1956; Sastry, 1974; Christie, 1982, 1987; Kates, 1986).

1.3.2. Stereochemistry and Nomenclature of glycerolipids

The rules of stereospecific numbering were put forward by the IUPAC-IUB Commission on Biochemical Nomenclature (1978) and is now favored and universally adopted. In order to designate the stereochemistry of glycerol derivatives, the carbon atoms of glycerol are numbered stereospecifically. If a glycerol molecule is drawn in the

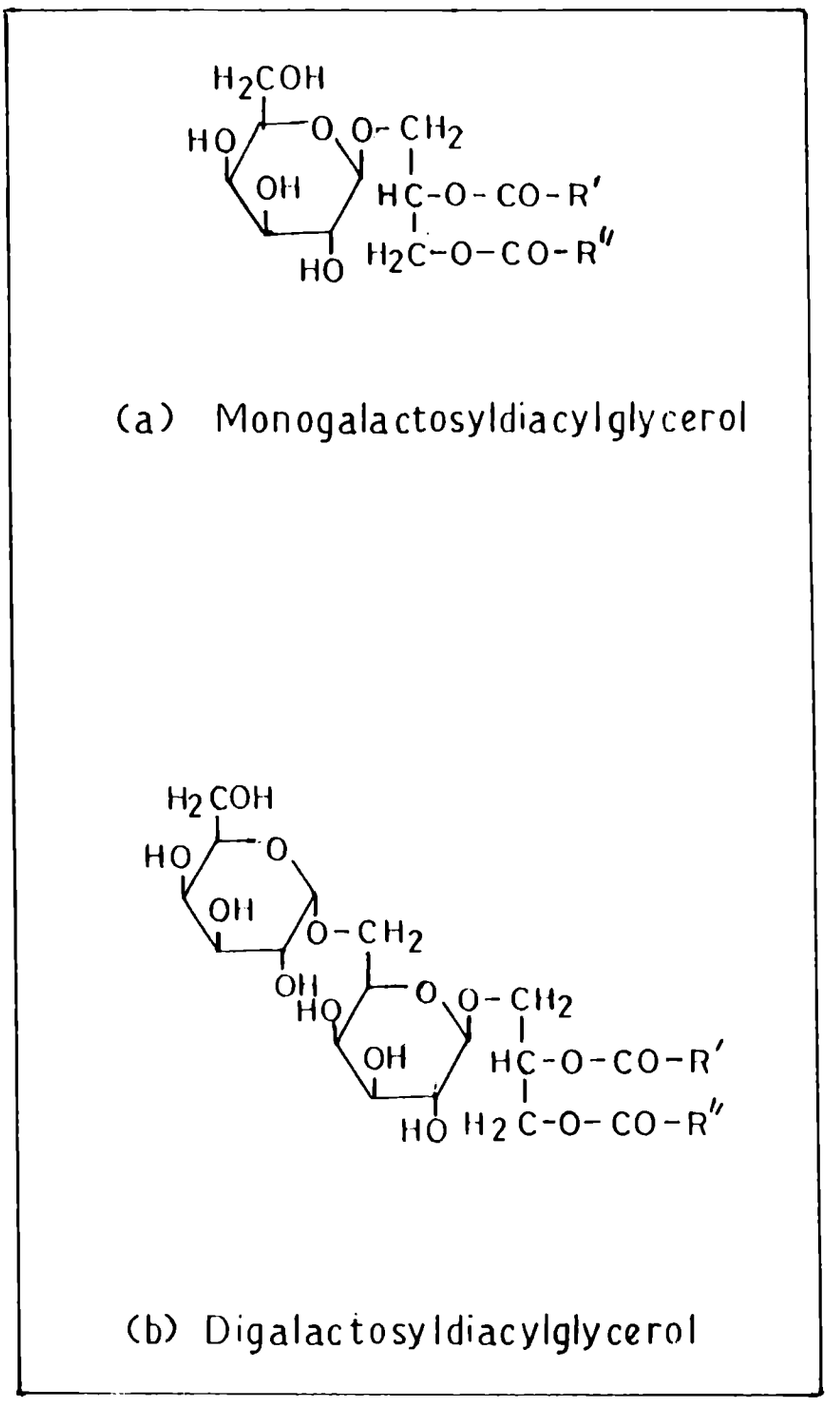


Figure 4. The structures of commonly occurring plant glycoglycerolipids.

Fischer projection with the secondary hydroxyl group to the left of the central prochiral carbon atom, then the carbons are stereospecifically numbered 1,2 and 3 from top to bottom (Figure 2). Molecules that are stereospecifically numbered in this fashion have the prefix 'sn' (for stereochemically numbered) immediately preceeding the term 'glycerol' and separated from it by a hyphen, and differentiates such numbering from conventional numbering conveying no steric information. The prefix 'rac' precedes the full name if the product is an equal mixture of both antipodes whereas prefix "X" is used if the configuration is unknown or unspecified. Any glycerolipid will be chiral when the substituents at the sn-1-and sn-3-positions are different (Litchfield, 1972; Christie, 1982).

1.3.3. Complexity of naturally occurring triacylglycerol mixtures

The glycerol molecule, with its three hydroxyl groups, on esterification with three fatty acids can lead to a complex mixture of simple and mixed triacylglycerols. When a mixture of 'n' different fatty acids is esterified to glycerol, the number of possible triacylglycerols that can be formed may be calculated using the formulas in Table 2 (Litchfield, 1972). The triacylglycerol mixtures found in plant seeds contain only five to ten different fatty acids (mainly C_{16} and C_{18} acids) which can generate 125 to 1000 possible molecular species of triacylglycerols. However, biological syntheses are often selective and not all the theoretically possible combinations will be present in

Table 2 Formulas for Calculating the Possible Number of Triacylglycerols That can be Formed from 'n' Different Fatty Acids (Litchfield, 1972)

Isomers distinguished	Formula	Triacylglycerols
all isomers distinguished	n^3	SSS, sn-SSU, sn-USS, sn-SUS, sn-UUS, sn-SUU, sn-USU, UUU
optical isomers distinguished	$\frac{n^3+n^2}{2}$	SSS, β-SSU, β'-SUS β-SUU, β'-USU, UUU
no isomers distinguished	$\frac{n^3+3n^2+2n}{6}$	SSS, SSU, SUU, UUU

appreciable proportions. Three factors make the analytical chemistry of natural triacylglycerol mixture difficult; the great complexity of these mixtures and the very similar chemical and physical properties of these molecules.

1.3.4 Distribution of Fatty Acids in Natural Triacylglycerol Mixtures

Fatty acid distribution patterns: Analyses of natural fat triacylglycerol mixtures by porcine pancreatic lipase and various stereospecific analyses procedures have revealed a number of regular patterns in the distribution of fatty acids between the sn-1, sn-2, and sn-3 positions of the triacylglycerols. These positional distribution patterns are distinctly different in plant and animal triacylglycerols. The general trends for the commonly occurring fatty acids of plant seeds is briefly discussed.

Saturated fatty acids of high molecular weight. Palmitic acid (16:0) in esterified almost exclusively at the combined 1,3-position of seed triacylglycerols (Mattson and Volpenhein, 1961a, 1961b, 1963; Gunstone et al, 1965a). From the data of Mattson and Volpenhein (1961a, 1961b, 1963), it followed that the 16:0 content of the combined 1,3-positions can be estimated by the empirical formula, % 16:0 in 1,3-position = 1.47x, where x is mole percent 16:0 in total triacylglycerol and $0<x<30$. Stereospecific analyses by Brockerhoff and Yurkowski (1966) have shown that the 16:0 content of the sn-1- and sn-3- positions are approximately equal in rapeseed oil, soybean oil and cocoa butter, but

slightly different in peanut, linseed, corn and olive oils. In peanut and soybean, sn-1-position contains slightly more 16:0 than sn-3-position (Fatemi and Hammond, 1977a, 1977b; Hokes, 1979; Sanders, 1979; Van Pee et al , 1979; Pan and Hammond, 1983).

Studies on the positional distribution of stearic, arachidic, behenic and lignoceric acids indicate that these acids are esterified almost totally at the combined 1,3-position in the same manner as 16:0 (Mattson and Volpenhein, 1961a, 1963; Hokes, 1979; Sanders, 1979; van Pee et al, 1979; Manganaro et al, 1981).

Unsaturated fatty acids: Gunstone (1962) and Mattson and Volpenhein (1963) first suggested that oleic acid (18:1), linoleic acid (18:2) and linolenic acid (18:3) are randomly distributed among the free hydroxy groups remaining after 16:0, 18:0 and C_{20}, C_{22} and C_{24} acids are esterified at the 1,3-positions. Further study of lipolysis results by Gunstone et al (1965a, 1965b) however indicate that among the C_{18} unsaturated acids, 18:1 and 18:3 show slight preference for the 1,3-position while 18:2 shows a slight preference for the sn-2- position. In the oils of maize, soybean, linseed, olive, cocoa, rapeseed and peanut position-2 is almost exclusively occupied by unsaturated fatty acids and sn-1-position contains more unsaturated fatty acids than the sn-3 (Brockerhoff and Yurkowski, 1966; Evans et al 1969, Arunga and Morrison, 1971; Fatemi and Hammond, 1977a, 19677b; Hokes, 1979; Sanders, 1979; van Pee et al 1979; Manganaro et al , 1981; Pan and Hammond, 1983). These results were taken into account by Evans et al (1969) when they proposed the following rules for estimating the positional distribution

of 18:1, 18:2 and 18:3 in seed triacylglycerols: (i) Saturated acids and those with chain length greater than eighteen carbons are first distributed equally at the sn-1- and sn-3- positions. (ii) 18:1 and 18:3 acids are then distributed equally and randomly at the unfilled 1-, 2- and 3- positions, with any excess from the 1- and 3- positions being added to the sn-2- positions. (iii) All remaining positions are filled by 18:2. Gunstone (1962) has suggested a pattern of distribution of fatty acids in natural triacylglycerol mixtures based on his observation that the 2-position is preferentially esterified with C_{18} unsaturated acids. Litchfield (1970a, 1971a) has studied the positional distribution of Cruciferae seed triacylglycerols and determined an equation for estimating the percentages of 18:1, 18:2 and 18:3 in the sn-2-position as a function of overall fatty acid composition. According to Litchfield (1972), the best available methods for estimating the positional distribution of 18:1, 18:2 and 18:3 are the Evan's hypothesis (1969) for non-Cruciferae species and the Litchfield correlation formula (Litchfield, 1971a) for the Cruciferae species. Both methods however, provide only estimates which may deviate slightly from the actual fatty acid distribution (Litchfield, 1972).

Other Acids: Positional distribution of 10:0, 12:0 and 14:0 vary between major taxonomic groups but are consistent within each individual group (Litchfield, 1970b, 1971b). Unusual fatty acids are found exclusively at the sn-3- positions of seed triacylglycerols (Gunstone, 1965a; Conacher et al, 1970). Erucic acid, eicosenoic and tetracosenoic acids are also esterified almost exclusively at the

combined 1,3-positions of seed triacylglycerols (Mattson and Volpenhein, 1961a, Brockerhoff and Yurkowski, 1966; Grynberg and Szczepanska, 1966; Litchfield, 1970a, 1971a, 1971b).

Triacylglycerol patterns: The theoretical approach to estimating triacylglycerol composition assumes that the composition of natural triacylglycerol mixtures are the net result of (i) the fatty acids available for triacylglycerol biosynthesis and (ii) the substrate specificities of the enzymes that construct the triacylglycerols. There has been several efforts to devise empirical mathematical formulas relating triacylglycerol composition to fatty acid composition (Litchfield, 1972). These "fatty acid distribution hypotheses" generally assume that chance plays a key role in the enzymatic construction of triacylglycerols from fatty acids and glycerol within the framework of certain restricting conditions.

The 1-random-2-random-3-random hypothesis first proposed by Tsuda (1962) assumes that three different pools of fatty acids are separately distributed to the sn-1-, sn-2- and sn-3- positions of the glycerol molecules in a fat. Within its respective positions, each pool of fatty acid in distributed at random. The mole % of any triacylglycerol, sn-XYZ having fatty acid X, Y and Z at positions 1, 2 and 3 respectively can be calculated from the general equation,

$$\% \text{ sn-XYZ} = \left[\begin{matrix}\text{mole \% X at} \\ \text{sn-1-position}\end{matrix}\right] \left[\begin{matrix}\text{mole \% Y at} \\ \text{sn-2-position}\end{matrix}\right] \left[\begin{matrix}\text{mole \% Z at} \\ \text{sn-3-position}\end{matrix}\right] \quad (10^{-4})$$

To make a 1-random-2-random-3-random calculation, it is necessary to have stereospecific analysis data of the fatty acid composition (expressed as mole %) of the sn-1, sn-2 and sn-3- positions of a fat.

The 1,3-random-2-random hypothesis proposed independently by Vander Wal (1960) and Coleman and Fulton (1960) assumes that two different pools of fatty acids are separately and randomly distributed to the 1,3- and 2-positions of the glycerol molecules in a fat. Since the sn-1- and sn-3- positions are both randomly esterified from the same pool, their fatty acid compositions are equivalent. The amount of each component triacylglycerol can be calculated from the equation,

$$\% \quad XYZ = \begin{bmatrix}\text{mole \% X at} \\ \text{1,3-position}\end{bmatrix} \begin{bmatrix}\text{mole \% Y at} \\ \text{sn-2-position}\end{bmatrix} \begin{bmatrix}\text{mole \% Z at} \\ \text{1,3-position}\end{bmatrix} \quad (10^{-4})$$

The 1,3-random-2-random distribution is calculated from pancreatic lipase hydrolysis data.

In addition to the 1-random-2-random-3-random and the 1,3-random -2-random hypotheses, a number of fatty acid distribution hypotheses for natural fats have been proposed and abandoned over the years. The 'Even or Widest Distribution' of fatty acids in natural triacylglycerol mixtures was proposed by Hilditch and co-workers (Collin and Hilditch, 1929; Bhattacharya and Hilditch, 1930; Hilditch and Meara, 1942; Hilditch, 1956) to describe a pronounced tendency of fatty acids to form mixed acid triacylglycerols i.e., to be distributed as widely as possible among the triacylglycerol molecules of a fat. The 1,2,3-random hypothesis proposed by Longenecker (1941) and elaborated by Mattil and

Norris (1947) assumes that one pool of fatty acids is randomly (by chance) distributed to all the three positions of the glycerol molecule of a fat. Thus the fatty acid composition of all positions are equivalent. However, modern techniques of triacylglycerol analysis have shown that the 1,3- and 2-positions always have different compositions (Litchfield, 1972). Several restricted random distribution hypotheses have appeared in the literature (Bernstein, 1946; Daubert, 1949; Kartha 1953a, 1954a, 1954b; Youngs, 1959; Coleman, 1963; Tsuda, 1968). None of these have been proven accurate, when compared with experimental results.

1.3.5. Glyceride analysis - techniques and applications

All animal and plant tissues contain lipid mixtures of such complexity that no single analytical method for isolation and identification is adequate and a combination of techniques is required. There are several chromatographic techniques and systems which lend readily to a sequential application resulting in progressively simpler mixtures of lipids. A preliminary separation into two or three well defined groups of lipid classes has been found to facilitate the ultimate resolution of the mixture. The specific combination of methods depends on the complexity of the lipid mixture and on the needs of the analysis. The most popular and effective combination of complementary chromatographic systems is provided by thin-layer chromatography (TLC) and argentation thin-layer chromatography (Ag^{+}TLC) followed by gas-

liquid chromatography (GLC) or liquid - liquid partition chromatography prior to positional analysis using enzymatic methods (Litchfield, 1972). The principles and general methodology of the chromatographic and enzymatic techniques of lipid analysis (employed in the course of this investigation) are briefly discussed.

1.3.5.1. Separation of Acylglycerols

Thin-layer chromatography (TLC) by virtue of its sensitivity and versatility is the method of choice in many laboratories for the separation of lipid classes. It is also rapid and simple. Not only is TLC useful for lipid fractionation, but it is also a good method for monitoring samples obtained from preparative separations or the products of a reaction mixture. TLC of lipids is usually carried out on silicic acid as adsorbent with or without calcium sulfate as binder, but other adsorbents such as alumina, ion-exchange cellulose, Sephadex, cellulose or silica gel impregnated with silver nitrate or boric acid may be used (Kates, 1986). TLC may be used for adsorption chromatography, ion-exchange chromatography, and partition (liquid-liquid) chromatography (normal or reverse phase).

Separation of neutral lipids: The analysis of neutral lipids by TLC has been described in a number of reviews (Mangold, 1969; Skipski and Barclay, 1969; Litchfield, 1972; Snyder, 1973; Blank and Snyder, 1975; Mahadevan, 1976; Myher, 1978; Christie, 1982; Kates, 1986). In most systems, the separation of lipid classes is performed by adsorption

chromatography on plates with silica gel G layers. The lipids are separated as classes by virtue of their differences in type and number of functional groups present in the lipid molecule.

A variety of solvent systems have been used to separate simple lipids by TLC on silica gel in a single dimension. Those used most frequently contain hexane or petroleum ether, diethyl ether and small amounts of acetic (or formic) acid in various proportions; the most popular solvent system being petroleum ether-diethyl ether-acetic acid, 90:10:1, by volume (Litchfield, 1972; Myher, 1978; Christie, 1982; Kuksis, 1983; Kates, 1986) Hydrocarbons and sterol esters migrate with the solvent front. Phospholipids, glycolipids and monoacylglycerols remain at the origin, and cholesterol and diacylglycerols do not separate. Nevertheless, this system is useful when separation of only the major lipid classes is desirable. A slight alteration of the development solvent to petroleum ether (hexane) - diethyl ether - acetic acid, 80:20:1, by volume (Blank and Snyder, 1975) or heptane-isopropylether - acetic acid, 60:40:1, by volume, (Kuksis, 1983) greatly improves the separation of neutral lipid classes of low R_f values. However, under such condition, the separation of lipids with high R_f values will be obscured. Skipski and Barclay (1969) have developed a two-step system for the complete separation of a complex mixture of neutral lipids. Cholesterol can be separated from diacylglycerols using a solvent system of chloroform - methanol-acetic acid, 98:2:1, by volume (Snyder, 1973). Separation of 1,2-and 1,3-isomers of diacylglycerols can be prevented by the use of hexane-diethyl ether-aqueous ammonia,

40:60:1, by volume (Snyder, 1973). The use of borate - impregnated plates is recommended for resolution of isomeric monoacylglycerols (Thomas et al, 1965). According to Skipski and Barclay (1969) the effect of the degree of unsaturation and/or the chain length of the fatty acids in the lipid molecules on the separation is negligible. On the contrary, Myher (1978) states that chain length of the fatty acids making up an acylglycerol can have a considerable effect on the R_f values. Acylglycerols containing short-chain fatty acid are more polar than their long-chain counterparts (Kuksis and Beckenridge, 1968). The effect of polar functional groups has been illustrated (Mikolajczak and Smith, 1967).

TLC of Phospholipids: One dimensional TLC procedures are recommended for the analysis of natural mixtures of phospholipids with relatively simple compositions, for rapid group separations, and for small-scale preparative purposes (Skipski and Barclay, 1969; Renkonen and Luukkonen, 1976). The major phospholipid classes are separated mostly with solvents consisting of chloroform, methanol and water, with or without added ammonia or acetic acid. A very satisfactory system has been developed by Skipski et al (1964). Silica gel plates are prepared with 0.001 M aqueous solution of sodium carbonate, silica gel H adsorbent and chloroform-methanol-acetic acid- water (25:15:4:2 by volume) solvent system. Under these chromatographic conditions, diphosphatidylglycerol and phosphatidic acid move to the solvent front and phosphatidylserine and phosphatidylinositol migrate between phosphatidylethanolamine and phosphatidylcholine. The neutral lipids do

not interfere since they migrate to the solvent front. In most circumstances, it is preferable to employ silica gel H (without binder) as sharper separation of most of the individual phospholipid classes, especially of minor acidic components are obtained in the absence of metal ions. When acidic phospholipids are present in a sample at low levels only, the common phospholipids may be separated on layers of silica gel G (with calcium sulfate as binder) by using chloroform-methanol-water, 25:10:1 by volume (Skipski et al , 1964) as the solvent for development. For lipid samples with high content of neutral lipids and low content of phospholipids, prewashing of the chromatogram with acetone-hexane (1:3, by volume) prior to separation of the phospholipid is recommended (Skipski and Barclay, 1969). The prewashing solvent moves all neutral lipids to the top of the chromatogram and permits good separation of phospholipids with high R_f values. Modifications of Skipski's system have been described in the literature, often to compensate for local conditions of temperature and humidity or experiment. Procedure for the improved separation of phosphatidylserine and phosphatidylinositol involve incorporation of ammonium sulfate, EDTA or boric acid into the adsorbent (Allan and Cockcroft, 1982; Fine and Sprecher, 1982). One dimensional TLC systems have been used less often with plant lipid extracts, as glycolipids tend to co-chromatograph with phospholipids when the common elution systems are used. Nonetheless, some valuable separations have been described (Nicols, 1963; Pohl et al , 1970; Morrison et al , 1980).

In most instances, many more distinct phospholipid classes can

be separated by two-rather than one-dimensional TLC procedures. In the former technique, the sample is applied as a spot to the bottom left-hand corner of the plate, which is developed in one direction as normal, before the plate is dried, turned anticlockwise through 90^{o} and developed again with a different mobile phase. The most useful separations are achieved when two contrasting solvent mixtures are employed, one neutral or basic and the other acidic. Rouser et al. (1969, 1976) have used a neutral mixture of chloroform-methanol-water (65:25:4, by volume) in the first direction, and an acidic mixture of butanol- acetic acid-water (60:20:20, by volume) in the second direction. These workers also report the use of a basic system, chloroform-methanol- 28% ammonia (65:35:5, by volume) in the first direction and an acidic system, chloroform-acetone-methanol-acetic acid-water (5:2:1:1:0.5, by volume) in the second direction.

The principal disadvantages of two - dimensional TLC techniques is that precise orientation of the spots of lipids on the plate can vary greatly with small changes in the properties of the adsorbent, or in the temperature or humidity of the laboratory. The technique is recommended by many research groups only for preliminary investigation of samples or for checks on purity of lipid isolated by other methods while one-dimensional techniques are preferred for routine analysis (Christie, 1987).

Apart from the common detecting reagents for lipids, a variety of different spray reagents can be employed to detect and identify particular structural moieties in phospholipids (Skipski and Barclay,

1969; Renkonen and Luukkonen, 1976; Kates, 1986): phosphate group (Ryu and MacCoss, 1979); choline moiety (Wagner et al , 1961); free amino groups (Skipski et al , 1962); and vicinal diol groups (Shaw, 1968). Phospholipids separated chromatographically can be estimated by determining the phosphorus content (Kates, 1986). The water - soluble moieties, such as choline, ethanolamine, serine, glycerol and inositol may be determined by analysis of the water - methanol phase of the hydrolysates of the parent lipids (Dittmer and Wells, 1969; Kates, 1986).

Argentation or Silver ion chromatography is based on the formation of reversible complex between silver ions and the π-electrons of ethylenic and acetylenic bonds (Litchfield, 1972; Myher, 1978; Christie, 1982). Silver ion chromatography is accomplished by impregnating silver nitrate into normal lipid adsorbent. After development with a suitable solvent system, a given class of lipids can be fractionated according to the number of double bonds per molecule. The Ag^+/olefin complex is of sufficient low energy that it can be made or broken during standard lipid chromatographic procedures. Exposure to silver nitrate does not produce any chemical alteration of normal fatty acids or triacylglycerols; hence the fractions separated can be recovered unaltered from the impregnated adsorbent for further analysis.

Argentation chromatography of lipids was first described simultaneously by Morris (1962) who used thin layers and by de Vries (1962) and Barrett et al (1962) who used columns. They demonstrated that a given class of compounds can be separated according to the number

of isolated double bonds and the cis/trans geometry of those bonds. Subsequently, de Vries and Jurriens (1963, 1964) and Wessels and Rajagopal (1969) showed that positional isomeric dienes and cis and trans molecules could be separated. Separation of lipids by argentation chromatography and its practical aspects have been discussed by Morris (1966), Guha and Janak (1972), Litchfield (1972), Christie (1982) and Kates (1986).

The procedures for argentation thin-layer chromatography (Ag+TLC) differ little from that of conventional TLC. Generally the adsorbent consists of 5-20% silver nitrate and 15-80% silica gel (Litchfield, 1972; Myher, 1978). Other authors (Wood and Snyder, 1966; Wessels and Rajagopal, 1969; Bottino, 1971) have recommended various levels of silver nitrate for better resolutions. Litchfield (1972) has reviewed the various methods of impregnating the plate with silver nitrate. However, plates are commonly prepared by spreading a slurry of the adsorbent in water containing the desired level of silver nitrate in the usual manner. Precautions should be taken to protect the plates from sun light. Renkonen (1967) and Akesson (1969) have recommended the activation of TLC plates impregnated with silver nitrate at 190-195^{o}C for 2-4 hours for better resolution. Two general types of solvent mixtures are widely used for separation of triacylglycerols (i) chloroform containing 0-6% methanol, ethanol or acetic acid and (ii) various benzene-diethyl ether mixtures, depending on the polarity of the triacylglycerol mixture (Litchfield, 1972). In general, a maximum of five to ten triacyglycerol bands can be resolved on a single TLC plate.

For more complex mixtures two separations may be necessary using solvent systems of different polarity. Iodine is unsuitable for locating triacylglycerol bands in the presence of silver nitrate (Renkonen, 1967). The most convenient non-destructive method for locating triacylglycerol bands is spraying with 2',7'-dichlorofluorescein and viewing under UV light (Kuksis et al, 1968). Elution and recovery of the triacyglycerol bands from the TLC plates have been described by Hill et al (1968). Identification and quantitation of the components of each band can be made on the basis of fatty acid composition, position of the band and comparison of R_f values of simple triacylglycerol standards of different degrees of unsaturation and from a knowledge of the relative proportion of the fractions on the chromatoplate (Blank et al, 1965).

Blank et al (1965) and Gunstone and Padley (1965) have studied the separation of triacylglycerols containing saturated, monoene, linoleic and linolenic acids. Each fraction obtained by Ag^+TLC was treated as a ternary or quarternary mixture. In each band one triacylglycerol was found to predominate and form 80% or more of the fraction. For calculating the composition of the bands, Gunstone and Padley (1965) determined the order of elution of triacylglycerols given by the following sequence in which 3,2,1 and 0 indicate the number of double bonds in the three acyl groups of each triacylglycerol: 333, 332, 331, 330, 322, 321, 320, 311, 222, 310, 221, 300, 220, 211, 210, 111, 200, 110, 100 and 000. They have assigned arbitrary values for the complexing power of each fatty acid chain, viz., saturated: 0, oleic : 1, linoleic : 2+a, and linolenic : 4+4a, where 'a' is some fraction less than one.

Studies on the separation of triacylglycerols by Ag^+TLC have indicated that separations are not strictly due to unsaturation alone, but is influenced by the nature of the mixture to be separated, experimental conditions like silver nitrate concentration, activation temperature and type of solvent system and by the types of fatty acid as well as total unsaturation. Overlapping of triolein (111) and monolinoleo-dipalmitin (002) is common (Blank et al, 1965). Under certain experimental conditions 011 and 002 have identical R_f values (den Boer, 1964; Jurriens, 1965); 002 can elute before 011 (de Vries, 1964) and 003 can be adsorbed more strongly than 122 (Wessels and Rajagopal, 1969). Although major separation characteristics are determined by the Ag^+/olefin complex, three minor factors also influence the R_f of a triacylglycerol (Litchfield, 1972). Larger molecules travel ahead of smaller molecules due to their poorer adsorption, particularly for samples having fatty acids shorter than C_{14} or longer than C_{20} (Barrett et al, 1962); the position of the double bond in the fatty acid chain has some influence on R_f values (Wessels and Rajagopal, 1969) and cis/trans isomers can be separated (de Vries and Jurriens, 1963, 1964; Wessels and Rajagopal, 1969). Yet another factor which influences the fractionation is the concentration of components; a component will have a higher relative R_f when it is present as a major component than when it is a minor one (Blank et al, 1965). Generally, the fractionation of triacylglycerol mixtures on the basis of total unsaturation alone have been reported presumably according to Blank et al (1965), because the systems were not efficient or the finite separations within classes went unrecognized.

1.3.5.2 Separation of fatty acids

The determination of fatty acid composition is an essential part of lipid analysis. Gas-liquid chromatography (GLC) is the technique that is preferred and used extensively. The procedure involves the hydrolysis of the lipid sample followed by conversion of the fatty acids thus released into volatile derivatives, usually methyl esters suitable for GLC analysis.

Fatty acid methyl esters suitable for GLC can be prepared from lipid samples by a number of procedures (Christie, 1992). Methanolysis using acidic catalysts (hydrogen chloride, acetyl chloride, sulfuric acid and boron trifluride) or base-catalyzed transesterifications (using potassium hydroxide, sodium methoxide) are commonly employed (Litchfield, 1972; Myher, 1978; Christie, 1982, 1992; Kates, 1986). Alternatively, the lipid can be saponified and then acidulated to yield free fatty acids which are converted to methyl esters with methanol/HCl, methanol/H_2SO_4, methanol/BF_3 or diazomethane. Christie (1992) has reviewed the use of other less commonly used reagents - N,N-dimethylformamide dimethyl acetyl/ pyridine, sodium or potassium salt of fatty acid with methyl iodide/ dimethylacetamide; and pyrolysis of tetramethylammonium salts of fatty acids in aqueous solution. Special procedures are required for preparing chromatographable methyl esters of fatty acids containing functional groups (Christie, 1982). Volatile derivatives of fatty acids, other than methyl esters are prepared also in certain cases (Myher, 1978; Christie, 1982).

For analysis of fatty acid methyl esters, 1.5 to 3.0 m length glass or stainless steel packed columns containing 3-20% (w/w) of liquid phase coated on an inert acid washed and silanized support material of uniform grade (100-200 mesh) are generally useful (Litchfield, 1972). Open-tubular or capillary columns constructed from fused silica are also popular (Kuksis, 1984; Ackman, 1986).

The liquid phases in use for GLC of fatty acid methyl esters are either non-polar silicones and hydrocarbons or polar polyesters. Silicone liquid phases (SE-30, OV-1, JXR, QF-1) permit the separation of fatty acid components mainly on the basis of their molecular weights. High molecular weight hydrocarbon liquid phases separate saturated and unsaturated components of the same chain length, unsaturated esters eluting before the related saturated compounds. These phases are useful in the analysis of oxygenated or high molecular weight fatty acids. Low polarity phases are utilized principally in open tubular and support coated open tubular columns.

The polar polyester phases, classified according to their degree of polarity, from those of relatively low polarity (NPGS, neopentylglycol succinate; EGSP-Z, copolymer of ethyleneglycol succinate and a phenyl silicone) to those of higher polarity (BDS, butanediol succinate; PEGA, polyethyleneglycol adipate; EGSS-Y, copolymer of ethyleneglycol succinate and methyl silicone) and to highly polar phases (EGS, ethyleneglycol succinate; DEGS, diethyleneglycol succinate; EGSS-X, copolymer of ethyleneglycol succinate and lesser proportion of methyl silicone than EGGS-Y), allow the separations of esters of the same chain

length, unsaturated components eluting after the saturated ones. In general, polyesters of high polarity are the most useful since they give best resolution of component fatty acids and EGSS-X and EGSS-Y have become widely accepted as the most useful representatives of the polyester phases particularly in packed columns.

Alkyl polysiloxanes are often used under various trade designations viz., Silar 10C, Silar 9CP, SP2340 and OV275. They are more polar than EGSS-X and stable at temperatures above those possible for EGSS-X and afford excellent separations of polyunsaturated fatty acids. Acidic liquid phases like DEGS containing 3% phosphoric acid (Kuksis, 1977) or Carbowax 20M-terephthalic acid or the structurally related phase SP-1000 (Supelco, Inc., USA) are also useful.

The gas chromatographic characteristics of the methyl esters of commonly occurring fatty acids has been the subject of several reviews (Ackman, 1969; Jamieson, 1970; Litchfield, 1972; Myher, 1978; Christie, 1982, 1987; Kates, 1986). The analysis of the less common fatty acids have also been extensively investigated. Cis-trans isomers of unsaturated fatty acid (Jaeger et al, 1976; Heckers et al, 1977; Ackman and Eaten, 1978; Dittmar et al, 1978), conjugated octadecadienoic (Christie, 1973) and conjugated octadecatrienoic acids (Morris et al, 1960; Mikolajczak and Bagby, 1964) isomeric methyl branched octadecanoates (Abrahamsson, 1964; Ackman, 1967; Smith and Lough, 1975) cyclopropane (Christie, 1968) and cyclopropene fatty acids (Recourt et al, 1967), vicinal diols (Wood, 1967), polyhydroxy (Wood et al, 1966),

epoxy (Emken, 1971, 1972) and a series of hydroxy-, acetoxy-, and oxo-stearates (Tulloch, 1964) have been separated by GLC.

Generally fatty acids are identified by using standards or retention time relationships. The absolute retention times have very little value for identification purposes since they are dependent on a number of instrumental parameters, on the nature and age of the packing material and on operation conditions. Hence, retention time of a fatty acid methyl ester relative to that of a chosen standard commonly occurring component (usually 16:0 or 18:0) is more significant (Christie, 1982) and is given as:

$$\text{Relative retention time, r (18:0)} = \frac{\text{retention time of ester}}{\text{retention time of 18:0}}$$

Authentic standard mixtures, available from commercial suppliers containing accurately known amounts of methyl esters of saturated, monoenoic and polyenoic fatty acids are useful for checking quantification procedures and for the identification of fatty acids by direct comparison of the retention times of their methy esters with those of the unknown esters on the same column under identical conditions (Litchfield, 1972). Secondary external reference standards consisting of a natural fatty acid mixture of known composition is also used for identification of fatty acids (Ackman and Burgher, 1965; Holman and Hofstetter, 1965; Holeman and Rahn, 1966; Christie, 1982).

For a homologous series of long chain methyl esters, there is a linear relationship between the logrithm of retention time and the number of carbon atoms in the aliphatic chain of each acid (Litchfield, 1972; Christie, 1982). The retention time of any member of a series can be predicted if the retention time of any one member of that series is known (Litchfield, 1972). Systematic interrelationships between the retention times of different unsaturated homologous series have also been reported, allowing the retention time of one series to be predicted from the retention time of a related series (Ackman, 1969; Jamieson, 1970).

Equivalent chain lengths (ECL), representing the carbon number of the aliphatic chain of the fatty acid esters (Miwa et al, (1960) are used for identification of fatty acids. ECL value of an unknown compound can be calculated from an equation (Myher, 1978),

$$ECL_X = ECL_M + \frac{\log R_X - \log R_M}{\log R_N - \log R_M} (n-m)$$

where m and n are the carbon numbers of two saturated reference compounds, M and N, respectively and the R values are the retention of the subscripted compounds. Generally for fatty acid analysis, ECL values are usually found by reference to the straight line obtained by plotting the logarithms of the retention times of a homologous series of saturated, straight chain fatty acid methyl esters against the number of carbon atoms in the aliphatic chain of each acid. The retention time of

the unknown esters are measured under identical operating conditions and the ECL values are read directly from the graph.

The increment in ECL values of a given ester over that of the saturated ester of the same chain length, known as Fractional Chain Length (FCL) is dependent on the structure of the fatty acid ie, the number of double bonds in the aliphatic chain and the distance of the double bond from the carboxyl and terminal ends of the molecule. The retention characteristics of the methyl esters of the complete series of monoenoic C_{18} acids (Gunstone et al, 1967), the methylene interrupted cis-cis-dienoic acids (Christie, 1968), of acids with more than one methylene group between the double bonds (Gunstone and Jie, 1970) and polyunsaturated fatty acids (Ackman, 1963; Hofstetter et al, 1965) on a number of liquid phases and the effect of double bond position on ECL values (Jamieson, 1970) have been investigated. With suitable secondary reference standards, the ECL values of the esters of most of the fatty acids occurring naturally can be predicted.

In the first analysis of any new sample, confirmation of the fatty acid structure should be obtained by unequivocal chemical degradation and spectroscopic procedures (Christie, 1982). However, by a judicious choice of two or more liquid phases differing in polarity, a complete analysis of fatty acids separated both by chain length and degree of unsaturation can be achieved. The greatly increased resolution of capillary columns makes overlapping peaks less likely and identification by retention times more accurate. Occasionally, other

chromatographic techniques such as Ag^{+}TLC or liquid-liquid partition chromatography in combination with GLC may be required to completely separate and identify all components (Litchfield, 1972).

1.3.5.3 Determination of Positional Distribution of Fatty Acids in Triacylglycerols

A lipase (EC. 3.1.1.3) that is completely specific for the primary ester bonds of acylglycerols is present in crude pig (mammalian) pancreas extracts. It is used to catalyze the hydrolysis of the fatty acids esterified at the primary positions of the triacylglycerols to produce sn-1,2,(2,3)-diacylglycerols, sn-2-monoacylglycerols and fatty acids (Figure 5). The fatty acid composition of the 2-monoacylglycerol accurately reflects that of the position 2 in the original triacylglycerol (Litchfield, 1972; Christie, 1982).

Porcine pancreatic lipase was introduced in 1956 by Mattson and Beck and by Savary and Desnuelle for the selective deacylation of triacylglycerol. Since then, the purification and characterization, specificities, optimum reaction conditions and reaction products of porcine pancreatic lipase have been widely studied and it is more fully charaterized than any other lipase. The properties of the enzyme have been reviewed by Litchfied (1972) and Brockerhoff and Jensen (1974).

Specificity: Porcine pancreatic lipase possess near-absolute specificity for the primary ester linkage. All straight chain saturated fatty acids are apparently hydrolyzed from the primary positions at approximately the same rate (Litchfield, 1972; Christie, 1982). Ester

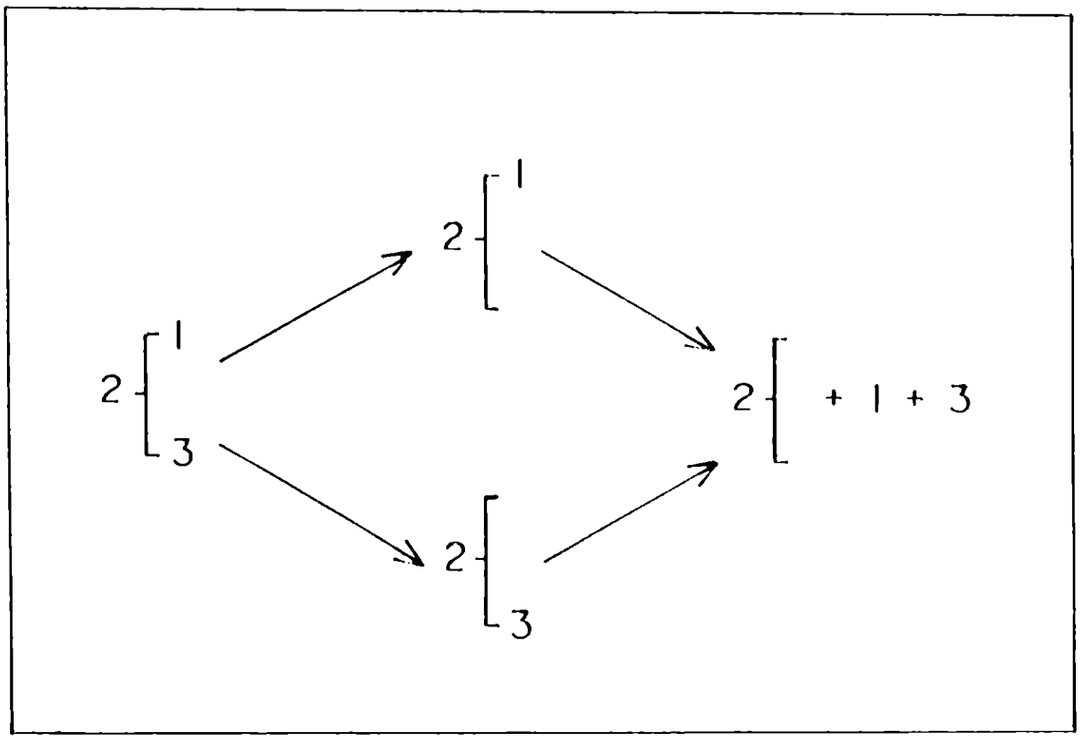


Figure 5. Course of the reaction during hydrolysis of a triacylglycerol by pancreatic lipase. 1, 2 and 3 refer to the fatty acids at the sn-1-, sn-2- and sn-3- positions of the original triacylglycerol. 1,2(2,3)-diacylglycerols, sn-2-monoacylglycerols and free fatty acids are formed on hydrolysis of the triacylglycerol.

bonds of long-chain polyunsaturated fatty acids in the primary positions of fish oils (Bottino et al, 1967) and trans-3-hexadecenoic acid and phytanic acid (Christie, 1982) are hydrolyzed more slowly while triacylglycerol molecules that contain short-chain fatty acids of milk fat are hydrolyzed more rapidly (Jensen et al, 1967) than molecules that contain only long-chain fatty acids. However, when the enzyme is used on triacylglycerols that contain a more normal range of fatty acid components, little fatty acid specificity is evident (Litchfield, 1972; Christie, 1982).

Reaction conditions: Calcium ions are essential for the reaction. It is necessary that the triacylglycerols are well dispersed and in micellar form for hydrolysis to occur. Hexane may be used as carrier for solid, highly saturated fats. In triacylglycerol structural studies, the concentration of the various cations, bile salts, enzyme, pH of the medium and temperature are adjusted so that the optimum degree of hydrolysis is obtained (50-60 percent) with minimum undesirable acyl migration in the shortest possible time. The optimum reaction conditions for porcine pancreatic lipase include a pH near 8, a 0.5 to 1.5 M electrolyte concentration, the presence of calcium ions, a high enzyme-sample ratio, vigorous agitation, an emulsifier to maximize interfacial surface area, and a reaction temperature of 37-40^{o}C. A semimicro method developed by Luddy et al (1964) has been widely used and found to give the best results.

Hydrolysis products: The sn-1,2 (2,3)-diacylglycerols obtained by deacylation may or may not be representative of the original triacylglycerols depending on fatty acid and triacylglycerol

specificities of the enzyme. If representative diacylglycerol products are required for further analysis, randomness of the reaction should be checked. The fatty acids released are generally not representative of the combined sn-1-and sn-3 positions of the original triacylglycerol as hydrolysis may not be completely random, or there may be contamination of fatty acids liberated from the lipids of the enzyme preparation or by fatty acids released from position-2 after migration to the primary positions. However, the sn-2-monoacylglycerols produced by porcine pancreatic lipase are representative of the sn-2-position and the fatty acid composition of the monoacylglycerol fraction represents that of the 2-position of the original triacylglycerols (Coleman, 1963, 1964). The composition of each fatty acid in positions 1 and 3 can be calculated from its concentration in the intact triacylglycerol and in position-2.

Brockerhoff (1965) published a **stereospecific analysis** technique based on the stereospecific hydrolysis of derived diacylphosphatidylphenol by phospholipase A. This method permitted the fatty acid composition at the sn-1-, sn-2- and sn-3- position to be distinguished. The following year Lands et al, (1966) introduced an alternative method for distinguishing the sn-1-and sn-3- fatty acids utilizing the stereospecific phosphorylation of derived diacylglycerols by diglyceride kinase.

Subsequently, other enzymes from various sources have been studied for structure elucidation of triacylglycerol molecules of which milk lipase (Jensen et al, 1962), Rhizopus arrhizus lipase (Mazliak, 1967) and Geotrichum candidum lipase (Sampugna and Jensen, 1968) have been sufficiently well characterized for analytical applications.

1.4 OIL PALM FRUIT LIPIDS

Fully developed oil palm fruit mesocarp (see Section 1.2 for the anatomy of the fruit) contains 45 to 50% of lipids by fresh weight (75-80% by dry weight) (Hartley, 1977). The oil palm therefore is the most efficient plant in converting energy into lipid reserve. This reserve is tapped by the large scale cultivation of oil palm to produce commercial palm oil for edible use. The oil palm fruit lipids are largely composed of triacylglycerols (95-98%) and therefore the lipids are commercially known as palm oil (Sonntag, 1979b).

Palm oil from unbruised, ripe oil palm fruits with free fatty acid content below 0.1% consists nearly of 98% triacylglycerols and 2-3% diacylglycerols and 0.1% monoacylglycerols (Jacobsberg and Ho, 1976; Jacobsberg, 1983). Any deviations from these values reported by these authors for the composition of palm oil as it occurs in the ripe unbruised fruit cells, may be attributed to deterioration or modification during extraction and processing.

In general, most studies available on palm oil composition (lipid profile) pertain to commercial grade palm oil that has been extracted from the fruit by the wet processing method. Commercial grade palm oil has a lower triacylglycerol content (88-92%)and higher free fatty acid and diacylglycerol content than the fruit lipids attributed to enzymatic hydrolysis or process conditions. The constituents of crude palm oil are represented schematically in Figure 6.

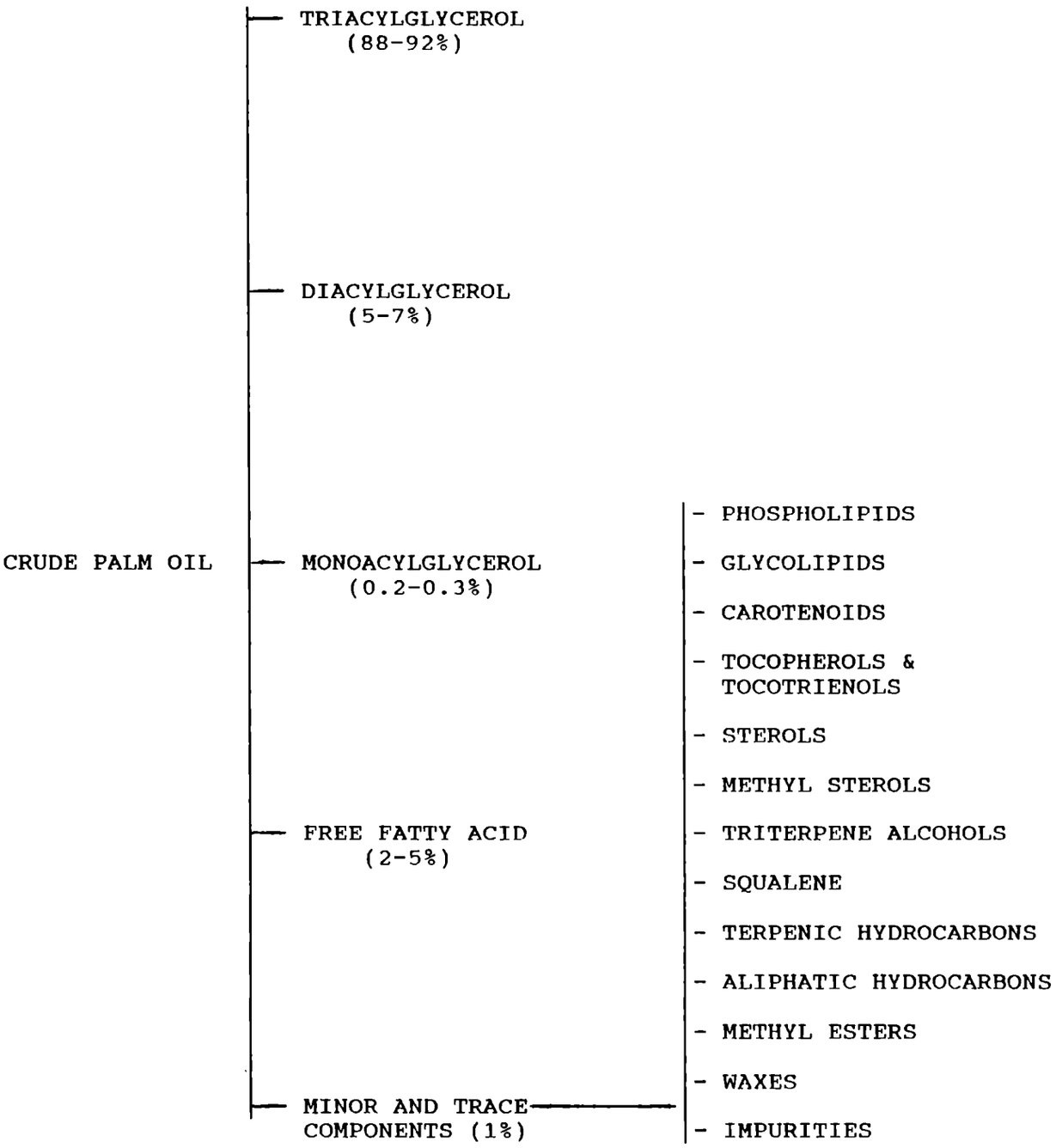


Figure 6 The Constituents of Crude Palm Oil

1.4.1. Partial Glycerides and Free Fatty Acids

D'Alonzo and co-workers (1982) established a procedure to determine the carbon number profile analysis of refined and bleached fats directly by high temperature glass capillary column gas chromatography after derivatization of the fatty acids, monoacylglycerols and diacylglycerols with BSTFA [(N,O)-bis (trimethylsilyl) trifluoroacetamide]. Quantitative information on mono-, di-and triacylglycerols as well as free fatty acids was obtained for palm oil. Their results indicated 93.1% triacylglycerol, 5.8% diacylglycerol and 0.1% free fatty acid. Monoacylglycerol was not detected. The diacylglycerols consisted of C32, C34 and C36 at 0.6%, 3.0% and 2.2% levels respectively.

Goh and Timms (1985) described a method for the routine determination of partial glycerides in crude, fractionated and refined palm oils by GLC of the TMS (trimethylsilyl) derivatives. They studied the effect of fractionation and refining on these constituents. Levels of 5.3-7.7%, 0.21-0.34% and 2.4-4.5% diacylglycerol, monoacylglycerol and free fatty acid respectively were reported by these authors for commercial palm oil.

Other studies on the diacylglycerol and monoacylglycerol content of palm oil have been reported during crystallization studies of palm oil (Berger, 1975, 1979; Naudet and Faulkner, 1975; Okiy, 1978; Okiy *et al*, 1978; Tan, 1985; van Putte and Bakker, 1987).

Jacobsberg and Ho (1976), Jacobsberg and Jacqmain (1977) and

Goh and Timms (1985) found no correlation between free fatty acid and diacylglycerol content. They concluded that most of the diacylglycerol found in crude palm oil was not formed by hydrolysis of triacylglycerol but was a residual by-product of the biosynthesis of triacylglycerol. Thus, even an unbruised fruit with free fatty acid content of only 0.32%, contained 5.66% diacylglycerol.

Changes in the content of diacylglycerol, monoacylglycerol and free fatty acid classes during development of the oil palm fruit have been investigated by Oo et al (1986) and Bafor and Osagie (1988a). Thin-layer chromatographic analysis showed that diacylglycerol, monoacylglycerol and free fatty acid levels were significantly high in the immature fruit mesocarp, but diminished to trace levels during the period of active lipid (triacylglycerol) formation.

A lipolytic enzyme present in the oil palm fruit which is activated by bruising or damage to cellular structure of the ripe fruit, leads to rapid hydrolysis of the triacylglycerol at the site of rupture resulting in the formation of free fatty acid and partial glycerides (Chin, 1983). Chemical hydrolysis, a reaction of zero order kinetics is brought about in the presence of water and is fatty acid catalyzed (Loncin, 1956). Certain lipolytic fungi can also enhance the rate of chemical hydrolysis (Coursey, 1961). All these factors lead to an increase in the level of free fatty acid and partial glycerides in palm oil compared to the content present in fresh unbruised fruits (Jacobsberg, 1983,1988).

1.4.2. Polar Lipids

Phospholipids and glycolipids are the polar lipids of palm oil (Khor et al, 1980; Goh et al, 1982) with the former receiving considerable attention because of the suspected deleterious effect of phosphorus on oil quality (Jacobsberg, 1983; Maclellan, 1983). Although the polar lipids constitute only a minor percentage of the total lipids, they often play an important role in oil quality. They can facilitate the dispersion of microparticulate impurities like iron and other undesirable materials. Phospholipids can also act as pro-oxidants. Both lipids are almost completely removed in the refining process, (Jacobsberg, 1983; Maclellan, 1983).

Khor et al (1980) have reported that fractionation of total lipids of Malaysian palm oil on an acid-treated florisil column gave 96.2% neutral lipids, 1.4% glycolipids and 2.4% phospholipids. Goh et al (1982) have estimated the phospholipid content of mesocarp of oil palm fruit to be 1000-2000 ppm. Malaysian crude palm oil contains relatively lower levels of phospholipids since the wet milling process ensures that only an estimated 4% of the phospholipids of the fruit remain in the crude oil (Goh et al, 1984, 1985; Gee et al, 1985). The major phospholipid components of oil palm fruit mesocarp, have been found to be phosphatidylcholine (36%), phosphatidylethanolamine (24%) and phosphatidylinositol (22%) with minor components as phosphatidylglycerol (9%), phosphatidic acid (3%), diphosphatidylglycerol (4%) and lysophosphatidylethanolamine (2%). An artifact from enzymatic transphosphatidylation in methanolic solvent has been isolated and

characterized as phosphatidylmethanol from unsterilized fruit mesocarp (Goh et al, 1982).

Goh et al (1985, 1988) report the content of glycolipids as 1000-3000 ppm in oil palm fruit mesocarp. A study of the glycolipids of Malaysian palm oil by Khor et al (1980) have shown the presence of esterified sterylglycosides (15.8%), monoglycosyldiacylglycerol (26.8%), cerebrosides (5.6%), sterylglycosides (4.9%) and diglycosyldiacyl-glycerol (23.1%).

The phospholipids and glycolipids of tenera variety of oil palms grown in Maharashtra State of India have been reported by Kulkarni et al (1988, 1991). Their results are comparable to other values (Khor et al, 1980; Goh et al, 1982).

Changes in the phospholipids and glycolipids of developing oil palm fruit of dura variety have been studied by Bafor and Osagie (1986, 1988b, 1989). In the early stages of fruit development, phospholipids and glycolipids were the major lipid constituents of the mesocarp. As fruit development progressed, the content of these lipid classes decreased and reduced to trace levels at maturity. The individual phospholipid and glycolipid classes have also been investigated by Bafor and Osagie (1988b) during development of oil palm fruits.

1.4.3 Minor Constituents

Crude palm oil contains apart from major amounts of triacylglycerols, around 1 percent minor constituents (Goh et al, 1985, 1988) (Figure 6) Apart from fatty acids, partial glycerides,

phospholipids and glycolipids the other minor components of palm oil are carotenoids, tocopherols and tocotrienols, sterols, methylsterols, triterpenic and aliphatic alcohols, terpenic and aliphatic hydrocarbons and wax esters (Goh et al 1985, 1988; Young, 1987; Jacobsberg, 1988; Choo et al, 1990; Goh and Hew, 1990). The minor constituents of palm oil have been studied with respect to their effects on oil quality and stability and optimized conditions for their elimination or reduction (Berger, 1979; Ong, 1981; Jacobsberg, 1983; Maclellan, 1983; Goh et al, 1985). The physiologically active minor components (carotenoids, tocopherols and tocotrienols) have been investigated with respect to their dietary and nutritional importance (Elson, 1992).

Carotenoids are the pigments responsible for the red color of crude palm oil. The carotene content of crude palm oil from Malaysia and Zaire varied between 500-700 ppm (Jacobsberg, 1974). Dura species from Nigerian sources had larger amounts (800-1600 ppm) (Ames et al, 1960). Choo et al (1990) reported the concentration of carotenoids of oil palm fruit varieties. Analysis showed that α and β carotenes constitute approximately 90% of the total carotenoid content, the rest being δ-carotene, phytofluene, phytoene, lycopene, neurosporene and α-and β-zeacarotene., A High Performance Liquid Chromatographic method for analysis of palm oil carotenoids has been developed by Tan (1987). There have been many attempts to recover carotenes from crude palm oil by saponification of the oil, fractional crystallization or adsorption on a variety of adsorbents (Loncin, 1975; Ong, 1975, 1978; Chan et al, 1976; Mamuro and Kubota, 1986; Ooi and Ong, 1986).

Tocopherols and tocotrienols are natural antioxidants and have

been found in crude palm oil at 600-1000 ppm levels (Tan and Oh, 1981; Gapor and Berger, 1982). The main constituents were ca. 44% γ-tocotrienol, 22% α-tocopherol and 12% δ-tocotrienol, the remaining being α and β-tocotrienol and β-, γ, and δ-tocopherols.

The major phytosterols of palm oil have been identified as sitosterol, campesterol and stigmasterol, and trace amounts of cholesterol. Gas- liquid chromatographic analysis also showed the presence of minor sterols, viz., Δ5-avenasterol, Δ7-stigmasterol and Δ7-avenasterol at 0-25 ppm (Weir, 1975; Rossell et al, 1983). Dimethylsterols, triterpene alcohols (40-80 ppm) and methyl sterols (40-80 ppm) have also been determined from the unsaponifiable fraction of palm oil by Itoh (1973a,1973b). Cycloartenol, 24-methylene cycloartenol, cycloartanol and α-amyrin, gramisterol, obtusifoliol and cetrostadienol have been detected by gas-liquid chromatography (Itoh, 1973a, 1973b; Huyghebeart, 1977). Triterpenoid methyl ethers, squalene (200-350 ppm), methyl esters (ca 50 ppm), wax esters (5 ppm), sesqui-and di-terpene (ca 30 ppm) and aliphatic hydrocarbons (ca. 50 ppm) have been identified (Goh and Gee, 1986; Young, 1987).

Exogenous impurities present in trace levels in palm oil include metallic contaminants, phenolic acids, dirt and moisture (Goh et al, 1988). Trace amounts of organic and inorganic contaminants can arise during extraction of oil from the fruit. Most of the phosphorus estimated in palm oil is present as inorganic phosphate (Gee et al, 1985). There are a number of investigations on iron and copper contamination and their effects on oil quality and their removal or reduction (Wong, 1977; Yeoh, 1977; Segers, 1983; Swaboda, 1983; Goh et

al, 1984; Amelotti et al, 1986). Other trace impurities such as phenolic compounds, tannins, lignin, carbohydrates and trace flavanoids have not been well studied (Goh et al, 1985) but most of these originate from sepals, exocarp, fibre and shell of the fruit.

1.4.4. Fatty Acid Composition

The major component fatty acids of palm oil are palmitic acid (16:0) and oleic acid (18:1) followed by linoleic acid (18:2), stearic acid (18:0) and myristic acid (14:0) and trace amounts of lauric (12:0) and linolenic (18:3) acids. The presence of palmitoleic (16:1) and arachidic (20:0) acids are also reported (Chin, 1979; Tan and Oh, 1981; Chin et al, 1982; Tan et al, 1983a, 1983b). The fatty acid composition ranges for typical commercial samples of bonafide palm oil and its products have been given in Table 3.

The individuality of palm oil stems primarily from its high content of 16:0 which, at about 40-45% in commercial palm oil, is almost twice, the quantity found in other common oils rich in 16:0 (Sonntag, 1979b; Padley et al, 1986). This feature, together with the other fatty acids present in significant quantities, (18:1, 18:2 and 18:0), is responsible for the triacylglycerol composition and the consequent solid fat content (SFC) of the oil.

The influence of geographic origin on fatty acid composition has been shown to be minimal. Rossell et al (1983, 1985) have reported the fatty acid composition of palm oils from various geographic regions to establish a reliable purity criteria for palm oil and to verify oil

Table 3 Fatty Acid Composition Ranges Specified by CODEX, SIRIM, MARDI and PORIM for Whole Palm Oil and Fractionated Palm Oil Products

Fatty acid %	Palm oil				Palm olein	Palm stearin
	CODEX (1981)a	SIRIM (1979)b	MARDI (1980)c	PORIM (1980)d,e	PORIM (1980)d,e	PORIM (1980)d,e,
12:0	<12	0-0.5	<0.05-0.4(0.1)	0.1-1.0(0.2)	0.1-1.1(0.1)	0.1-0.6
14:0	0.5-5.9	0.5-5.9	0.6-1.7(1.0)	0.9-1.5(1.1)	0.9-1.4(1.0)	1.1-1.9
16:0	32-59	32.0-51.0	41.1-47.0(43.7)	41.8-46.8(44)	37.9-47.7(39.8)	47.2-73.6
16:1	<0.6	0-0.6	<0.05-0.6(0.1)	0.1-0.3(0.1)	0.1-0.4(0.2)	0.05-0.2
18:0	1.5-8.0	1.5-8.0	3.7-5.6(4.4)	4.2-5.1(4.5)	4.0-4.8(4.4)	4.4-5.6
18:1	27-52	34.6-52.0	38.2-43.5(39.9)	37.3-40.8(39.1)	40.7-43.9(42.5)	15.6-37.0
18:2	5.0-14.0	5.0-11.8	6.6-11.9(10.3)	9.1-11.0(10.1)	10.4-13.4(11.2)	3.2-9.8
18:3	<1.5	0-0.6	<0.05-0.5(-)	<0.05-0.6(0.4)	0.1-0.6(0.4)	0.1-0.6
20:0	<1.0		0.05-0.8(0.3)	0.2-0.7(0.4)	0.2-0.5(0.4)	0.1-0.6

Figures in parenthesis are mean values

a - CODEX standard, 1981

b - Chin, 1979

c - Chin et al, 1982

d - Tan et al, 1983a

e - Tan and Oh, 1981

SIRIM - Standards and Industrial Research Institute of Malaysia

MARDI - Malaysian Agricultural Research and Development Institute

PORIM - Palm Oil Research Institute of Malaysia

authenticity. In general, the composition of palm oil was within a narrow range, with only minor differences due to geographical origin, eg., higher 18:0 contents for Ivory Coast samples, and higher 16:0 content for Sumatran oils.

Jacobsberg (1975) has analyzed the overall fatty acid composition of the oil from mesocarp of the fruit forms of oil palm, viz., dura, pisifera and tenera. Her studies indicated that there were only slight differences in fatty acid composition among the three fruit forms. Ng et al (1976) determined variations in the fatty acid composition of dura, pisifera, tenera, virescent (dura) and albescens (dura) mesocarp oils. Oil from pisifera and albescens palms appeared to have a higher 18:2 content and lower 16:0 content than oils from dura and tenera palms.

Apart from the variation of fatty acid composition between fruit forms of E. guineensis, Ng et al (1976) have also investigated the variations in fatty acid composition between bunches, between palm trees and within a single bunch. The outer, middle and inner fruits from the equatorial regions of a bunch were analyzed. Outer fruits were found to have less 16:0 and more 18:2 than the inner fruits. Significant variations were observed in the fatty acid composition of oil from different bunches of a single palm and also between different palms of a single fruit form. These authors have attributed variations within and between bunches of a palm to be due to the degree of ripeness of the fruit.

The variability of fatty acid composition within the species E. guineensis has been studied by Rajanaidu and Tan (1983) mainly to

investigate the possibilities of improving fatty acid composition. Oil palm progenies were screened to select elite palms for breeding studies. They analyzed outer and inner fruits of the apical, middle and basal regions of a fruit bunch. No significant difference in fatty acid in the different regions was seen, however, 16:0 in some instance showed significant differences in region level. These authors concluded that a full spikelet from three regions of a bunch should be sampled for extraction of oil for fatty acid compositional studies.

The possibilities of modifying the level of unsaturation in palm oil by selection has been considered in several inter-specific hybridization programmes. The South American oil palm, E. oleifera (Kunth) has a mesocarp oil containing more unsaturated fatty acids than that of E. guineensis. The two palms hybridize readily. There are a number of reports on the fatty acid composition of oils from E. guineensis, E. oleifera and their hybrids and back crosses (Cornelius, 1975, 1976, 1977; Gascon and Wuidart, 1975; Macfarlane et al, 1975a, 1975b; Naudet and Faulkner, 1975; Wuidart and Gascon, 1975; Meunier and Hardon, 1976; Ng et al, 1976; Noiret and Wuidart, 1976; Ong et al 1981, 1983; Rajanaidu et al, 1982, 1983, 1985; Meunier, 1985; Tan et al, 1985a, 1985b) Hybrid palm oils have been found to have an unsaturation higher than ordinary palm oil, being intermediate between that of the oils of the two species. Ong et al (1981) have determined the inheritance of fatty acid in the hybrids. While most of the fatty acids and total unsaturation indicate a co-dominant effect, for 18:2, E. guineensis was dominant and dictated the level of 18:2 in the hybrid. Available breeding studies for fatty acid composition in oil palm have

focussed on the 18:1 content since 18:2 appeared limited in the oil palm and 16:0 and 18:1 were negatively correlated (Rajanaidu and Tan, 1983, 1985). While the emphasis on oil palm breeding was towards unsaturation, palms giving specific types of oils (e.g. higher 18:0 levels) have been evaluated further in order to expand on the usage of palm oil (Berger, 1985; Teah, 1985).

Crombie and Hardman (1958), Oo et al, (1985, 1986) and Bafor and Osagie (1986) have studied the changes in the fatty acid composition of the total lipids of the mesocarp of oil palm fruits at progressive stages of development. According to Crombie and Hardman (1958) oil from the immature fruit contained only trace levels of 18:1, but significant levels of 16:0, 18:2 and 18:3. On the other hand, other studies have revealed the presence of 18:1 throughout fruit development, though its relative proportion changed. Oo et al (1985, 1986) and Bafor and Osagie (1986) have reported that in the early stages of development, higher levels of 18:2 and 18:3 were present with a correspondingly lower level of 16:0 and 18:1. With the onset of fat formation (generally estimated at 16 weeks after anthesis in these reports), the content of 18:2 and 18:3 decreased significantly while there was a gradual increase in 16:0 and 18:1 content.

The effects of various processing conditions, viz., extraction of palm oil in the mill, refining, bleaching and deodorization and crystallization and fractionation on the fatty acids of palm oil have been investigated. Chin and Tan (1977) have made a comparative study of the fatty acid composition of palm oil extracted from the fruit with solvent and that of pressed oil from normal mill production. Fatty acid

composition showed only slight differences. In attempting to improve oil recovery during palm oil extraction, palm oil retained in the centrifuge sludge was analyzed by Chow et al (1987). The fatty acid composition of oil extracted from the sludge waste did not differ significantly from the normal composition of palm oil. Arumughan et al (1985) have analyzed the possible changes that may occur to the physical and chemical properties of palm oil under different refining and bleaching conditions. Their data on fatty acid composition indicate that neutralization and bleaching did not alter the fatty acid profile of the oil. The effects of bleaching and deodorization on oxidative properties and possible isomerization of the fatty acids of palm oil were investigated by Siew and Mohammad (1989). Deffense (1985) has studied the effect of fractionation on fatty acid composition of palm oil and observed that the 16:0 tends to migrate in the stearin while the fatty acid composition of the triacylglycerols of palm oil and that of the oleins remain relatively similar inspite of fractionation. Jacobsberg and Ho (1976) and Jacobsberg and Jacqmain (1977) have compared the fatty acid composition of crude palm oil fractions from industrial fractionation processes in Malaysia. Other studies on the fatty acid composition of palm oil during fractionation have been reported by Martinenghi (1972), Taylor (1976), Bek-Nielsen and Krishnan (1977), Berger (1977), Ong and Boey (1979), Tan et al (1983, 1983a, 1983b) and Oh et al, (1988) PORIM specifications for palm stearin and palm olein are given in Table 3.

Available studies on fatty acid composition are reported on whole palm oil. Only a few have been reported on the triacylglycerol

class. Fatty acid composition of the triacylglycerols of palm oil have been investigated by Jurriens et al (1964), Jurriens and Kroesen (1965), Naudet and Faulkner (1975), Berger et al (1978) and Vela (1988). Berger et al (1978) and Naudet and Faulkner (1975) have compared the fatty acid composition of the triacylglycerols from E. guineensis, E. malanococca and their hybrids. The triacylglycerol fatty acid composition closely resembled the fatty acid composition of whole palm oil. This can be attributed to the fact that triacylglycerols account for more than 90% of the lipid class in palm oil. Oo et al (1986) have investigated the changes in the fatty acid composition of the triacylglycerols during development of oil palm fruits. The triacylglycerols of mature oil palm fruits harvested 22 weeks after anthesis had a fatty acid composition similar to reports by other authors for commercial palm oil. However, in the early stages of development, the triacylglycerol contained a higher percentage of unsaturated fatty acids particularly 18:2 and 18:3.

The fatty acid composition of the triacylglycerol classes separated from the total triacylglycerol on the basis of their unsaturation have been determined by Jurriens et al (1964), Jurriens and Kroesen (1965) and Tan et al (1981). These studies revealed that the saturated triacylglycerol fraction contained 86-90% of 16:0. Fraction containing one double bond per molecule contained 33.3% 18:1 and 56-61% of 16:0. Fractions with two and three double bonds contained high levels of 18:1 and 18:2,18:3 was present only in the polyunsaturated fraction.

Positional distribution of fatty acids in the triacylglycerols of palm oil have been determined by lipase hydrolysis technique. The earlier studies on the positional distribution of fatty acids in palm

oil triacylglycerols were reported by Coleman and Fulton (1961), Mattson and Volpenhein (1963), Jurriens et al (1964) and Jurriens and Kroesen (1965). From these investigations it became evident that unsaturated fatty acids were preferentially esterified at the sn-2-position of the triacylglycerol molecules while there was a corresponding higher content of saturated fatty acids in the 1,3 positions of palm oil triacylglycerols.

Investigation by Jacobsberg (1975) on the fatty acid composition of the sn-2-position of the triacylglycerol of the three fruit forms of the oil palm, viz., dura, pisifera and tenera revealed that while fatty acid composition itself varied to a certain extent, the stereospecific distribution on the glycerol radical showed little fluctuations. Hence, it was concluded that the differences in analytical data of commercial palm oil drawn from storage and ship tanks could be attributed rather to deterioration than to genetic differences.

The fatty acids located in the 2-position of the triacylglycerol of palm oils from various geographic regions have been determined by Rossell et al (1983, 1985). Sumatra palm oil sample showed the highest levels of 16:0 at the 2-position and the lowest level of 18:1 as compared to the other oils. These authors have estimated the palmitic enrichment factor (PEF), ie., the ratio of percentage of 16:0-2-position and 16:0-overall in an attempt to determine adulteration of crude palm oil with stearin fractions.

Vela (1988) determined the fatty acids at the sn-2-position of palm oil and its fractions in an attempt to detect the presence of palm oil or its liquid fraction in mixtures with other oils, in particular,

olive oil, based on the fact that the sn-2-position of an oil always contains a small residue of saturated acids which is constant for each oil.

Jurriens et al (1964) and Jurriens and Kroesen (1965) have reported the fatty acid composition of the sn-2-positions of the triacylglycerol classes (separated on the basis of unsaturation by Ag^{+} TLC) of Congo and Sumatra palm oils respectively.

Naudet and Faulkner (1975) have determined the fatty acid composition of triacylglycerols of palm oils from E. guineensis, E. malanococca and their hybrid. 16:0 and 18:1 contents at sn-2-positions were found to be intermediate between the oils of the parental varieties for the hybrid.

Apart from the lipase hydrolysis method, C^{13}NMR spectroscopic methods have been used to determine the positional distribution of fatty acids in the triacylglycerols of palm oil (Ng, 1983, 1984, 1985; Ng and Ng, 1983). The C^{13} NMR spectrum of the carbonyl carbons of the acyl groups of the triacylglycerol has been shown to give the composition of the saturated, 18:1 and 18:2 acyl groups at the 1,3 positions and at the sn-2-positions of the glycerol moiety. A shortcoming of this technique is that there is no differentiation of the various saturated fatty acids. However the results are comparable with enzymatic hydrolysis techniques.

The composition of the positions sn-1-, sn-2-, and sn-3- of the triacylglycerols of palm oil were determined by Christie (1991) by partial hydrolysis with ethyl magnesium bromide, derivatization of the total products with (s)+(+)-1-(1-naphthyl) ethylisocyanate and isolation

of the diacyl-sn-glycerol urethane derivatives by chromatography on solid phase extraction columns containing an octadecylsilyl bonded phase. The diastereomeric sn-1, 2-and 2,3-diacylglycerol derivatives were separated by HPLC on silica followed by determination of their fatty acids by GLC. Fatty acid compositions at the sn-1, -2- and -3-positions were calculated from the fatty acid compositions of the triacylglycerols, 2,3-sn-diacylglycerol derivatives and the 1,2-sn-diacylglycerol derivatives. The results of the sn-2- composition were comparable with other reports in the literature. sn-3-position contained more 16:0 while 18:1 was present at the sn-1 position..

Apart from a report by Okiy et al (1978) there are no available reports on the fatty acid compositions of the diacylglycerol, monoacylglycerol and free fatty acid fractions of palm oil. However, Oo et al (1986) have investigated the changes in the fatty acid profile of the lipid classes of the developing oil palm fruit and reported that the diacylglycerol, monoacylglycerol and free fatty acid fractions are characteristically higher in 16:0 content and lower in 18:1 and 18:2 content than the triacylglycerol and polar lipid classes.

Goh et al (1982) have reported the fatty acid composition of the total phospholipids of palm oil. The phospholipids were characterized by a high content of 18:2. Kulkarni et al (1988) have determined the fatty acid composition of the various phospholipid classes of palm oil. They also observed the presence of higher levels of 18:2 in the phospholipids. Phosphatidylcholine was higher in 16:0 and lower in 18:1 than the other phospholipids. All other phospholipids had comparable fatty acid compositions with characteristic low levels of

16:0 and high 18:0 and 18:2 content than found in crude palm oil. The fatty acid composition of the phospholipids of developing oil palm fruit have been investigated by Bafor and Osagie (1986). The major fatty acids of phospholipids of immature fruit were 16:0 and 18:2 with low levels of 18:1. 18:1 became a prominent fatty acid in the phospholipids in ripe fruits.

The fatty acid composition of the glycolipids of palm oil indicate a significantly higher content of 18:3 (11%) (Goh *et al*, 1982). The 18:2 level was also higher than that of crude palm oil while 16:0 was found to be lower (Goh *et al*, 1982). Similar trend was reported by Kulkarni *et al* (1991) for the fatty acid composition of various glycolipid classes of palm oil. The fatty acid composition of maturing oil palm fruit mesocarp revealed that the unsaturated fatty acids were relatively high in the immature fruit glycolipids and the major component fatty acids were 18:1, 18:2 and 18:3 (Bafor and Osagie, 1986). The mature fruit glycolipids were higher in saturated fatty acids.

1.4.5. Triacylglycerol Composition

The earlier studies on the nature and type of the triacylglycerols of palm oil were investigated by the permanganate-acetone oxidation method of Hilditch and co-workers (1956) and Kartha (1953a, 1953b, 1954), progressive hydrogenation method (Banks *et al*, 1935), transition and melting point studies (Meara, 1948) and by the systematic crystallization technique from a suitable solvent of Hilditch and co-workers (1956). Hilditch and Maddison (1940), Hilditch and Meara

(1942), Hilditch et al (1947) and Meara (1948) have resolved palm oil from various geographic origins by exhaustive crystallization from acetone-diethyl ether and have determined the triacylglycerol composition of palm oil. From these early oxidation and crystallization studies it became clear that the chief component triacylglycerols of palm oil were 'oleo'-dipalmitin and palmito-di-'olein' in amounts which varied according to the proportions of 16:0, 18:1 and 18:2 in the whole fat. Together, these two groups of triacylglycerols usually amounted to 70-75 percent of palm oil, 'oleo' - dipalmitin preponderating in oils with high 16:0 content and conversely. The other minor components were 10-12 percent 'oleo' - palmitostearin, and 3-12 percent linoleo-diolein according to the 18:1 and 18:2 content of the palm oil; and about 3-9% tripalmitin plus dipalmitostearin, varying with the 16:0 content of the palm oil (Hilditch, 1956). These early workers concluded that the triacylglycerol composition of palm oil did not conform very accurately to the rules of 'Even' Distribution (Hilditch and Meara, 1942) and also did not agree with either those calculated on the 'Random' distribution or with those calculated by Kartha's formula (Luddy et al, 1954).

The earlier oxidation methods have been modified subsequently by several authors and used to determine the triacylglcyerol composition of palm oil. The trisaturated triacylglycerols of palm oil and its fractions were determined by combining the classical oxidation method with gas chromatography (Bouvron, 1979). After oxidation of the fat with potassium dichromate in acetone medium, the unmodified trisaturated triacylglycerols were extracted and analyzed directly by gas chromatography. By a controlled oxidation method with potassium -

periodate in t-butanol (von Rudlov, 1956) the same author (1981) converted the monounsaturated triacylglycerols of palm oil and its fractions into azelaoglycerides which were quantitated by gas chromatography of the methyl esters of the azelaoglycerides. Dasgupta et al (1981) have devised a method for the determination of triacylglycerol composition of palm oil by oxidation with periodate - permanganate (Youngs, 1961), fractionation of the derived azelaoglycerides by thin-layer chromatography and quantitation of the fractions obtained by colorimetry using Rhodamine 6G reagent at 535 nm. The results compared well with lipase hydrolysis data.

A number of chromatographic techniques have been used for the determination of triacylglycerol composition of palm oil from Congo (Jurriens et al, 1964), Sumatra (Jurriens and Kroesen, 1965) and Malaysia (Jacobsberg, 1975; Kifli, 1975; Tan et al, 1981). These investigations involved (i) the isolation of the triacylglycerols from palm oil by column or thin-layer chromatography, (ii) separation of the triacylglycerols according to their degree of unsaturation by TLC on silica gel impregnated with silver nitrate (Jurriens et al, 1964; Jurriens and Kroesen, 1965; Tan et al, 1981) followed by (iii) gas chromatographic analysis of the triacylglycerols according to their carbon number (Jurriens and Kroesen, 1965; Tan et al, 1981) (iv) determination of fatty acid composition of total triacylglycerols and their fractions and or (v) positional analysis of the fatty acids at the sn-2- positions by lipase hydrolysis technique (Jurriens et al, 1964; Jurriens and Kroesen, 1965; Jacobsberg, 1975; Kifli, 1975) and (vi) computation of the triacylglycerol species from the data obtained by

lipase hydrolysis according to Vander Wals' hypothesis (Vander Wal, 1960) based on the method of Coleman and Fulton (1960) or Kanichi-Hayakawa (1967) or (vii) from argentation and gas chromatographic data (Tan et al, 1981).

From these studies, it was observed that palm oil contains two major triacylglycerols of the types oleodipalmitin and palmitodiolein, minor amounts of the types linoleodipalmitin and oleopalmitolinolein together with smaller quantities of many other triacylglycerols. Approximately 85% of the unsaturated fatty acids are located at the central ester bond of the triacylglycerols. Dipalmitoyl-2-oleoylglycerol (POP) is the major triacylglycerol of palm oil with values ranging from 20.0% (Kifli, 1975) to 31.6% (Tan et al , 1981) followed by 1,3-rac-palmitoyl-oleoyl-2-oleoylglycerol (POO) accounting for 18.9% (Jurriens et al , 1964; Jurriens and Kroesen, 1965) to 25.77% (Tan et al, 1981). 26% (Jurriens and Kroesen, 1965) to 35.8% (Kifli, 1975) of the triacylglycerols consist of tripalmitin (PPP), 1-3-rac-palmitoyl-oleoyl-2-palmitoylglycerol (PPO),dipalmitoyl-2-linolylglycerol (PLP), triolein (OOO) and 1,3-rac-palmitoyl-linolyl-2-oleoylglycerol (POL). The distribution of fatty acid in the triacylglycerols of palm oil was found to agree well with that of 1,3-random, 2-random distribution hypothesis of Vander Wal (1960) according to Jurriens et al (1964), Jurriens and Kroesen (1965) and Tan et al (1981) based on the computation method of Coleman and Fulton (1961).

The triacylglycerols of palm oil have been analyzed based on their carbon number profile, ie., the number of carbon atoms in the acyl chains of a triacylglycerol molecule, by direct GC of solutions of the

triacylglycerol mixtures on packed columns under temperature programmed conditions. Palm oil contains C46, C48, C50, C52 and C54 triacylglycerols. As an aid to the identification of genuine palm oil, Palm Oil Research Institute of Malaysia (PORIM) and Malaysian Agricultural Research and Development Institute (MARDI) have published typical ranges for the levels of C46 through C54 triacylglycerols of crude palm oil. C50 and C52 are the major triacylglycerols of palm oil comprising 40-45.2% and 38.2-43.8% respectively of the total triacylglycerols. Minor amounts of C48 and C54 (4.7-10.8% and 6.4-11.4% respectively) are present (Tan et al, 1983b). Rossell et al (1985) have calculated the carbon number profile of the triacylglycerols of palm oil from different geographic regions. Carbon number analysis of palm oil triacylglycerols by high temperature progammed GC has been reported by several authors (Jurriens and Kroesen 1965; Chin, 1979; Karleskind, 1981; Perron, 1983; Tan et al, 1983b; Tan, 1985). Jurriens and Kroesen (1965) and Tan et al, (1981) have determined the carbon number profile of the triacylglycerol fractions obtained by Ag^+ TLC.

A High Performance Liquid Chromatographic (HPLC) method was developed and tested on palm oil triacylglycerol by Peterson et al (1981). The triacylglycerols were separated into four fractions on the basis of Partition Number, ie, having the same carbon numbers and number of double bonds, under the conditions described. These authors have determined the triacylglycerol class and type within each group and the fatty acid components. El-Hamdy and Perkins (1981) and Kimmey and Perkins (1984) have separated the triacylglycerols of palm oil according to their theoretical carbon number values (TCN) by High Performance

Reverse phase chromatography on Supelcosil LC-18 columns using acetone-acetonitrile as mobile phase and refractive index detector. Takahashi *et al* (1984) have separated the triacylglycerols of palm on a Lichrosorb RP- 18 column with acetone- acetonitrile as mobile phase. Deffense (1985) and Lago and Hartman (1986) have reported the triacylglycerol composition of palm oil by HPLC. The results of these authors agree well with those obtained by argentation and lipase hydrolysis procedures of Jurriens and Kroesen (1965) and Jacobsberg (1975).

Comparative studies on the triacylglycerol composition of oils from *E. guineensis*, *E malanococca* and their hybrids have been reported by Naudet and Faulkner (1975), Berger *et al* (1978), Rajanaidu *et al* (1983, 1985) and Tan *et al* (1985a, 1985b). They have concluded that hybridization modifies not only fatty acid composition but triacylglycerol composition as well. The results of Berger *et al* (1978) indicate that the major triacylglycerols of *E. guineensis* (tenera variety) are SUS and UUS (39.9% and 34.3%) respectively, while the hybrid contains predominantly UUS (39.5%) and UUU (44.4%). Tan *et al* (1985b) have determined the relationship between fatty acid and triacylglycerol composition of hybrid oils; a positive correlation exists between 16:0 and C50 and C54 and between 18:1 and C54 triacylglycerol while 16:0 and C54 and 18:1 and C50 and C52 are negatively correlated. Ong *et al* (1981) have determined the triacylglycerol compositions of the F1 (*E. guineensis* X *E. oleifera*) and the backcross hybrid mesocarp oils and demonstrated that the triacylglycerol composition of hybrid palms are intermediate between those of their respective parentals. The results from this study were

used to develop the "Co-Dominance theory of Elaeis Palm Hybridization".

The effects of various processing conditions on the triacylglycerol composition of palm oil have been investigated. Several workers have reported the triacylglycerol composition of palm oil during crystallization and fractionation studies (Kellens, 1972; Taylor, 1973, 1976; Koslowsky, 1975; Deroanne et al, 1976; Bek-Nielsen and Krishnan, 1977; Berger, 1977; Berger et al, 1978; Anon, 1983; Tirtiaux, 1983; Deffense,1985; van Putte and Bakker, 1987; Oh et al, 1988) of interesterified and randomized palm oil (Babin, 1974; Braae, 1977; Macrae, 1983; Duns, 1985; Lago and Hartman, 1986; Oh et al, 1988) and for food application (Duns, 1985; Pease, 1985; Berger and Ong, 1985; Yap et al, 1989; Berger, 1990).

1.5 SCOPE OF THE PRESENT INVESTIGATION

From the forgoing review, it is evident that oil palm fruit is the most important commercial source of edible oil in the world. The significance of oil palm, vis-a-vis, edible oil situation in India has also been brought out. A cursory perusal of the literature would reveal that the basic information on the oil palm fruit lipids is not commensurate with the importance of palm oil in the world edible oil trade. In view of the growing importance of oil palm as a source of edible oil for India, a comprehensive study on the lipids of oil palm fruit produced under the Indian agro-climatic conditions was conceived. The problem has also been designed to gather basic information on the oil palm fruit lipids to provide scientific explanation to the technological problems that currently and actively are being pursued at RRL(T). The scope of the present investigation include the following:

i. Lipid profile of the mature oil palm fruit mesocarp

The oil palm fruits of tenera variety grown under the climatic conditions prevailing in the southern part of Kerala State were subjected to detailed investigation with respect to the composition of the lipids, fatty acid composition of the lipid classes, positional distribution of the fatty acids in the triacylglycerols, distribution of fatty acids among the triacylglycerol species (obtained by Ag^{+}TLC), triacylglycerol composition and lipid profile of the anatomically distinct regions of the oil palm fruit and of oil palm fruit varieties.

ii. Composition and structure of lipids in the developing oil palm fruit

The lipids extracted from the fruits of different developmental stages were analyzed for their constituent lipid classes and fatty acids, positional distribution of fatty acids in the triacylglycerols and triacylglycerol composition.

iii. Lipid profile of process streams of palm oil mill

The information gathered on the lipid composition and structure of the oil palm fruit was extended to the commercial process conditions of palm oil extraction with a view to understand the changes occurring in the lipids and on the quality of palm oil. The total lipids obtained from various process steps were subjected to detailed characterization and the results were correlated with palm oil quality problems under commercial process conditions.

CHAPTER 2

MATERIALS AND METHODS

2.1 MATERIALS AND METHODS OF SAMPLING

2.1.1 Source of Materials

Oil palm fruits used in the course of this investigation were collected from the germ plasm garden of the Central Plantation Crops Research Institute (C.P.C.R.I), Indian Council of Agricultural Research (I.C.A.R), Palode, Trivandrum.

2.1.2 Design of Experiment and Sample Collection

2.1.2.1 Studies on the Developing Oil Palm Fruit

Oil palms of the tenera variety (15 year old) were randomly selected for the study. Tenera is a hybrid variety and therefore the

palms selected were matched for their age, and hybrid combinations were selected from the germ plasm record of the C.P.C.R.I. The oil palm is monoecious and the female inflorescences were selected, pollinated and tagged. The fruit bunches were harvested at two week intervals, (4,8,10,12,14,16,18,20,22 and 24 weeks after anthesis, WAA). Fruits from an entire bunch were separated from the bunch stalk and spikelets, and mixed well. 500g of a random, representative sample was then removed from the bulk for analysis. Samples from each developmental stage were collected from three different palms separately and analyzed in duplicate.

2.1.2.2 Studies on oil palm fruit forms

Mature fruits of dura, pisifera and tenera fruit forms (or varieties) were collected. Exocarp (outer, waxy skin) and mesocarp (middle, oil bearing fibrous tissue) regions (and pericarp from the whole fruit) were identified, peeled and separated. Tissues from the respective regions or zones were obtained from a number of whole fruits taken from different regions of a single bunch (as recommended by Rajanaidu and Tan, 1983) and mixed to obtain a homogenous sample. Three bunches were analyzed for each fruit form. Samples were analyzed in duplicate and the results were subjected to statistical analysis.

2.1.2.3 Studies on process streams of palm oil mill

The different process streams, viz., sterilizer condensate, sludge water, press fibre residue, and crude palm oil were sampled by operating the Demonstration plant for palm oil established at C.P.C.R.I having a capacity of 1 ton fresh fruit bunches (FFB) per hour. The process details were reported earlier by Sundaresan et al (1990). The plant was operated specifically to collect samples at various stages of operation. Condensate of approximately 300kg from the sterilizer was collected from an entire batch of 1000 kg FFB. Representative samples were taken from the bulk. The method of extraction of oil was hydraulic pressing and samples were collected from the press fibre after it was subjected to 75 kg/cm^2 pressure as optimized for the demonstration plant. The oil water mixture (400 kg) from the press with an oil-water ratio of 1:2 (v/v) was clarified. The sludge from the bottom was removed for sampling. Oil from the clarifier was further purified by a high-speed centrifuge for the crude palm oil sample. Two trials were conducted for collection of samples as described and they were analyzed (in duplicate) separately.

500 g of a random sample of fresh fruits collected at the mill for processing were taken (prior to sterilization step) for extraction of total lipids by solvent extraction method.

2.2 ANALYTICAL METHODS

2.2.1 Extraction of Lipids

2.2.1.1 Extraction of lipids from mesocarp of oil palm fruits

Total lipids were extracted with chloroform-methanol, 2:1 (v/v) solvent mixture from fresh mesocarp as described by Goh et al (1982) for oil palm fruits. This is essentially the procedure of Folch et al (1957).

Fresh fruits were steam sterilized for 20 min at approximately 15 psi in an ordinary household pressure cooker to inactivate lipase and to facilitate the blending of mesocarp tissue with solvent. The mesocarp of the steam sterilized fruits was peeled and 500g was blended with approximately 500 ml of chloroform- methanol, 2:1 (v/v) solvent mixture in a blender. The homogenized mesocarp-solvent mixture was left overnight (ca. 16-20 hrs). The extract was filtered and the residue was further reextracted with 3x100 ml aliquots of fresh solvent mixture (or until the residue was completely devoid of lipid). The extracts were combined and washed once with one quarter of the total volume of the filtrate of ca. 0.6% sodium chloride solution. The phases were separated in a separatory funnel. The lower chloroform layer was carefully removed, passed through anhydrous sodium sulfate to remove any moisture and concentrated under vacuum in a rotary evaporator at 60^{o}C. Lipids were dissolved in a few ml of benzene and solvent was concentrated to dryness. The total lipid thus obtained was immediately redissolved in a

minimum volume of chloroform and stored in glass vials at 4°C for further analyses.

2.2.1.2 Extraction of lipids from different regions of the oil palm fruit

The three regions or zones viz., exocarp, mesocarp and pericarp of the oil palm fruit were separated from the three varieties viz., dura, pisifera and tenera. Chloroform-methanol, 2:1 (v/v) was immediately added to the freshly peeled tissues and total lipids were extracted as described in section 2.2.1.1.

2.2.1.3 Extraction of lipids from process streams of palm oil mill

Total lipids were extracted from the process streams of the palm oil mill, viz., sterilizer condensate, sludge water and press fibre residue.

Total lipids from fresh fruits collected at the mill site prior to processing were extracted with chloroform-methanol, 2:1 (v/v) as described in section 2.2.1.1.

The press fibre residue obtained from the hydraulic press was dried in an air oven at 100±5°C for 1 hour to remove moisture. Kernels were removed from the nut-fibre residue and 500g of the fibrous material was extracted with chloroform-methanol, 2:1 (v/v) as detailed in section 2.2.1.1.

250 ml of chloroform was added to 500 ml of sterilizer condensate taken in a separatory funnel and shaken well repeatedly at intervals of 10-15 min for about 1 hour. The two layers were allowed to separate and the lower chloroform layer was carefully removed. Extraction was repeated thrice with 100 ml aliquots of chloroform. The chloroform layers were combined, washed once with one quarter of its volume of 0.6% aqueous solution of sodium chloride. The lower chloroform layer was passed through anhydrous sodium sulfate and the total lipids were concentrated as given in section 2.2.1.1. Total lipids from sludge was also extracted similarly.

2.2.2 Thin-Layer Chromatography

2.2.2.1 Scheme of analysis

Lipid samples obtained from tissues by solvent extraction process are complex mixtures of several lipid classes. Commonly, a combination of chromatographic techniques are used to separate the total lipids into small and simpler fractions for further analysis (Christie, 1982). The scheme of separation of the total lipids of oil palm fruit is given in Figure 7. The total lipids obtained from the mesocarp of the oil palm fruit and from the various process streams were separated into the component lipid classes viz., triacylglycerol, 1,3-diacylglycerol, 1,2-diacylglycerol, monoacylglycerol, free fatty acid and polar lipids by thin-layer chromatography (T.L.C). Phospholipids and glycolipids were separated from the total polar lipids. Triacylglycerols were further

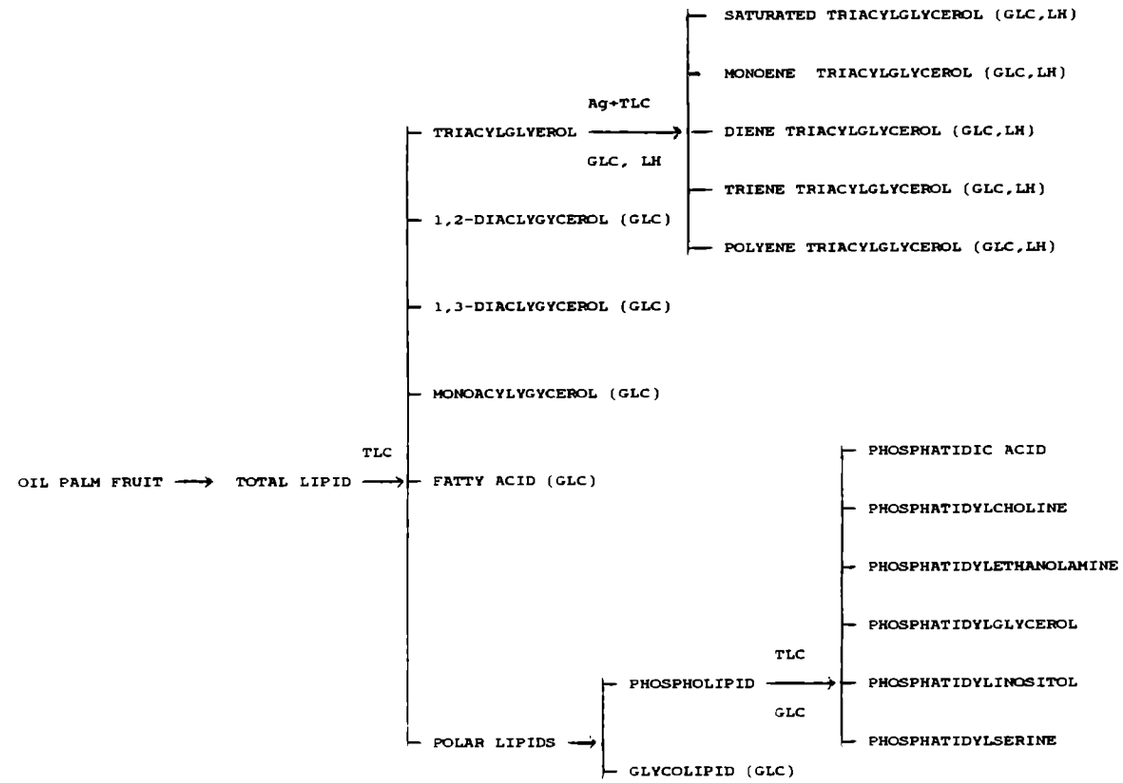


Figure 7 **Flow sheet indicating the fractionation of total lipids of oil palm fruit mesocarp into various lipid classes by thin-layer chromatography.** TLC - thin-layer chromatography; Ag^{+} TLC - argentation thin-layer chromatography; GLC - gas - liquid chromatography; LH - lipase hydrolysis.

fractionated by argentation thin-layer chromatography (Ag^+ TLC) into various triacylglycerol classes. The total phospholipids of mature oil palm fruits were separated into individual classes by TLC. Each lipid class was quantitated spectrophotometrically or by gas-liquid chromatography. Fatty acid composition of each lipid class was determined. Fatty acids at the sn-2-position of the triacylglycerols of the developing oil palm fruit were determined by hydrolysis with pancreatic lipase. The triacylglycerol classes of mature oil palm fruits were also subjected to lipase hydrolysis.

Preparation of chromatoplate: An aqueous slurry of adsorbent was prepared by vigorously shaking a specific amount of silica gel (usually 50g) with approximately double the volume of distilled water (100 ml). The adsorbent slurry was applied evenly onto clean 20 x 20 cm glass plates using an applicator (spreader) adjusted to give an appropriate adsorbent thickness. Each plate was allowed to dry in air at room temperature and then activated for 1 hr at 110^{o}C. The plate was used as soon as it had cooled to room temperature.

Microslide TLC plates were prepared to monitor methanolysis reaction products and to check the recovery of lipids from adsorbents. A slurry of silica gel adsorbent was made with chloroform. Microslides were dipped into the slurry and the layer of adhering adsorbent was allowed to dry in air. The chromatograms were developed as usual.

Application of sample: A known weight of lipid sample dissolved in a solvent was applied as a narrow band, ie., a row of discrete spots close to each other, with a Hamilton syringe, 2-3 cm from the bottom edge of the plate. Appropriate mixtures of authentic lipid standards that migrated in a known order was applied to each plate along-side the unknown mixture so that direct comparison of R_f values was possible.

Development of chromatogram: The developing tank was lined with filter paper and the appropriate solvent system poured into the tank. Filter paper lining was wetted with the developing solvent to saturate the atmosphere inside the tank with solvent vapor at least 30 minutes prior to immersion of the plate. The chromatogram was developed in the suitable solvent system. The plate was removed from the tank when the solvent front moved to about 2-3 cm below the upper edge of the plate. The chromatogram was quickly dried and lipid bands/spots detected. A number of plates were developed for preparative work.

Detection of lipids on thin-layer chromatogram: The following methods were used to detect lipid spots/bands on a developed chromatogram:

i. Sulfuric acid spray: The chromatogram was sprayed lightly with 50% sulfuric acid-methanol (by volume) followed by heating in an oven at 180^{o}C for 30 minutes to 1 hour. All lipids formed dark brown or black spots on a white background. Since this is a destructive method, sulfuric acid spray was used only when the lipids were not required for further analysis.

ii. Iodine vapor: The chromatogram was placed in a development tank containing a few crystals of iodine. In several minutes brown spots appeared on a pale yellow background. Areas of the required lipids were identified and marked. Iodine was allowed to evaporate off. The adsorbent was then scraped out and lipids eluted from the gel.

iii. 2',7'- dichlorofluorescein: Non-polar lipids became visible in UV light after the chromatogram was sprayed with a solution of 0.2% 2',7'-dichlorofluorescein in 95% methanol-water. Lipids appeared as yellow bands on a purple background.

iv. Ninhydrin spray 0.2% Ninhydrin in butanol saturated with water was sprayed on the developed chromatogram, followed by heating of the chromatogram for about 5 minutes at 100-105^{o}C in an atmosphere saturated with water. Lipids with free amino group showed up as red spots on a white background.

Recovery of lipids from adsorbent: Each lipid spot/band was carefully scraped out and extracted with 5ml of appropriate solvent. The silica gel and solvent was shaken well and a short centrifuge spin was used to speed the settling of the gel as recommended by Weber et al (1971) and Christie (1982). The supernatent extract was decanted and the procedure was repeated with 4x5 ml aliquots of solvent. The combined extracts were concentrated under vacuum in a rotary evaporator. Lipids were used immediately for analyses or dissolved in a minimum volume of chloroform and stored in glass vials for later use. Extraction of the lipids from the gel was found to be complete using micro-TLC plates. For spectrophotometric estimations, a portion of the developed chromatogram,

devoid of any sample was also scraped out and eluted in a manner similar to the sample and used as the blank during estimations to compensate for absorbance due to adsorbent (Chapman and Robertson, 1977).

2.2.2.2 Thin-layer chromatography of total lipids

Chromatoplates: Total lipids were subjected to thin-layer chromatography on 1mm thick silica gel G adsorbent prepared as described in section 2.2.2.1.

Sample application: Approximately 50 to 80 mg (known, accurately weighed quantity) of total lipid was applied per plate. Standard tripalmitin, dipalmitin, monopalmitin and palmitic acid were co-chromatographed with the sample.

Solvent system: Solvent system used for development was petroleum ether - diethyl ether-formic acid, 60/40/1.6, (v/v/v) as per an earlier report of Ong *et al* (1981) for the separation of total lipids of oil palm fruit mesocarp.

Detection of lipids: Lipid class bands were detected by spraying with sulfuric acid or by a brief exposure to iodine vapor for preparative work. Lipid bands were identified with respect to R_f values of standard lipids (Table 4).

Recovery of lipids from silica gel: Tri-, di-, and monoacylglycerols and fatty acid bands were eluted with chloroform. The polar lipid band was first eluted with acetone to obtain the glycolipids followed by methanol to extract the phospholipids.

Table 4 Separation of Total Lipids of Oil Palm Fruit Mesocarp by Thin-layer Chromatography. R_f values of standard neutral lipid classes separated on 1mm thick silica gel G with petroleum ether - diethyl ether-formic acid (60/40/1.6, v/v/v) solvent system

Lipid class	R_f value
Carotene	10.0
Tripalmitin	8.4
Palmitic acid	4.9
1,3-Dipalmitin	2.9
1,2 (2,3)-Dipalmitin	2.2
Monopalmitin	0.4

2.2.2.3 Thin-layer chromatography of phospholipids

Chromatoplate: The total phospholipid obtained from TLC of total lipids were fractionated into the various phospholipid classes by thin-layer chromatography on 0.5mm thick silica gel H adsorbent.

Application of sample: Approximately 5 mg of total phospholipid was applied per plate. Standard phosphatidic acid, phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol, phosphatidylinositol and phosphatidylserine were chromatographed alongside the sample for identification.

Solvent system: A single development using chloroform - methanol - acetic acid - water, 170/25/25/6 (v/v/v/v) system (Bafor and Osagie, 1988b) separated the total phospholipid of oil palm into various classes.

Detection: Spots were visualized with iodine vapor. Ninhydrin reagent was sprayed for the detection of phospholipids containing amino groups. R_f values of standard phospholipids were compared with the phospholipid spots of the sample (Table 5).

Elution of phospholipids: Spots were scraped and eluted from the adsorbent with chloroform-methanol, 1:2 (by volume).

Table 5 Separation of Phospholipids of Oil Palm Fruit Mesocarp.

R_f values of standard phospholipids separated by thin-layer chromatography on 0.5 mm thick silica gel H and chloroform-methanol - acetic acid - water (170/25/25/6; v/v/v/v) solvent system

Phospholipid	R_f value
Diphosphatidylglycerol	9.7
Phosphatidic acid	8.0
Phosphatidylethanolamine	3.5
Phosphatidylglycerol	2.3
Phosphatidylcholine	1.8
Phosphatidylserine	0.8
Phosphatidylinositol	0.4

2.2.2.4 Argentation thin-layer chromatography

Preparation of plates: 10% silver nitrate impregnated plates were prepared by spreading a slurry made with 20g silica gel G and a solution consisting of 2.2 g of silver nitrate in 40 ml distilled water to a thickness of 0.5 mm. The plates were dried in air at room temperature in the dark.

Activation of plates: Silica gel plates impregnated with silver nitrate were activated at 195^{o}C for two hours prior to use (Renkonen, 1967 and Akesson, 1969). Plates were cooled to room temperature in the dark before use. Precautions were taken to minimize the exposure of the plates to light.

Application of sample: About 2mg of triacylglycerol, previously separated by TLC of the total lipids were applied as a narrow band to the plate. Standard tripalmitin, monooleodipalmitin, monopalmitodiolein and triolein were also chromatographed alongside the triacylglycerol sample.

Solvent system: The solvent system used for the separation of triacylglycerol was benzene-petroleum ether-diethyl ether, 90/10/3 (v/v/v) as reported by Tan *et al* (1981) and Bafor and Osagie (1989).

Detection of bands: The plates were sprayed with 2'-7'-dichlorofluorescein and viewed under UV light. Bands were identified according to their position on the plate with respect to the R_f values of standards and according to their fatty acids compositions (Gunstone and Padley, 1965) (Tables 6 and 7).

Table 6 Argentation Thin-layer Chromatography of Standard Triacylglycerols. R_f values of standard triacylglycerols separated on 0.5 mm thick silica gel G adsorbent impregnated with 10% (w/w) silver nitrate and solvent system of benzene-petroleum ether-diethyl ether (90/10/3, v/v/v)

Triacylglycerol	R_f value
Tripalmitin	6.4
Dipalmitoolein	5.4
Dioleopalmitin	4.4
Triolein	3.1

Table 7 Argentation Thin-layer Chromatography of Triacylglycerols of Oil Palm Fruit Mesocarp. R_f values of triacylglycerol of palm oil. TLC conditions as in Table 6.

Band	R_f value
1 (Saturated)	6.5
2 (Monoene)	5.6
3 (Diene)	4.5
4 (Diene)	3.9
5 (Triene)	3.2
6 (Polyene)	2.4
7 (Polyene)	0.3

Recovery of triacylglycerols from adsorbent: Each triacylglycerol band was carefully scraped out and 4 x 5ml aliquots of a solution of 1% sodium chloride in methanol-water (90/10, v/v) was added to the gel with vigorous mixing to destroy the characteristic red color of the silver - dichlorofluorescein complex (Hill et al, 1968). The adsorbent gel was then eluted with 4x5ml portions of chloroform until the triacylglycerols were completely extracted.

2.2.3 Gas-Liquid Chromatography

2.2.3.1 Preparation of fatty acid methyl esters

The determination of fatty acid composition of a lipid sample involves the conversion of the lipid to volatile fatty acid methyl esters suitable for gas-liquid chromatographic analysis. Fatty acid methyl esters were prepared according to IUPAC method (2.301) (Paquot and Hautefenne, 1987). The procedure involves saponification of the fat and acid catalyzed esterification by methanol of the fatty acids.

Reagents:

i. Hexane; redistilled, chromatographic quality

ii. Anhydrous methanol, prepared by refluxing dry magnesium turnings (5g) and iodine crystals (0.5g) with 50-75ml of freshly distilled methanol until the color of the iodine disappeared. 1 litre of freshly distilled absolute methanol was then added, refluxed for 2-

3 hours and then distilled off; excluding moisture from the system (Perrin and Armarego, 1988).

iii. 0.5N methanolic sodium hydroxide solution.

iv. 2% methanolic sulfuric acid.

v. Sodium chloride, saturated aqueous solution.

vi. Sodium sulfate, anhydrous.

Procedure: Approximately, 350 mg of palm oil was weighed into a round bottom flask. (Smaller quantities of lipids obtained from thin-layer chromatographic plates were also taken; the quantity of the reagents were proportionately decreased). 6ml of methanolic sodium hydroxide was added. Contents were refluxed for 10 minutes until the droplets of fat disappeared. 7ml of 2% methanolic sulfuric acid was added through the top of the condenser and boiling was continued for another 15 minutes. 2ml of hexane was added to the mixture through the top of the condenser and boiling was continued for 1 minute. The flask was cooled. A small amount of saturated sodium chloride solution was added and the flask shaken gently by rotating several times. More saturated sodium chloride solution was added in order to bring the level of the liquid into the neck of the flask. The hexane layer was allowed to separate. Maximum volume of the upper hexane layer was pipetted out and passed through anhydrous sodium sulfate to remove any traces of water. The esterification reaction was found to be complete by monitoring on micro TLC. The solution of the fatty acid methyl esters were injected directly into the gas chromatographic column. In case of

small quantities of lipid samples, the solution of methyl esters was concentrated very gently at room temperature under slight vacuum before injection.

2.2.3.2 Gas chromatographic column:

Two liquid phases were used during the course of this investigation for gas-liquid chromatography of fatty acid methyl esters:

i. EGSS-X (Methylsilicone-ethyleneglycol succinate polymer): 10% EGSS-X on Chromosorb W 100 (stationary phase support) metal, packed column (2m x 2mm i.d.) was prepared in the laboratory.
ii. Cross linked polyethyleneglycol-TPA phase: Commercially available HP-FFAP (crosslinked FFAP) US Patent No.4,293,415,USA, length x id x film thickness, 30m x 0.5 nm x 1.0 um was purchased from Hewlett-Packard, USA.

Preparation of EGSS-X column (Kates, 1986): 1 g of the liquid phase (EGSS-X) was dissolved in ca. 60 ml of warm chloroform in a 250 ml beaker. 9g of Chromosorb W 100 support material was slowly added with gentle stirring with a glass rod until a homogenous paste was obtained. The solvent was removed in vacuo at room temperature until a uniform free-flowing granular material was obtained. A small plug of pyrex glass wool was first inserted at the exit end of the column. A glass funnel was held to the inlet end. The column was held vertically and filled

slowly with packing material from the inlet end while vibrating the column by pressing gently against the tip of a vibro-graver. A gradually increasing vacuum at the inlet end was applied to ensure firm packing of the column. When column packing was complete, a plug of glass wool was inserted at the inlet end. Column was coiled into the desired size. Column was conditioned at 200^{o}C for 24 hours at 20 ml/min carrier gas flow rate before use. Authentic standard fatty acid methly esters were first injected to standardize chromatographic conditions prior to the experiment.

2.2.3.3 Separation of fatty acids

A Hewlett-Packard 5840 A model gas chromatograph equipped with a flame ionization detector was used. Methyl esters were analyzed on a 2m x 2mm id. 10% EGSS-X on Chromosorb W 100 metal column prepared in the laboratory. Injector and detector temperatures were 250^{o}C and 300^{o}C respectively. Column temperature was maintained isothermally at 180^{o}C. Carrier gas was nitrogen at a flow rate of 20 ml/min. The peaks were quantitated by electronic integration. Methyl esters were also analyzed (for some experiments) on a H-P FFAP column. (Column specifications are described elsewhere). Carries gas was at a flow rate of 10 ml/min. Other gas chromatographic conditions were similar to that for the EGSS-X column.

2.2.3.4 Identification of fatty acids

Since the component fatty acids of palm oil have been identified and widely reported in the literature, chemical and spectral analysis of individual fatty acids is not reported in this study. Instead, fatty acids were identified by standard methods involved in gas-liquid chromatographic procedures (Litchfield, 1972; Christie, 1982).

A series of standard methyl esters of normal carboxylic acids (10:0, 12:0, 14:0, 16:0, 18:0, 20:0, 18:1, 18:2 and 18:3) were injected and the retention times noted for each standard peak for a set of gas chromatographic conditions. Fatty acids of the unknown sample were identified by direct comparison of the retention times of standards with those of the unknown esters on the same column under identical conditions. Coconut oil and palm oil fatty acid methyl esters (of known composition) were also used as secondary standard mixtures.

Relative retention times were calculated for each fatty acid with respect to 16:0. The relative retention times of the esters separated on EGSS-X and FFAP columns under GC conditions described in section 2.2.3.3. are listed in Table 8. Since these values were found to be comparatively constant and similar to that of literature (Jamieson, 1970; Christie, 1982) they were used for identification of the fatty acids.

Equivalent chain length (ECL) values were also determined to express the elution sequence of the fatty acid methyl esters from the

Table 8 Gas-liquid Chromatographic Retention Time Relationships of Standard Fatty Acid Methyl Esters Used for Identification of Palm Oil Fatty Acids

Fatty acid methyl ester	Column			
	FFAP(a)		EGSS-X(b)	
	Relative Retention time*	ECL**	Relative Retention time*	ECL**
12:0	0.31	12.0	0.26	12.0
14:0	0.55	14.0	0.52	14.0
15:0	0.73	15.0	0.73	15.0
16:0	1.00	16.0	1.00	16.0
18:0	1.90	18.0	1.94	18.0
20:0	2.03	20.0	3.81	20.0
18:1	2.41	18.3	2.23	18.45
18:2	2.97	18.55	2.82	19.10
18:3	3.20	19.80	3.81	20.0

* Relative retention time ($r_{16:0}$) = $\frac{\text{retention time of ester}}{\text{retention time of 16:0}}$

** ECL = Equivalent chain length

GLC conditions: Injector and detector temperatures: 250°C and 300°C respectively; column temperature-isothermal, 180°C; carrier gas-nitrogen, flow rate (a)10ml/min, (b) 20ml/min.

gas chromatographic columns. ECL values were determined from a reference curve obtained by plotting the logarithms of the retention times of known, normal, saturated monocarboxylic fatty acid methyl esters against the number of carbon atoms in the aliphatic chain of each acid. ECL values of unknown sample esters chromatographed under identical operational conditions were then read from the reference curve using observed retention times. FCL values for individual double bonds were approximately additive as seen from the ECL values of 18:1, 18:2 and 18:3 (Table 8 and Figures 8 and 9).

2.2.4. Physico-Chemical Composition of Oil Palm Fruit

Bunch weight, fruit weight and percentages of mesocarp, kernel and shell were determined for oil palm fruits of different stages of development. Determination of moisture and volatile matter and oil content of fresh oil palm fruits and process streams of palm oil mill were by I.U.P.A.C methods 1.121 and 1.122 respectively (Paquot and Hautefenne, 1987).

2.2.5 Estimation of Phospholipids

The colorimetric determination of phosphorus was according to the method of Fiske and Subba Row (1925). This method for determination of phosphorus involves digestion of the phospholipid to liberate the organic phosphorus to free inorganic phosphorus. Inorganic phosphate is

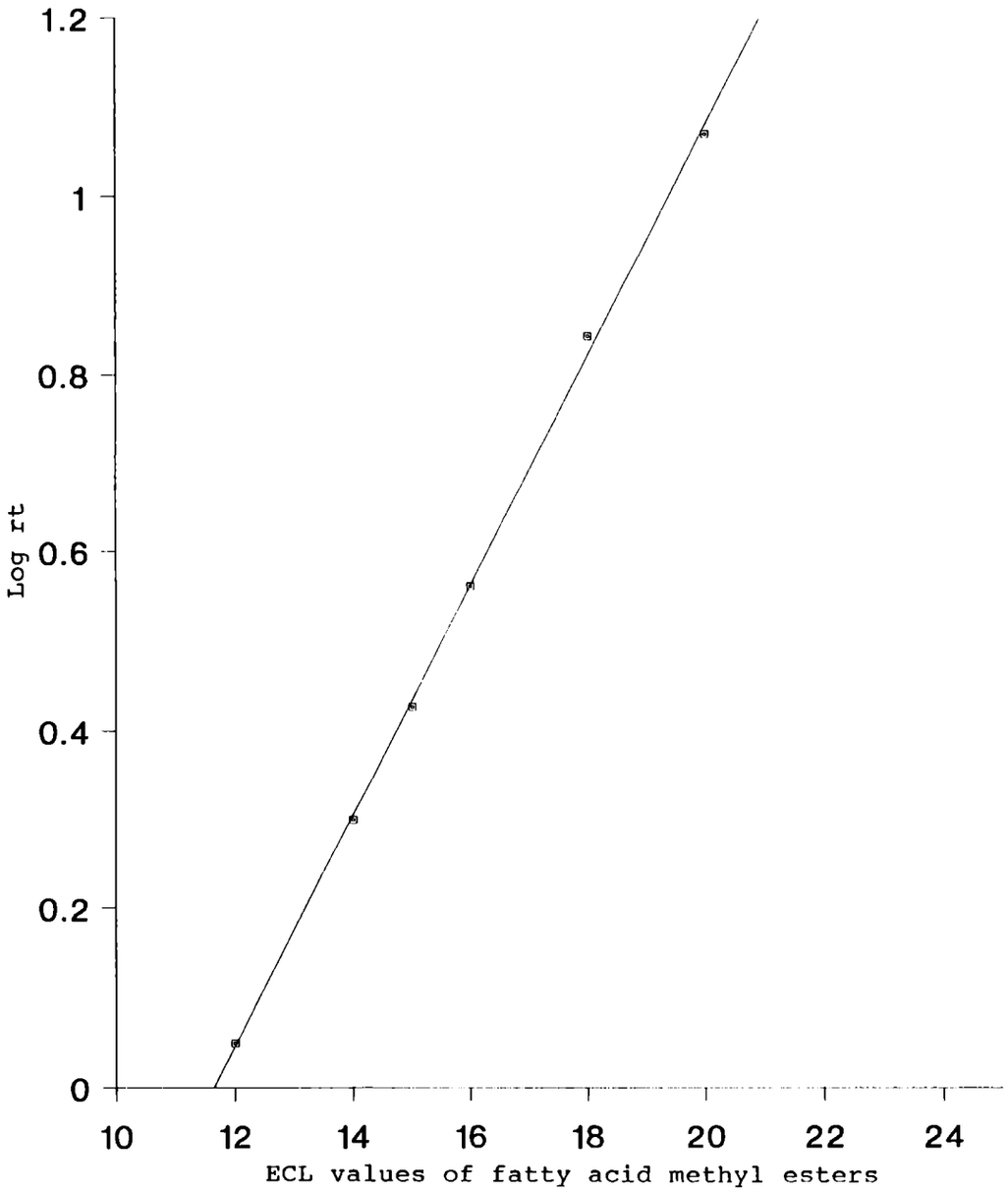


Figure 8. **Identification of fatty acids by GLC from retention time data of standard fatty acid methyl esters on FFAP column (GLC conditions as in Table 8).**

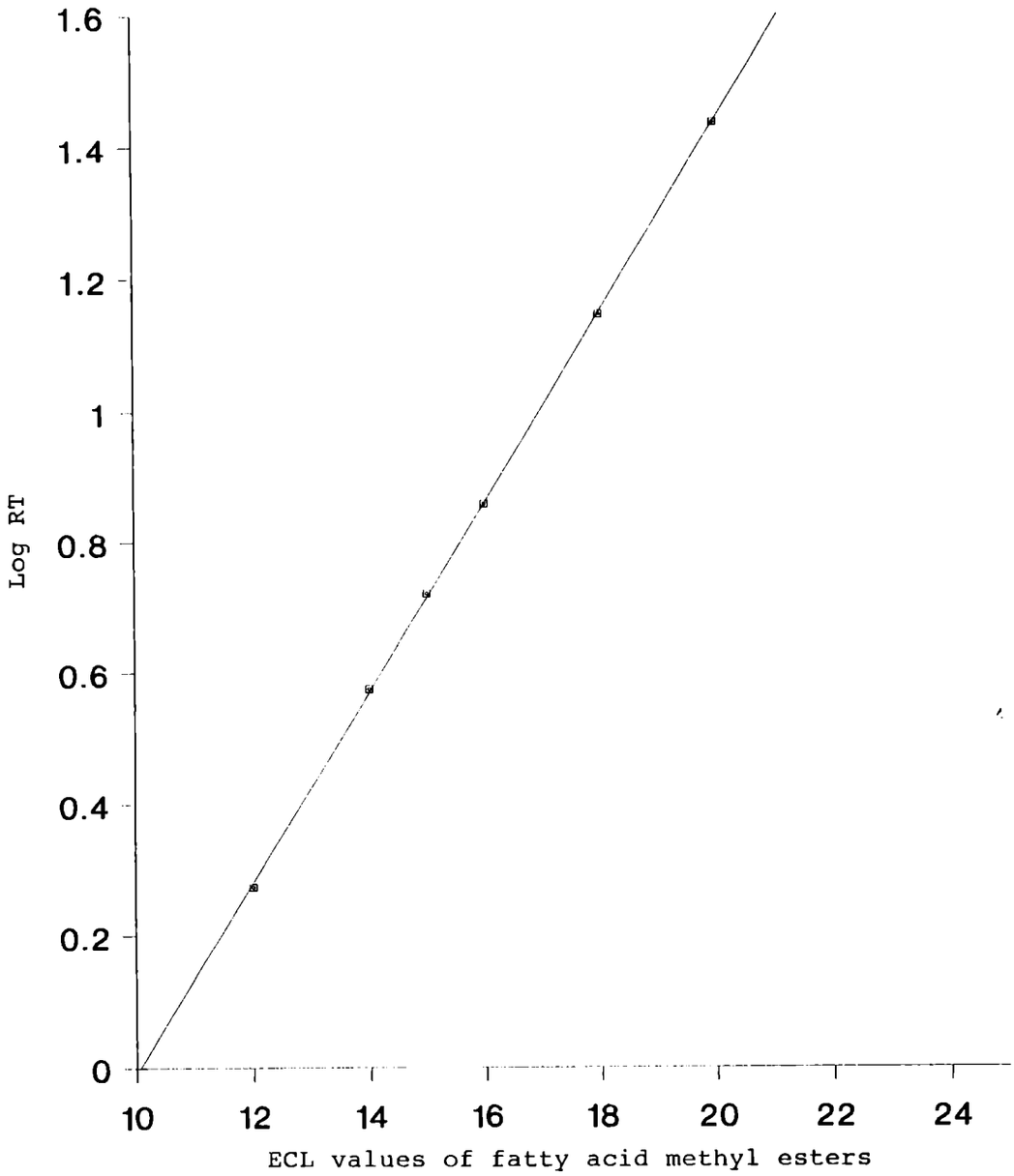


Figure 9. Identification of fatty acids by GLC from retention time data of standard fatty acid methyl esters on EGSS-X column (GLC conditions as in Table 8).

then estimated. Phosphorus reacts with ammonium molybdate to form phosphomolybdic acid which on treatment with ANSA reagent gets reduced. These reduction products, a mixture of lower oxides of molybdenum are determined spectrophotometrically at 660 nm. Molar amounts of phospholipids can be calculated since 1 mole phospholipid contains 1 mole phosphorus.

Reagents

i. 5 N sulfuric acid

ii. 2.5% ammonium molybdate

iii. 2N nitric acid

iv. ANSA reagent: This was made in powdered form and dissolved before use and not used for more than a week since it deteriorated slowly. 0.2g ANSA (1-amino-2-napthol-4-sulfonic acid), 1.2 g of sodium metabisulfite and 1.2 g sodium sulfite were mixed thoroughly. 0.25g of this mixture was weighed and dissolved in 10 ml of distilled water.

v. Standard solution:1.097 g of potassium dihydrogen phosphate was dissolved in 250 ml distilled water. A few drops of chloroform was added and the solution was refrigerated. For use 1 ml of this stock solution was pipetted out and made up to 10 ml in a standard volumetric flask (ie 1 ml = 99.96 µg P). Aliquots of this solution were used to prepare standard solutions of varying phosphate content ranging from 10 µg to 100 µg.

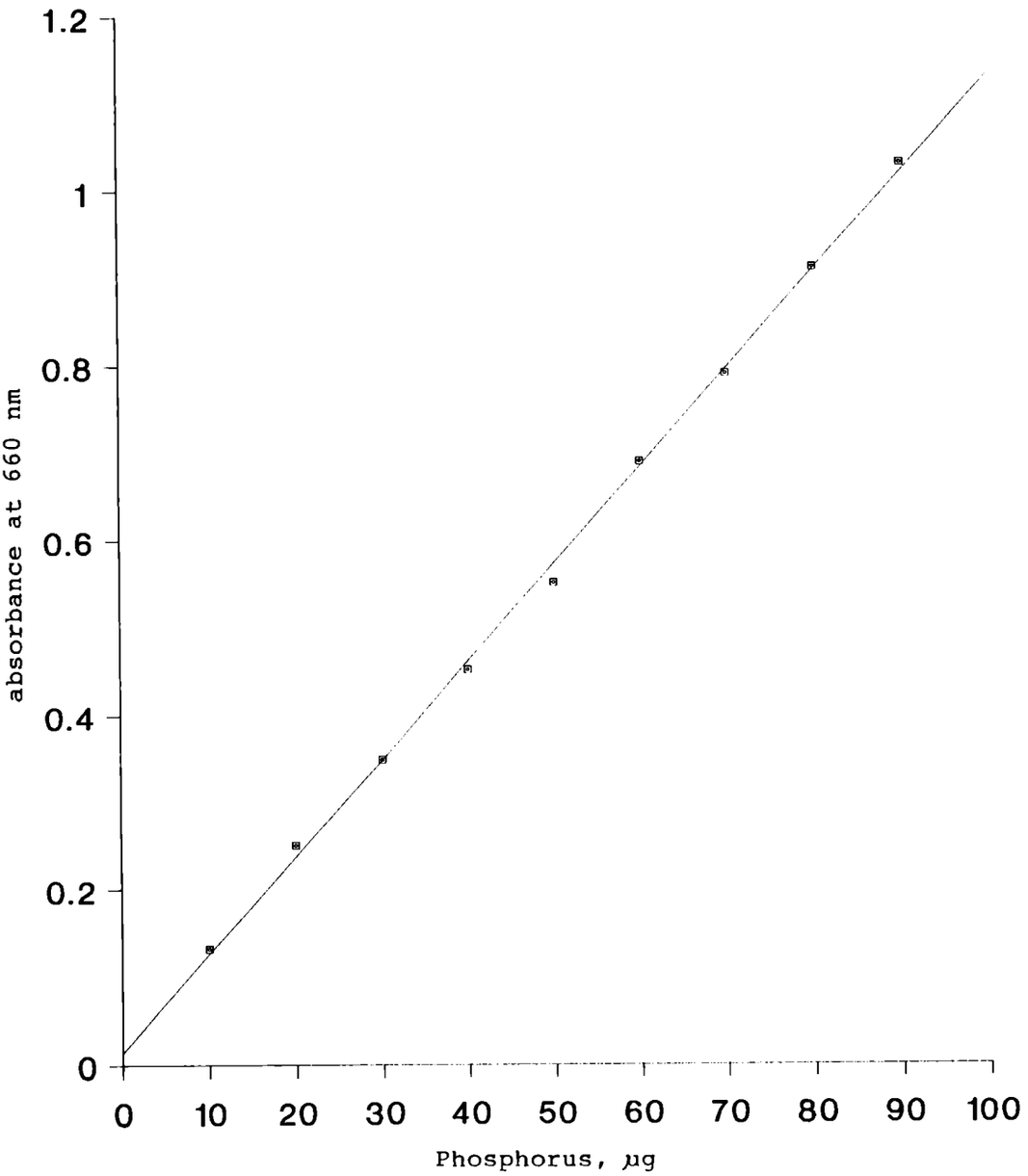


Figure 10. Standard curve for the estimation of phosphorus.

Procedure: A known quantity of lipid (equivalent to 10-100 µg of phosphorus) was weighed into a digestion flask. 1 ml 5N sulfuric acid was added and the mixture heated until digestion was complete and the contents turned brown. The digestion flask was cooled to room temperature. 1 to 2 drops of 2N nitric acid was added and digested once more until the contents became colorless. The digest was cooled. 1 ml of distilled water was added and contents boiled for 5 minutes. The digest was again cooled and quantitatively transferred to a 10 ml standard volumetric flask. 1 ml 2.5% ammonium molybdate followed by 0.1 ml ANSA reagent were added and made up to the mark with distilled water. A blank was also prepared simultaneously in a similar fashion. Color was also developed for the aliquots of standard phosphate solution. Optical density at 660 nm was measured using a Spectronic 20D.

The quantity of phosphorus (P) present was obtained from a plot obtained with standard phosphate solution (Figure 10). From the phosphorus content of the lipid, the total phospholipid content was calculated as 1,2-dipalmitoyl-sn-glycero-3-phosphorylcholine. The phospholipid equivalent factor of 24 was used to convert the elemental P content into total phospholipid, assuming that all analyzed P was derived from phospholipid (Goh *et al*, 1982). The conversion factor for individual phospholipids were calculated from their respective molecular weights since 1 mole P represents 1 mole phospholipid (for cardiolipin, 2 mole P represents 1 mole phospholipid).

2.2.5 Estimation of glycolipid

Glycolipid content may be determined by converting the hexoses to the furfural derivatives in strong acid and reacting them with anthrone, with which they form colored complexes that can be estimated spectrophotometrically. The procedure followed was essentially that of Southgate (1976).

Reagents

i. Anthrone-thiourea reagent (66% by volume) was prepared by adding 660 ml sulfuric acid cautiously with stirring and external cooling to 340 ml distilled water in a large beaker. 10g thiourea and 0.5 g anthrone (9,10-dihydro-9-oxoanthracene) were dissolved in one litre of this acid by slowly warming the mixture to 80-90^{o}C. The reagent was stored in a refrigerator.

ii. Standard glucose solution: 100 mg of glucose was dissolved in distilled water and made upto 1 litre in a standard volumetric flask. Aliquots of this standard solution were taken to give working standards of varying glucose content in the range 10-80 µg/ml.

Procedure: Solvent was evaporated from aliquots of lipid solution containing 10-80 µg sugar (as hexose). 10 ml of anthrone-thiourea reagent was added and swirled to mix the contents. The tubes were stoppered loosely and placed in a boiling water bath for 15 minutes; cooled to room temperature with cold running water for 7

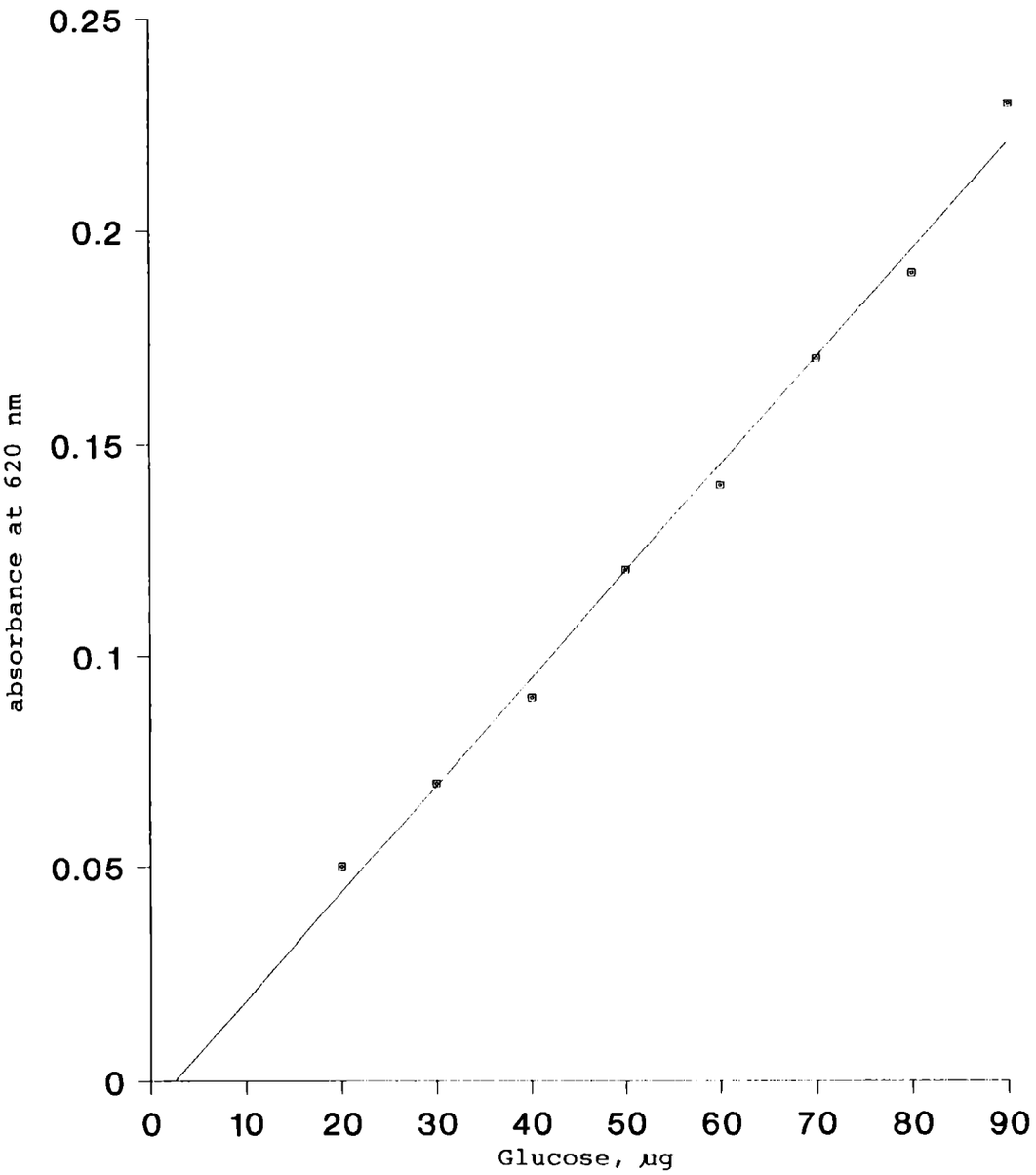


Figure 11. Standard curve for the estimation of glucose.

minutes and left in the dark for 30 minutes. After half an hour, absorbance at 620 nm was measured. A blank was also determined under the same experimental conditions. The quantity of hexose present was obtained from a plot obtained with standard glucose solution (Figure 11). The quantity of glycolipid was calculated as digalactosyldiacylglycerol by multiplying the a ount of hexose by a factor of 2.6, calculated from the molecular weight of 1,2-dipalmitoyl-3-O-(α-D-galactopyranosyl-(1′→6′)-O-β-D-galactopyranosyl)-sn-glycerol.

2.2.7 Estimation of Neutral Lipid Classes

2.2.7.1 Estimation of neutral lipids by oxidative dichromate method

The oxidative dichromate method of Bragdon (1951) is based on the oxidation of a suitable lipid extract by a potassium dichromate-sulfuric acid reagent. The dichromate is reduced by a proportionate amount that can be determined by absorbance measurements.

Reagents

i. Dichromate reagent: 20 g of potassium dichromate was finely powdered in a motar and added slowly to one litre of concentrated sulfuric acid and stirred well till the dichromate was fully dissolved.

ii. Standard lipid solutions: A stock solution of palmitic acid (16:0) was prepared by dissolving 100 mg of 16:0 in 10ml of chloroform.

Standard curve was drawn using different concentrations ranging from 1 to 10 mg of 16:0. Standard stock solutions of triacylglycerol, monoacylglycerol and diacylglycerol were also prepared.

Procedure: Aliquots representing 1 mg to 10 mg of lipid sample were taken in a 25 ml stoppered test tube. 5 ml of dichromate reagent was added to each and the tubes were placed in a boiling water bath for 30 minutes.The tubes were cooled. 20ml of distilled water was added. Optical density was read at 580 nm. Reagent blank was also prepared. Optical density for aliquots of standard 16:0 representing 1 to 10 mg were also determined and a curve was plotted with mg of 16:0 against absorbance (Figure 12). Similarly, standard curves for other acylglycerols were prepared (Figures 13 to 15). The oxidative dichromate method was used to determine the lipid profile of palm oil process streams (Section 2.2.1.3).

2.2.7.2 Estimation of neutral lipids by gas-chromatography

The lipid classes were also quantitated by conversion of the component fatty acids to methyl esters (section 2.2.3.1 and analysis of the fatty acid methyl esters by GLC (section 2.2.3.3) using an internal standard us described by Kates (1986).

An accurately weighted amount of internal standard, methyl pentadecanoate was added to the lipid sample prior to saponification and esterification of the lipid. The fatty acid methyl esters were subjected

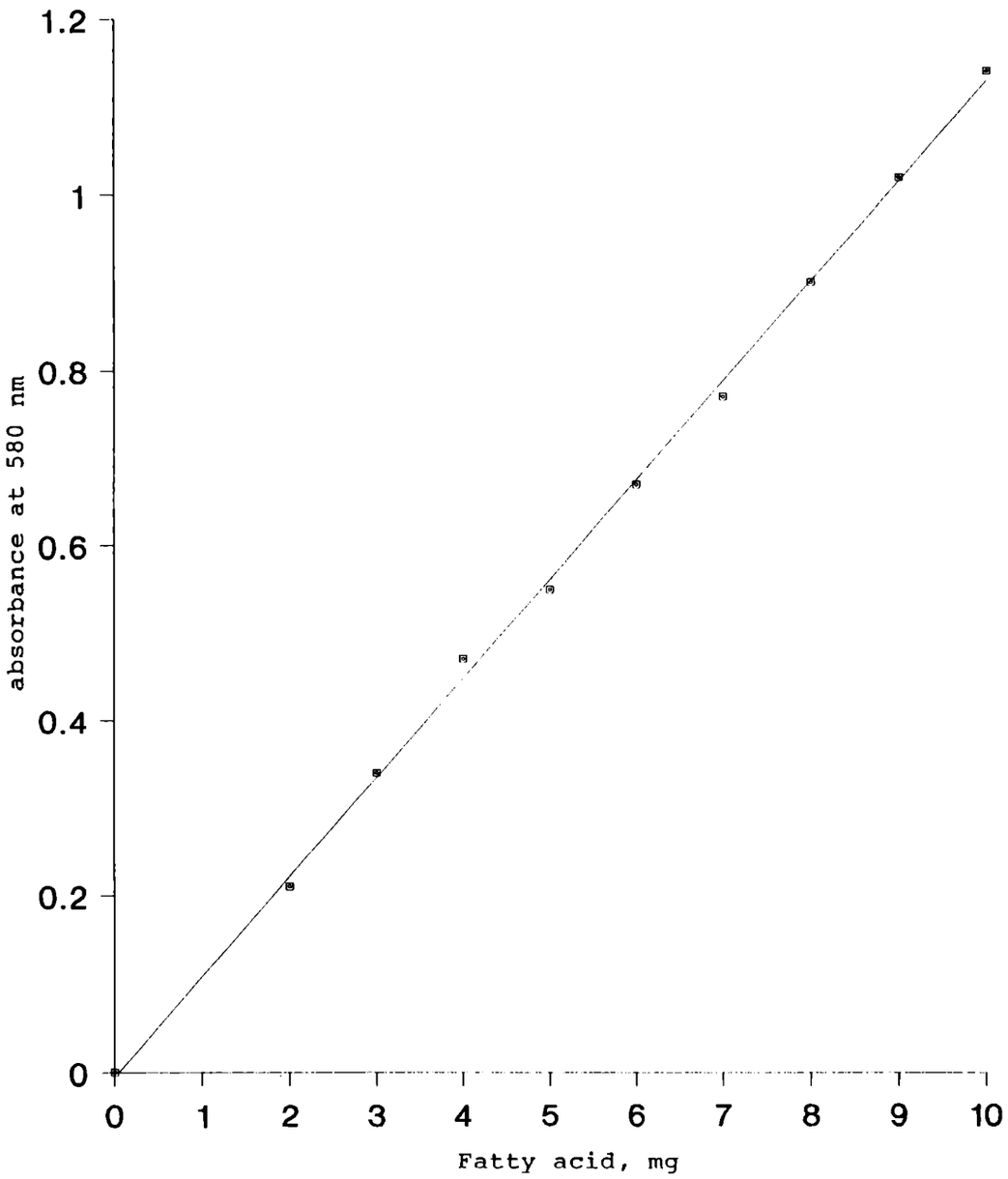


Figure 12. Standard curve for the estimation of fatty acids.

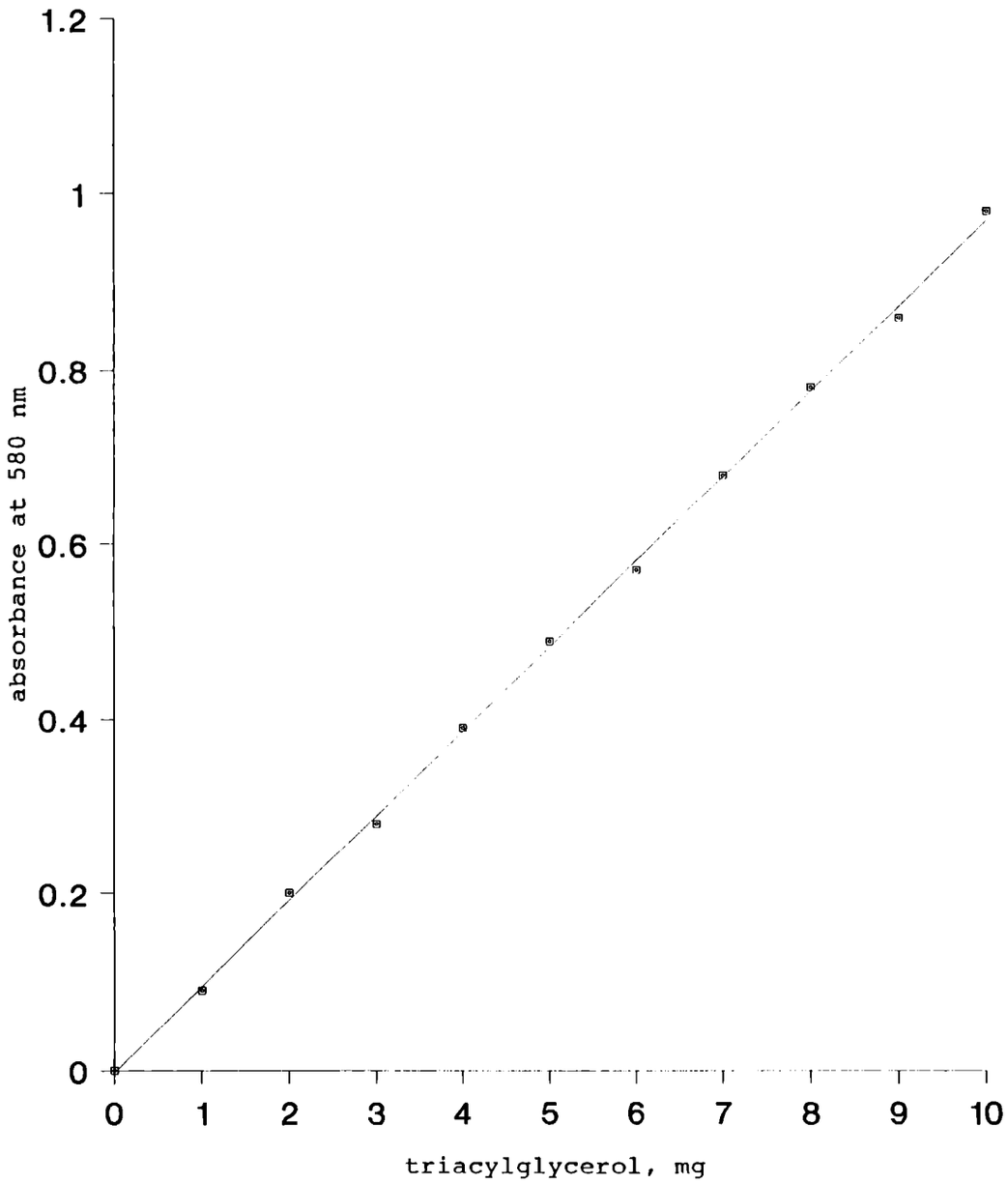


Figure 13. Standard curve for the estimation of triacylglycerols.

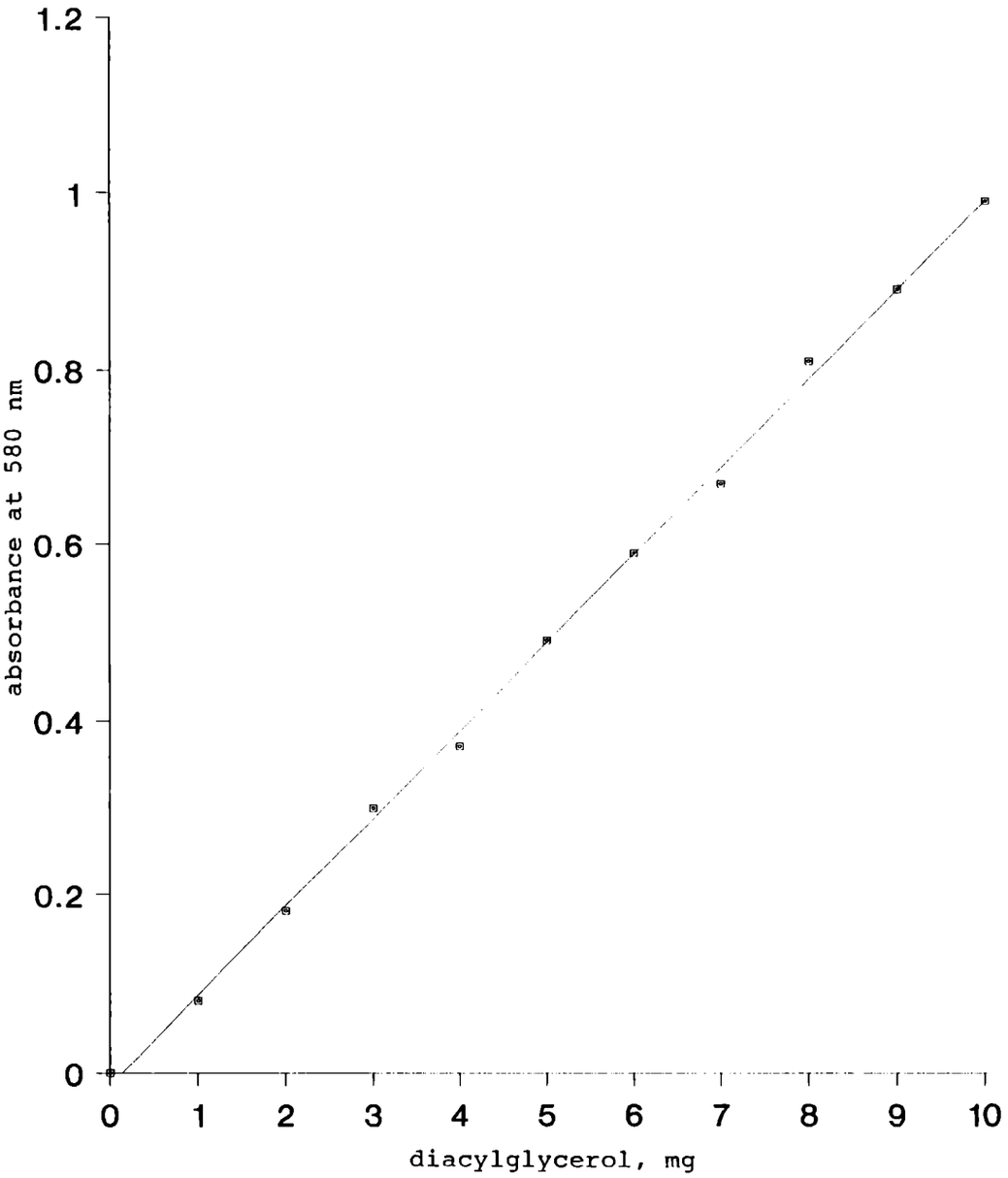


Figure 14. Standard curve for the estimation of diacylglycerols.

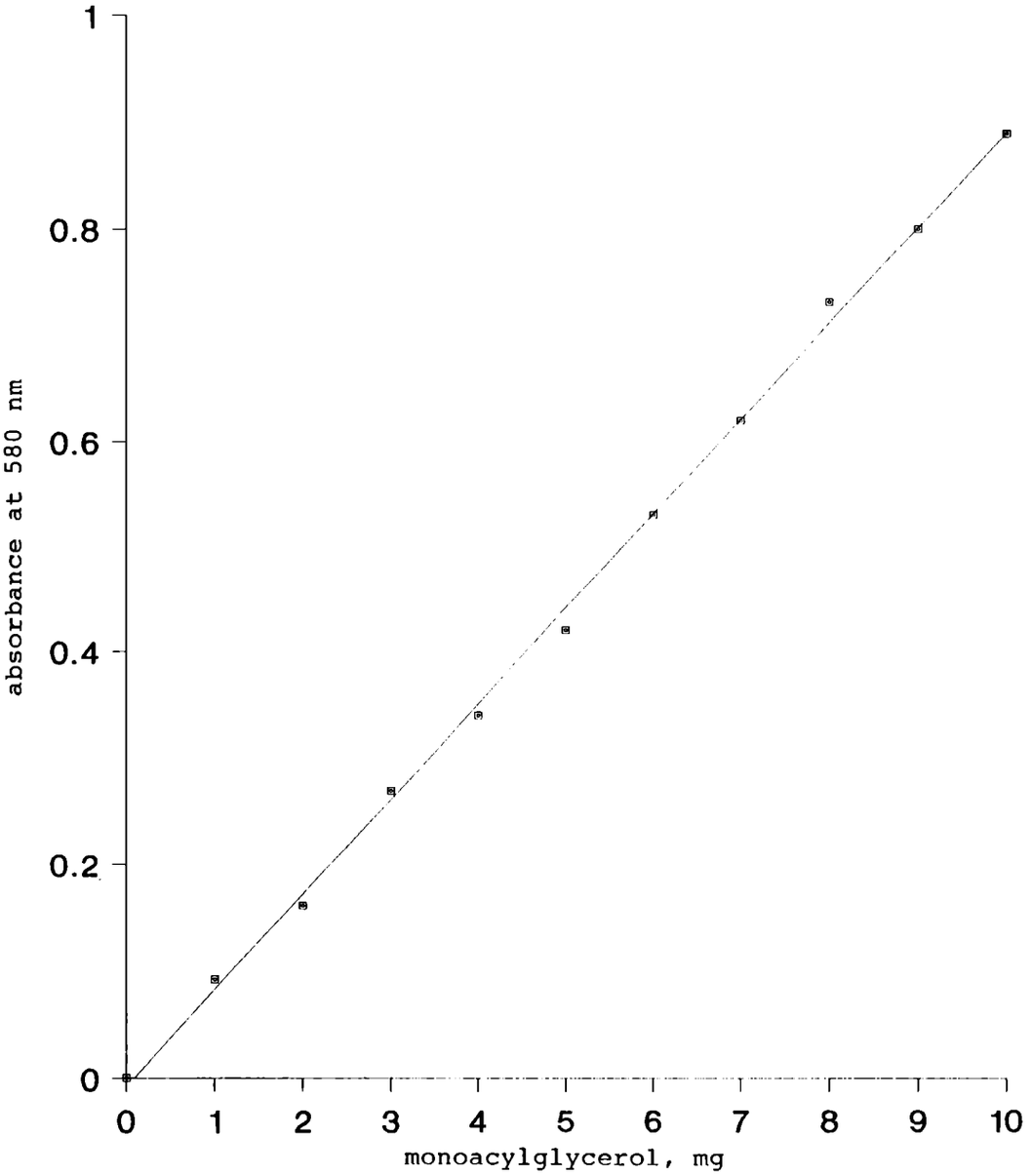


Figure 15. Standard curve for the estimation of monoacylglycerols.

to GLC. The amount of lipid sample was determined by relating the total area of the fatty acid methyl esters peaks to the area of the peak for internal standard. A correction factor was calculated from the molecular weight of the lipid class and the average molecular weight of the fatty acids determined from the fatty acid composition. This correction factor was used to convert the total amount of fatty acid to weight of the corresponding lipid class.

2.2.8 Determination of Positional Distribution of Fatty Acids in the Triacylglycerols

This procedure involves partial hydrolysis of triacylglycerols by crude lipase of mammalian pancreas extracts which exhibits a high degree of specificity for hydrolysis of acyl groups associated with the primary positions of glycerol; separation of the formed monoacylglycerols by thin-layer chromatography followed by methanolysis and gas-liquid chromatographic analysis of the fatty acid methyl esters formed. The semi-micro procedure of Luddy et al (1964) was used for deacylation of triacylglycerols with pancreatic lipase. The modifications recommended by Rossell et al (1985) for hydrolysis of palm oil triacylglycerols were also incorporated.

Reagents

i. 1M tri(hydroxy methyl)methylamine (TRIS) buffer adjusted to pH8.

ii. 22% calcium chloride solution.

iii. 1.0% bile salt solution.

iv. Porcine pancreatic lipase.

v. Freshly distilled diethyl ether.

vi. anhydrous sodium sulfate.

Procedure: 5 mg of triacylglycerols (isolated by thin-layer chromatography of total lipids) were weighed into a 5 ml screw cap vial. 1.0 ml TRIS buffer (at pH8), 0.1 ml 22% calcium chloride solution and 0.25 ml of 1% bile salts solution were added. The contents were warmed in a water bath for 1 minute at 40^{o}C. 5 mg of pancreatic lipase was added. The contents were shaken for 4 min with a vortex cyclo-mixer. At the end of the reaction time, the mixture was immediately transferred to a separatory funnel and extracted thrice with 5 ml portions of diethyl ether. The extracts were combined, washed with distilled water, dried over anhydrous sodium sulfate, filtered and solvent evaporated. Hydrolysis was found to be around 30% under the conditions. The reaction products were immediately isolated by preparative TLC on silica gel G with petroleum ether-diethyl ether-formic acid, 60/40/1.6, (v/v/v) as the developing solvent system. Monoacylglycerol band was isolated from the gel as described (section 2.2.2.1), converted to fatty acid methyl esters and analyzed by gas-liquid chromatography. The positional distribution of fatty acids in the combined 1,3-positions of the triacylglycerols was computed according to the method of Coleman (1964).

i. The % fatty acid X at sn-2-position is given by:

% X at sn-2-position	=	% X in monoacylglycerol produced by hydrolysis with pancreatic lipase

ii. The % fatty acid X at the 1,3-position is given by:

$$\%\ X \text{ at combined 1,3-position} = \frac{3\,[\%\ X \text{ in original triacylglycerol}] - [\%\ X \text{ at 2-position}]}{2}$$

iii. The percent proportion of a fatty acid X at sn-2-position is given by the following equation (Mattson and Volpenhein, 1963):

$$\%\ \text{proportion of X} = \frac{\%\ X \text{ in 2-position}}{3 \times \%\ X \text{ in total triacylglycerol}} \times 100$$

iv. Calculations for the component triacylglycerols are given by the following equations of Vander Wal (1964):

For molecules composed of a single type of acyloxy group A:

$$\%\ AAA = (\%A_1)(\%A_2)(\%A_3)/10{,}000$$

For molecules composed of two acyloxy groups A and one acyloxy group B:

$$\%\ ABA = (\%A_1)(\%B_2)(\%A_3)/10{,}000$$

$$\%\ AAB = (\%A_1)(\%A_2)(\%B_3) \times 2/10{,}000$$

For molecules composed of three different acyloxy groups, A,B and C:

$$\%\ ABC = (\%A_1)(\%B_2)(\%C_3) \times 2/10{,}000$$

$$\%\ ACB = (\%A_1)(\%C_2)(\%B_3) \times 2/10{,}000$$

$$\%\ BAC = (\%B_1)(\%A_2)(\%C_3) \times 2/10{,}000$$

2.2.9 Statistical Analysis

The values given in the tables are the mean values of duplicate determinations of three samples taken for a given experiment. Standard deviation, S of the sample was calculated using the following expression:

$$S = \sqrt{\frac{\Sigma x^2 - (\Sigma x)^2/n}{n-1}}$$

2.2.10 Chemicals

All chemicals used in the study were either Analar grade of BDH, India, GR grade of Merck, India or S.D. Fine Ltd., India. Porcine pancreatic lipase (steapsin) was obtained from Sigma Chem. Co., St. Louis, USA. 2',7'-Dichlorofluorescein was purchased from Fluka, Switzerland. Silica gel G used for TLC was from Merck, India; silica gel H from Astra-IDL, India.

All solvents used in this investigation were either Analar grade of BDH, India or GR grade of Merck, India or S.D. Fine Ltd., India. The solvents used for chromatographic analyses were distilled prior to use.

Lipid standards: The authentic reference compounds, viz., fatty acid methyl esters (10:0, 12:0, 14:0, 16:0, 18:0, 20:0, 18:1, 18:2, 18:3), triacylglycerols (PPP, POP, POO, OOO) and phospholipid

standards were procured from Sigma Chemical Co., St. Louis, U.S.A.

Gas chromatographic phases: The liquid phase, EGSS-X was purchased from Supelco, Inc., PA, U.S.A. The inert support material, Chromosorb W HP 100/120 was procured from Hewlett-Packard, Arondale Division, U.S.A.,

2.2.11. Instruments

The following general laboratory appliances/instruments were used in the study:

A Sumeet mixer/homogenizer (Power Control and Appliances, Bombay) was used for blending and homogenizing purposes.

A 'Prestige' pressure cooker (12 litres capacity) having a steam pressure of 15 psi (equivalent to 120^{o}C) was used for sterilization of fresh fruits before solvent extraction.

All centrifuging operations were performed on a 'Remi' Heavy Duty Centrifuge (Model 570).

pH measurements were made on an 'Elico' (model LT-10T) pH meter with an accuracy of $\pm$ 0.05. The pH meter was calibrated with standard pH solutions (4.0, 7.0 and 9.2).

All heat treatment and activation of chromatography plates were carried out in a 'Memert' (model TV10) air over with thermostatic control supplied by J.T. Jagatiani, National House Appliances, Bombay.

A Spectronic 20D (Milton Roy Company) spectrophotometer was used for all spectrophotometric estimations.

All solvent concentration was done with a Rotary Vacuum Evaporator, Buchi Type, 'Superfit' Model PBU-6, Continental Instruments, Bombay.

Gas-liquid chromatography was performed on a Hewlett-Packard 5840 A model gas chromatograph with in built electronic integrator and equipped with a flame ionization detector (FID)-Hewlett-Packard, Palo Alto, CA, U.S.A.

CHAPTER 3

RESULTS AND DISCUSSION

3.1 LIPID PROFILE OF OIL PALM FRUIT

The oil palm fruit is a drupe with a fleshy, fibrous mesocarp. There are three varieties of oil palm of commercial significance, viz., dura and pisifera are the parental varieties whereas tenera is the hybrid obtained by crossing the parentals (Photograph IV). In modern plantations, tenera variety is grown for palm oil. More details are given in section 3.1.3. Anatomically, the oil palm varieties can be differentiated by the size of their nut. Fruit of dura contains a large nut whereas the pisifera is conspicuous by the absence of it. The hybrid tenera has a higher proportion of mesocarp enclosing a smaller nut. Both mesocarp and kernel are the sites of lipid storage; the former being the larger reserve and source of commercial palm oil, whereas the latter reserve is smaller in proportion and primarily meant to meet the physiological requirement for seed germination. This section deals with

the results related to proximate composition of the mature oil palm fruit and the detailed investigation of the lipid profile of the mesocarp of the same. The distribution of lipids in the anatomically distinct regions of the oil palm fruit of the three varieties are also discussed. The objective of this section is to provide the basic information on the oil palm fruit and its lipid constituents.

3.1.1. Physico-chemical characteristics of oil palm fruit

The physico-chemical characteristics of the oil palm fruit of tenera variety are presented in Table 9 and the anatomical features of the same can be seen in Photograph IV. Oil palm fruit bunches collected for this study were mature, orange-red in color containing 1000-2000 fruits (Photograph II). The average weight of the fresh fruit was 6.63 g. As revealed from the Table, the mesocarp constituted the major share of the fruit (75.2%). The seed, enclosed by the mesocarp comprised 24.8% which was further composed of shell (16.6%) and kernel (8.2%). The fresh mesocarp, the major lipid reserve was largely occupied by oil or lipids (45.2%) in a fibrous matrix with the solubles such as sugar and protein constituting a minor proportion.

The physico-chemical parameters of the oil palm fruit are subject to great variations as they are influenced by variety, age of the palm and agro-climatic conditions. Even within the variety, for instance, tenera, these parameters are found to vary due to hybrid combinations. The mean values obtained for the physico-chemical

Table 9 Physico-chemical Characteristics of Oil Palm Fruit (Tenera variety)

Bunch weight, kg	17.5±1.7
Fruit weight, g	6.63±0.59
Mesocarp, %	75.16±0.08
Kernel, %	8.21±0.29
Shell, %	16.63±0.35
Mesocarp, moisture %	41.44±0.67
Mesocarp, fat, % (dry weight)	77.23±0.90
(fresh weight)	45.23
Kernel, moisture %	14.87±1.60
Kernel, fat, % (dry weight)	46.30±0.95
(fresh weight)	39.42

characteristics are by and large comparable with the range reported for tenera palms (Hartley, 1977; Maycock, 1987). The information recorded here is essentially to provide a basic understanding of the composition of the oil palm fruit bunch and the fruit which is the starting material for the present study.

3.1.2. Lipid Composition of oil palm fruit mesocarp

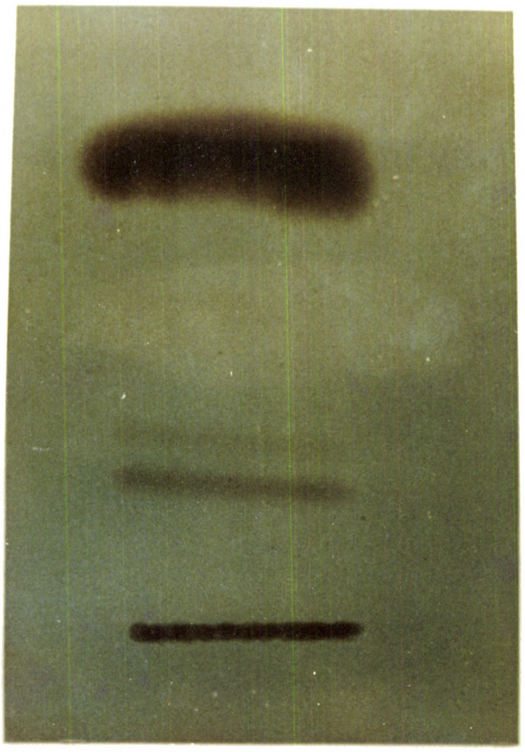
Lipid is the focus of the detailed investigation in this study and therefore further characterization was confined to this constitutent. The fresh mesocarp from mature oil palm fruit was subject to chloroform-methanol extraction following the method of Folch et al (1957) and modified by Goh et al (1982) for oil palm fruits to obtain the total lipids. Thin-layer chromatographic (TLC) technique (Figure 7) was employed to separate the total lipids into the various neutral lipid classes (triacylglycerol, diacylglycerol, monoacylglycerol and free fatty acid) and the polar lipids (Photograph V). The polar lipids thus obtained were further separated into phospholipids and glycolipids. The phospholipids were characterized for their individual components (Photograph VI). As seen in Photographs V and VI and Tables 4 and 5 the various lipid classes mentioned were well resolved. Individual bands were quantified and their concentrations are presented in Table 10. The total lipid was composed almost entirely of neutral lipid (99.17%). The triacylglycerol predominated the neutral lipid (96.07%) with partial glycerides (monoacylglycerol and diacylglycerol) and free fatty acid

LEGENDS TO PHOTOGRAPHS V-VII

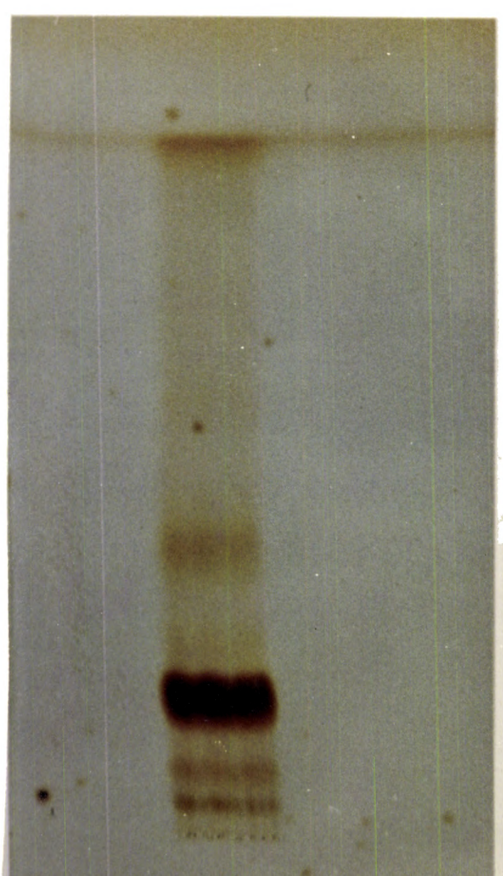
V : Thin-layer chromatographic separation of total lipids of oil palm fruit mesocarp. TLC conditions: adsorbent - 1mm thick silica gel G; sample size - 50 to 100mg; solvent system - petroleum ether - diethyl ether-formic acid (60/40/1.6, v); visualization of spots - exposure to iodine vapor;

VI: Thin-layer chromatography of phospholipids of oil palm fruit mesocarp. TLC conditions - adsorbent -silica gel H, 0.5 mm thick; single development with chloroform - methanol - acetic acid - water (170/25/25/6, v) solvent system; detection - exposure to iodine vapor.

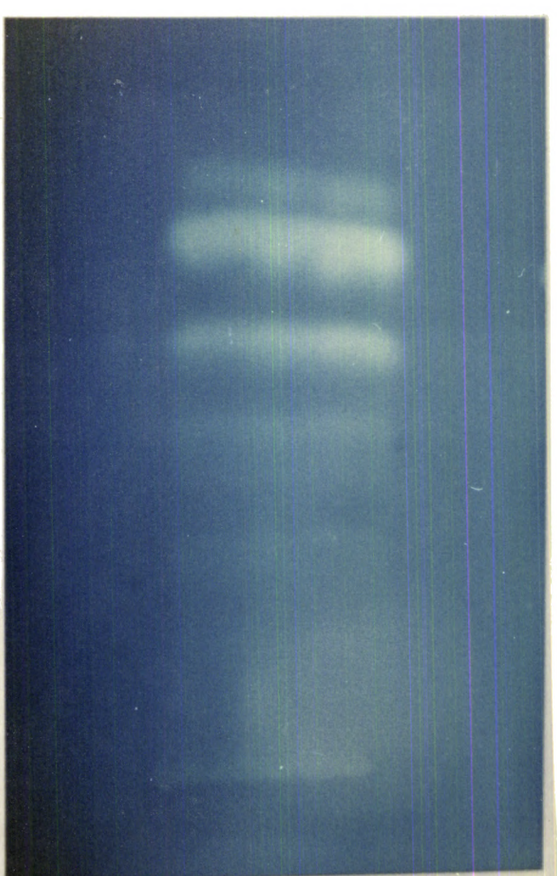
VII : Argentation thin-layer chromatography of triacylglycerols of oil palm fruit mesocarp. TLC conditions: adsorbent -silica gel G impregnated with 10% (w/w) silver nitrate, 0.5 mm thick; sample size - about 1-5 mg; solvent system - benzene -petroleum ether - diethyl ether (90/10/3, v); bands visualized under ultra-violet light after spraying with 2', 7'- dichlorofluorescien solution.



V. Thin-layer chromatographic separation of total lipids of oil palm fruit mesocarp



VI. Thin-layer chromatography of phospholipids of oil palm fruit mesocarp



VII. Argentation thin-layer chromatography of triacylglycerols of oil palm fruit mesocarp

Table 10 Lipid Composition of Oil Palm Fruit Mesocarp

Lipid	g/100g total lipid	g/100g fresh mesocarp
Neutral lipid	99.17	44.85
Triacylglycerol	95.27	48.45
Diacylglycerol	1.91	0.87
Monoacylglycerol	0.50	0.23
Free fatty acid	0.64	0.29
Polar lipid	0.83	0.37
Glycolipid	0.21	0.09
Phospholipid	0.62	0.28
Phospholipid (mole %)		
Phosphatidic acid	2.38	
Phosphatidylcholine	25.40	
Phosphatidylethanolamine	24.60	
Phosphatidylglycerol	12.70	
Diphosphatidylglycerol	3.97	
Phosphatidylinositol	22.22	
Phosphatidylserine/ Lysophosphatidylethanolamine	8.73	

contributing 3.93% of the neutral fraction. The polar lipids constituted a minor fraction of the total lipid and consisted of phospholipid (0.62%) and glycolipid (0.21%). When the total phospholipids were rechromatographed, they were resolved into seven fractions, phosphatidic acid, phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosophatidylglycerol (PG) diphosphatidylglycerol (DPG), phosphatidylinositol (PI) and phosphatidylserine (PS). Most of the phospholipids was contributed by PC, PI and PE (25.4, 22.2 and 24.6 mole % respectively).

As already indicated in the results for total lipid composition of oil palm fruit (Table 10), the total lipid is essentially made up of neutral lipid and that in turn is mostly contributed by triacylglycerol (which forms 95.3% of the total lipid). The total lipid of the oil palm fruit mesocarp, essentially triacylglycerol, is otherwise commonly known as palm oil. The concentrations of the neutral lipid classes generally indicate the soundness of the oil palm fruit. The presence of an extremely active lipase in the mesocarp and its activation due to bruising and aging of the fruit and improper process conditions, results in the alteration of the neutral lipid composition essentially by the hydrolytic action of the lipase on the triacylglycerol (Jacobsberg, 1983; Eng and Tat, 1985; Mohankumar *et al*, 1990). It has been reported that lipase is bound to the oil globule membrane and normally does not have access to the substrate triacylglycerol in a sound fruit. (Mohankumar *et al*, 1990; Mohankumar, 1992). Disruption of this membrane occurs due to aging, bruising of the

fruit while harvesting and improper process conditions and the free fatty acid generated and other hydrolytic products like diacylglycerol and monoacylglycerol consequent to this would depend on the extent of damage to the membrane. The free fatty acid under favorable conditions can be as high as 40% (Olie and Tjeng, 1974) with a corresponding decrease in triacylglycerols. Since free fatty acid is an index for the quality of the oil, in the commercial practise of palm oil extraction, care is taken to avoid damage to fruit by harvesting and transport and to ensure the complete destruction of the lipase by steam treatment of the fresh fruit before further processing (Berger, 1983; Eng and Tat, 1985; Arumughan et al, 1989). The total lipid obtained here was from oil palm fruits of right maturity and without any damage. The destruction of the lipase was ensured by heat treatment and therefore the neutral lipid composition presented here represented the actual values for sound oil palm fruits. The small amount of diacylglycerol and free fatty acid could be attributed to the metabolic intermediates of triacylglycerol biosynthesis. The polar lipids viz., phospholipid and glycolipid are the structural lipids of the cellular membranes, particularly of the fat globules in the case of oil palm fruit, which comprise of about 45% oil by fresh weight of the mesocarp. (Goh et al, 1985; Jacobsberg, 1988) Further detailed analysis of the phospholipids indicated the complexity of the polar fractions.

According to Jacobsberg and Ho (1976), Jacobsberg (1983) and Goh and Timms (1985) fresh, ripe, unbruised fruit of oil palm with a free fatty acid content below 0.1% consists almost entirely of

triacylglycerols (98%) with lower levels of diacylglycerols. They have found diacylglycerol level of 2.3% (equivalent to free fatty acid level of approximately 2.5%) to be rather high. Goh and Timms (1985) have concluded that the diacylglycerols present in fresh oil palm fruits are formed not by hydrolysis of the triacylglycerol but as a residual byproduct of the biosynthesis of triacylglycerol. Bafor and Osagie (1986) have not detected the presence of diacylglycerols, monoacylglycerols or free fatty acids in mature fruits. The high values for these lipid classes and low value of 78% for triacylglycerols, obtained by Oo et al, (1986) could be attributed to the hydrolysis of the triacylglycerols prior to extraction of the lipids from the fruit tissue.

The results obtained for the lipid profile of oil palm fruit mesocarp in this investigation are comparable with the results of Jacobsberg and Ho (1976) and Jacobsberg (1983) indicating that the lipids were extracted from sound oil palm fruits of correct maturity.

However, there are several reports on the composition (lipid class profile) of commercial grade palm oil. Commerical grade palm oil has a lower triacylglycerol content ranging from 88% (Berger, 1975, 1979) to 93% (D'Alonzo et al, 1982), higher free fatty acid ranging from 0.1% (D'Alonzo et al, 1982) to 3.2 (Berger, 1975, 1979) and diacylglycerol (5.8% (D'Alonzo et al, 1982) to 7.7% (Berger, 1975, 1979)) content than the fruit lipids. Higher levels of partial glycerides and free fatty acids in commercial palm oil could be attributed to enzymatic hydrolysis or deterioration during extraction and processing (Jacobsberg, 1983).

Khor et al (1980) have estimated 96.2% neutral lipid, 1.4% glycolipid and 2.4% phospholipid in oil palm fruits from Malaysia. Goh et al (1982) have reported 1000-2000 ppm phospholipid in solvent extracted palm oil from fresh fruits. Gee et al (1985) have estimated the glycolipid content as 1000-3000 ppm. The high values obtained by Khor et al (1980) was attributed to their method of extraction of the lipids from the oil palm fruit. The phospholipid and glycolipid content presented in this investigation for mature oil palm fruits are within the range reported by Goh et al (1982) and Gee et al (1985) respectively. The results reported in this study for the major phospholipid class are similar to the values of Goh et al (1982), Bafor and Osagie (1988b) and Kulkarni et al (1988, 1991) with small variations for the minor phospholipid classes.

3.1.2.1. Fatty acid composition of the lipid classes of oil palm fruit mesocarp

The various lipid classes were analyzed for their fatty acid compositions. The methyl esters of the fatty acid components of the lipid classes were prepared following the I.U.P.A.C method and separated on a HP-5840A model gas chromatograph with an electronic integrator (Section 2.2.3.). The relative proportion of the fatty acids of the individual lipid classes is presented in Table 11.

The various fatty acids quantitated by GLC are lauric acid (12:0), myristic acid (14:0), palmitic acid (16:0), stearic acid (18.0),

Table 11 Fatty Acid Composition of Oil Palm Fruit Lipid Classes

Lipid class	Fatty acid (wt %)							
	12:0	14:0	16:0	18:0	18:1	18:2	18:3	S/U
Neutral lipid	0.10	1.00	43.20	5.00	38.50	11.80	0.40	49/51
Triacylglycerol	0.59	1.75	42.56	4.08	41.71	8.96	0.35	49/51
Diacylglycerol	1.31	1.57	32.32	8.97	39.90	15.16	0.76	44/56
Monoacylglycerol	3.55	5.67	36.88	8.46	35.82	8.77	0.86	55/45
Free fatty acid	4.79	3.77	39.85	9.60	34.32	7.01	0.65	58/42
Phospholipid	0.34	1.37	32.60	2.81	38.22	23.19	1.47	37/63
Glycolipid	2.40	4.05	27.63	8.36	37.65	10.67	9.14	43/57
Total lipid	-	1.1	43.40	4.30	38.00	12.40	0.80	49/51

S/U =Saturated fatty acids/unsaturated fatty acids.

oleic acid (18:1), linoleic acid (18:2) and linolenic acid (18:3). It could be noted from the Table that oil palm fruit lipids had a simple fatty acid profile with a predominance of only three fatty acids, namely 16:0, 18:1 and 18:2 contributing more than 90%. Although these three fatty acids were responsible for the major share in all lipid classes, they were distributed unevenly among the individual classes as seen from Table 11. Of the neutral lipid fractions, the triacylglycerols had a similar pattern of distribution of the major as well as minor acids to that of the total lipids. The triacylglycerols, the most abundant of the lipid classes contained the major acids, 16:0, 18:1 and 18:2 in the proportion 42.56, 41.71 and 8.96% respectively with a total saturated/unsaturated ratio of 49/51. Of the neutral lipids, the diacylglycerols had a higher proportion of 18:2 and corresponding lower levels of 16:0. The other neutral lipids, viz., monoacylglycerols and free fatty acids contained a greater proportion of saturated acids and correspondingly lower proportion of unsaturated fatty acids. The differences in the fatty acid distribution profile between the neutral lipids and the polar lipid were more striking with higher proportion of saturated fatty acids associated with the former and higher level of unsaturated fatty acids in the latter with reference to the total lipids. Among the polar lipids, a further association of fatty acids could be seen, with glycolipids having exceedingly high level of 18:3 and phospholipids with greater proportion of 18:2. In contrast 16:0 was associated with the neutral lipids.

The various classes of lipids of the oil palm fruit as presented here contain various amounts of different fatty acids which are characteristic of a fat of particular origin. 16:0, 18:1 and 18:2 contributed more than 90% of the total acids of each lipid class. This means that palm oil essentially has a simple fatty acid profile as compared to other edible oils (Sonntag, 1979b; Padley et al, 1986). The fatty acid composition of each lipid class particularly between the neutral lipids, phospholipids and glycolipids exhibited differences only in terms of quantity. The salient features of the fatty acid composition of the lipid classes is the predominance of 16:0 and 18:1 and the association of 16:0, 18:2 and 18:3 with lipid class. While 16:0 tends to concentrate in the neutral lipids, 18:2 and 18:3 prefer phospholipids and glycolipids respectively. This is particularly significant for 18:3 which is found in trace amounts in the total lipids (0.8%) whereas its relative concentration is extremely high in the glycolipids (9.14%) showing a ten fold increase in this lipid class.

The fatty acid composition of commercial palm oil has been widely reported. However, there are only a few reports on the fatty acid composition of the total lipids and of the various lipid classes extracted with solvent from fresh oil palm fruits. Available studies on the fatty acid composition of the total lipids from fresh fruits reported in the course of fruit development studies by Crombie and Hardman (1958) and Bafor and Osagie (1986) indicate the similarity in fatty acid composition of total lipids from fresh fruits to that of commercial palm oil.

In general, fatty acid composition of palm oil falls within a narrow range with only minor differences due to geographical origin (Rossell et al, 1983, 1985), fruit varieties (Jacobsberg, 1975; Ng et al, 1976), variations within bunches and between palms (Ng et al, 1976; Rajanaidu and Tan, 1983), processing conditions such as refining, bleaching and deodorization (Chin and Tan, 1977; Arumughan et al, 1985; Chow et al, 1987). The fatty acid composition ranges for typical commercial samples of bonafide palm oil have been published. (Chin, 1979; Tan and Oh, 1981; Chin et al, 1982; Tan et al, 1983a, 1983b). Fatty acid composition of the total lipids of oil palm fruits reported here are within these ranges.

Fatty acid composition of the triacylglycerol class of palm oil have been reported by Jurriens et al (1964), Jurriens and Kroesen (1965), Naudet and Faulkner (1975), Berger et al (1978), Oo et al (1986) and Vella (1988). Okiy et al (1978) have reported the fatty acid composition of diacylglycerol, monoacylglycerol and free fatty acid classes of palm oil. Oo et al (1986) have investigated the changes in fatty acid profile of the lipid classes of developing oil palm fruit. The monoacylglycerol and free fatty acid classes were characterized by a higher level of saturated fatty acid and lower contents of 18:1 and 18:2 than the triacylglycerol classes. The fatty acid profile for the triacylglycerols, partial glycerides and free fatty acid classes presented here indicate a similar trend to the reported values.

According to Goh et al (1982), the phospholipids of palm oil were characterized by a high content of 18:2 while the fatty acid

composition of the glycolipids indicated a significantly high content of 18:3. Bafor and Osagie (1986) have also observed a similar trend for phospholipids and glycolipids of oil from African dura variety. The fact that phospholipids and glycolipid fractions from mature seeds have different overall fatty acid profiles from those of the corresponding triacylglycerol fractions have been reported in the literature for a number of oilseeds. Fatty acids are known to exhibit clear-cut tendencies to associate with specific lipids; glycolipids contain higher levels of 18:3 than the phospholipids from the same source which themselves possess high levels of 18:2 (Hitchock and Nichols, 1971; Harwood, 1980; Christie, 1987). Fatty acid composition of the phospholipid and glycolipid fraction of oil palm fruit mesocarp reported here are similar to the values given by Goh *et al* (1982). The association of 18:2 with phospholipids and 18:3 with glycolipids is also evident. On the other hand, 16:0 is seen to be associated with the triacylglycerols.

3.1.2.2. Positional distribution of fatty acids in the triacylglycerols of oil palm fruit mesocarp

The most abandant class of oil palm fruit lipid is the triacylglycerols and the functional properties of palm oil are largely due to the overall fatty acid composition as well as the distribution of the fatty acids between the positions in the triacylglycerol molecules (Deffense, 1985; Duns, 1985; Pease, 1985). To understand the positional

distribution profile of the fatty acids, the triacylglycerol fraction was isolated by TLC and subjected to pancreatic lipase hydrolysis (Section 2.2.8). The positional specificity of porcine pancreatic lipase is well known (Litchfield, 1972; Christie, 1982) and therefore its property was taken advantage of to distinguish the fatty acids associated with the primary and the secondary hydroxyl groups of the triacylglycerol molecule (Figure 5). Care was taken to restrict the hydrolysis to avoid acyl migration and subsequent non-random hydrolysis. The products of the lipolytic action on the triacylglycerols were isolated by TLC and the fatty acid composition of the monoacylglycerols were determined. The distribution profile was computed following the method of Coleman (1964). The results obtained thus are given in Table 12. There was a remarkable position specificity for the major fatty acids, viz., 16:0, 18:1 and 18:2. 16:0 was predominantly esterfied to the primary hydroxyl groups of the glycerol molecule, i.e., sn-1 and sn-3-positions (to the extent of 83.2%). 18:1 on the other hand, showed a preference for the secondary hydroxyl group (sn-2-position) to the tune of 52.1%. A more even distribution among the three positions of the triacylglycerols was observed for 18:2. The overall position specificity could also be distinguished in terms of saturated versus unsaturated fatty acids, the saturated being confined to the 1,3- positions in contrast to the preference of sn-2-position for unsaturated fatty acids.

The total triacylglycerols obtained by TLC of the total lipids of the oil palm fruit mesocarp were rechromatographed on silica gel impregnated with 10% silver nitrate (Ag^{+}TLC). Triacylglycerols were

Table 12 Positional Distribution of Fatty Acids in the Triacylglycerols of Oil Palm Fruit Mesocarp Determined by Lipase Hydrolysis

Fatty acid	Fatty acid position	Mole %	% proportion*
12:0	total	0.78	
	sn-2-	1.20	51.3
	**1,3-	0.57	
14:0	total	2.04	
	sn-2-	1.98	32.4
	1,3-	2.07	
16:0	total	44.53	
	sn-2-	22.38	16.8
	1,3-	55.61	
18:0	total	3.87	
	sn-2-	2.59	22.3
	1,3-	4.51	
18:1	total	39.81	
	sn-2-	62.27	52.1
	1,3-	28.58	
18:2	total	8.62	
	sn-2-	9.16	35.4
	1,3-	8.35	
18:3	total	0.34	
	sn-2-	0.42	41.17
	1,3-	0.30	

** calculated from the method of Colman (1964)

* Proportion at 2-position = w/3Tx100, where w is mole percent of the acid in the monoacylglycerol and T is the mole percent of the same acid in the original triacylglycerol (Mattson and Volephein, 1963). Hence, proportion at 1,3-positions = 100-proportion at 2-position.

separated on the basis of their total unsaturation. (Photograph VII; Tables 6 and 7). The total triacylglycerols of oil palm fruit mesocarp separated into seven bands or fractions under the chromatographic conditions described. According to the R_f values of standard triacylglycerols co-chromatographed with the sample, five fractions or triacylglycerol classes were identified and isolated. The first and second fractions were termed "saturated" and "monoene" triacylglycerols respectively. The third and fourth fractions were taken as "diene" triacylglycerols and the fifth fraction was called "triene" triacylglycerols. The remaining less mobile triacylglycerols were taken as "polyene". Each triacylglycerol class was quantitated and its fatty acid composition determined by GLC. Positional analysis of the triacylglycerol classes obtained by Ag^+TLC was also carried out following the lipase hydrolysis procedure as done for the total triacylglycerols. The fatty acid distribution profile among the triacylglycerol class is given in Table 13. As can be seen in the Table the saturated triacylglycerol class was mostly composed of 16:0 and 18:0 with minor concentration of 14:0 and also 18:1 to the extent of 3.49% as contaminant. In this fraction (accounting for 10.82%), 16:0, contributing 83.5%, was found to be evenly distributed among the sn-2- and 1,3-positions of the triacylglycerols. The largest class of triacylglycerols ie., monoene (32.3%) comprised of only two fatty acids namely, 16:0 and 18:1. In this class 16:0 predominantly occupied the 1,3-positions to the extent of 90.6%, whereas 18:1 was confined to the sn-2-position. In the case of diene triacylglycerols, which comprise the

Table 13 Fractionation of Total Triacylglycerols of Oil Palm Fruit by Argentation Thin-layer Chromatography. Mole % and fatty acid composition of the triacylglycerol classes. Positional distribution of the fatty acids at the sn-2-and 1,3-positions of the triacylglycerol classes obtained by lipase hydrolysis.

Triacylglycerol class	Triacyl-lycerol class mole %	Position of fatty acid	Fatty acid (mole %) 12:0	14:0	16:0	18:0	18:1	18:2	18:3
Saturated	10.82	total	0.99	4.89	83.53	7.09	3.49		
		sn-2	1.90	3.67	80.90	12.58	0.95		
			(64.0)	(25.0)	(32.3)	(59.1)	(9.1)		
		1,3-	0.54	5.5	84.85	4.35	4.76		
Monoene	32.32	total	0.48	2.73	58.99	3.56	34.24		
		sn-2	0.81	1.41	16.67	1.85	79.25		
			(56.3)	(17.2)	(9.4)	(17.3)	(77.2)		
		1,3-	0.32	3.39	80.15	4.42	11.74		
Diene	30.84	total	0.96	1.42	33.23	1.24	57.36	5.79	
		sn-2	1.88	1.28	15.63	1.39	71.94	7.88	
			(65.3)	(30.0)	(15.7)	(37.4)	(41.8)	(45.4)	
		1,3-	(0.50)	1.49	42.03	1.17	50.07	4.75	
Triene	11.19	total	0.48	2.53	22.19	5.49	42.80	26.51	
		sn-2	0.73	1.27	17.00	3.91	43.46	33.63	
			(50.69)	(16.73)	(25.54)	(23.74)	(33.85)	42.29	
		1,3-	0.36	3.16	24.79	6.28	42.47	22.95	
Polyene	14.83	total	0.58	1.75	26.00	2.42	28.32	39.39	1.54
		sn-2	1.05	2.05	15.87	1.48	24.64	52.36	2.54
			(60.3)	(39.0)	(20.3)	(20.4)	(29.0)	(44.3)	(55.0)
		1,3-	0.35	1.60	31.07	2.89	30.16	32.91	1.04

Figures in parenthesis represent relative abundance of a fatty acid at the sn-2 position. % proportion of a fatty acid at sn-2 position is calculated from w/3T x 100, where w is the mole % of the acid in the monoacylglycerol and T is the mole % of the same acid in the original triacylglycerol (Mattson and Volpenhein, 1963).

other major triacylglycerol class (30.84%), these were again composed of 16:0 and 18:1. While 16:0 showed an affinity for 1,3-positions, 18:1 preferred the sn-2-position, but to a lesser extent than the monoene. The major fatty acids of the triene and polyene triacylglycerols were 16:0, 18:1 and 18:2. Their distribution profiles were also similar, with 16:0 showing preference for 1,3-position and 18:1 and 18:2 occupying the sn-2-position, again to a lesser extent when compared to monoene, particularly in the case of 18:1 which showed an almost even distribution in these two classes.

Since the pattern of distribution of fatty acid in the triacylglycerol molecule is known to be correlated with the functional properties of fats (Formo et al, 1979; Manganaro et al, 1981; Wada and Koitumi, 1983; Pease, 1985; Neff et al, 1992), several fats have been analyzed for their fatty acid distribution profiles (Litchfield, 1972). The most well documented fat in this context is cocoa butter (Sonntag, 1979b; Padley et al, 1986). Its sharp melting point and related properties qualify cocoa butter as the best known confectionery fat. Like palm oil, cocoa butter also contains only a few fatty acids (18:0, 16:0 and 18:1) and the physical properties of cocoa butter is primarly due to the positioning of these fatty acids viz., 18:0 and 16:0 in the 1,3-positions and 18:1 in the sn-2-position (StOP, 1-3-rac-palmitoyl-stearoyl-2-oleoylglycerol). The distribution of fatty acids in the oil palm fruit triacylglycerols as presented here indicated the specificity of 16:0 to the 1,3-positions, giving rise to 19% of the triacylglycerol species POP (Table 14).

A few workers have attempted to fractionate palm oil to obtain a fraction rich in POP that could be used as a cocoa butter equivalent or substituent with comparable properties. Accordingly, Bernardini (1977), and Berger (1977) could obtain a fraction containing c.a. 70% SOS. The positional analysis of fatty acid of the triacylglycerols of palm oil derived from various geographic origins (Jurrens et al, 1964; Jurriens and Kroesen, 1985; Rossel et al, 1983, 1985) and fruit varieties (Jacobsberg, 1975) have been reported. The positional distribution of fatty acids between the sn-2-and 1,3-positions does not seen to vary under these conditions. The results obtained in the present study also are generally comparable with those values reported so far.

Recently, the positional specificity of the fatty acid in the triacylglycerol molecule has been correlated with nutritional consequences. Fatemi and Hammond, (1977a, 1977b; Myher et al, (1977) and Manganaro et al, (1981) have demostrated the positive correlation of atherogencity of peanut oil and the predominance of unsaturated fatty acids in the sn-1,3-position and a negative correlation with the saturated fatty acids in the sn-1, 3-position. The reverse was found to be true for the unsaturated fatty acids. On extrapolation of these findings to the positional analysis of the triacylglycerol of oil palm fruit lipids presented here, palm oil in expected to be less atherogenic as 16:0 in mostly occupying the 1,3-positions and 18:1 the sn-2-position. The hypocholesterolemic effect of palm oil in human volunteers has been recently shown (Elson, 1992).

The triacylglycerol composition based on the 1,3-random-2-random hypothesis of Vander Wal (1960) and calculated from the lipolysis

data (presented in Table 12) is shown in Table 14. The triacylglycerol composition as estimated by Vander Wals method (1964) was 11.07%, 34.68%, 31.25%, 16.55% and 6.44% for saturated, monoene, diene, triene and polyene triacylglycerols. POP or 1,3-dipalmitoyl-2-oleoylglycerol (19.26%) and PPO or 1,3-rac-palmitoyl-oleoyl-2-palmitoylglycerol (7.11%) dominated the monoene triacylglycerol class, whereas POO or 1,3-rac-palmitoyl-oleoyl-2-oleoylglycerol (19.79%) was the predominant triacylglycerol species in the diene fraction. The triene triacylglycerols comprised mostly of 5.09% triolein (OOO) and 1,3-rac-palmitoyl-linoleoyl-2-oleoylglycerol (POL), 5.78%.

A complex mixture of triacylglycerols can be resolved into sub-classes based on their degree of unsaturation and molecular weight (Litchfield, 1972; Christie, 1982; Kates, 1986). Triacylglycerol composition can be determined from fatty acid composition data of these triacylglycerol classes followed by the analysis of positional distribution of the fatty acids in the triacylglycerol. In the present study, the relative abundance of triacylglycerol species was estimated combining the data from Ag^{+}TLC, fatty acid composition of the triacylglycerol classes and from lipase hydrolysis of the triacylglycerols (Table 13) and the values are given in Table 15.

The component triacylglycerols of each fraction obtained by Ag^{+}TLC were determined from (i) the position of the band on the TLC plate (ii) order of elution of triacylglycerols as predicted by Gunstone and Padley (1965), (iii) by making the assumption of Jurriens et al (1964) and Jurriens and Kroesen (1965) that each fraction is composed of triacylglycerols with the same degree of unsaturation. A minor

Table 14 Triacylglycerol Composition (mole %) of Oil Palm Fruit Mesocarp Calculated According to the 1,3-Random-2-Random Distribution Theory - (Vander Wal, 1960). Values Calculated from Lipolysis Data by the Equations of Vander Wal (1964)

Triacylglycerol (mole %)									
Saturated		Monoene		Diene		Triene		Polyene	
PPP	6.92	POP	19.26	MOO	0.94	OOO	5.09	POLe	0.21
PMSt	0.16	StOSt	0.13	POO	19.79	MOL	0.27	PLeO	0.13
PPSt	1.12	MOP	1.83	StOO	1.61	POL	5.78	OOL	2.97
PPM	0.66	MOSt	0.15	OMO	0.26	StOL	0.47	OLO	0.78
PStSt	0.13	POSt	3.12	OPO	1.83	MLO	0.14	LLP	0.85
PStP	0.80	PPO	7.11	OStO	0.21	PLO	2.91	LPL	0.16
PMP	0.98	MPO	0.34	PPL	2.08	StLO	0.24	LOL	0.44
others	0.30	PMO	1.01	PLP	2.83	OML	0.15	LLO	0.43
		PStO	0.82	PML	0.30	OPL	1.07	OOLe	0.11
		StPO	0.58	MPL	0.10	OStL	0.12	others	0.39
		others	0.33	PStL	0.24	PLeP	0.13		
				StPL	0.17	others	0.18		
				MLP	0.27				
				PLSt	0.46				
				others	0.16				
Total	11.07		34.68		31.25		16.55		6.44

M = myristic; P = palmitic, St= stearic, O=oleic, L=linoleic,

Le= linolenic

correction was applied to the total fatty acid compositions of the fractions to bring the fatty acid composition into agreement with the supposition since the number of double bonds per molecule was not a whole number. This assumption was made to enable calculation of triacylglycerol isomers, and (iv) the triacylglycerol composition was deduced from the fatty acid composition both for overall and for the 2-position and from the percentages of the fractions. The polyene fraction was not analyzed completely. Calculations was based on the method followed by Jurriens et al (1964) and Jurriens and Kroesen (1965).

The triacylglycerol profile estimated by Ag^{+}TLC indicated the presence of 10.82% saturated triacylglycerols, 25.6% of SOS, 19.7% of SOO and the remaining 43.9% was contributed by several triacylglycerol species in the range 1-6% (Table 15).

A comparative evaluation of the triacylglycerol species quantitated by Vander Wals method (Table 14) and Ag^{+}TLC (Table 15) is presented in Figure 16. The values given in the histogram are for the comparable triacylglycerol species considering the limitations of the Ag^{+}TLC method presented here to resolve the positional isomers; nevertheless the triacylglycerols compared were the major species for palm oil. The values obtained by these two methods for the major triacylglycerol species (SSS, SOS and SOO) were comparable, with a few exceptions in terms of their relative abundance as well as in terms of the major species.

The close similarity of the data obtained by these two methods indicate that palm oil triacylglycerols obey the Vander Wals Hypothesis,

Table 15 Triacylglycerol Composition of Oil Palm Fruit Mesocarp Calculated from Ag+TLC and Lipase Hydrolysis Data

Saturated		Monoene		Diene		Triene		Polyene	
SSS	10.82	SOS	25.61 (79.25)	OSO	3.67 (11.90)	OOO	1.95 (17.47)	SSLe/SOLe*	0.69 (4.62)
		SSO	6.71 (20.75)	SOO	19.71 (63.91)	SLO	3.76 (33.63)	others	14.14 (95.38)
				SLS	2.88 (9.33)	SOL	2.91 (25.99)		
				SSL	2.48 (2.48)	LSO	2.56 (22.91)		
				OOO	2.10 (6.82)				
	10.82		32.32		30.84		11.19		14.83

S = saturated; O = oleic; L = linoleic; Le = linolenic

Figures in parenthesis are relative percent.

* No isomer distinguished.

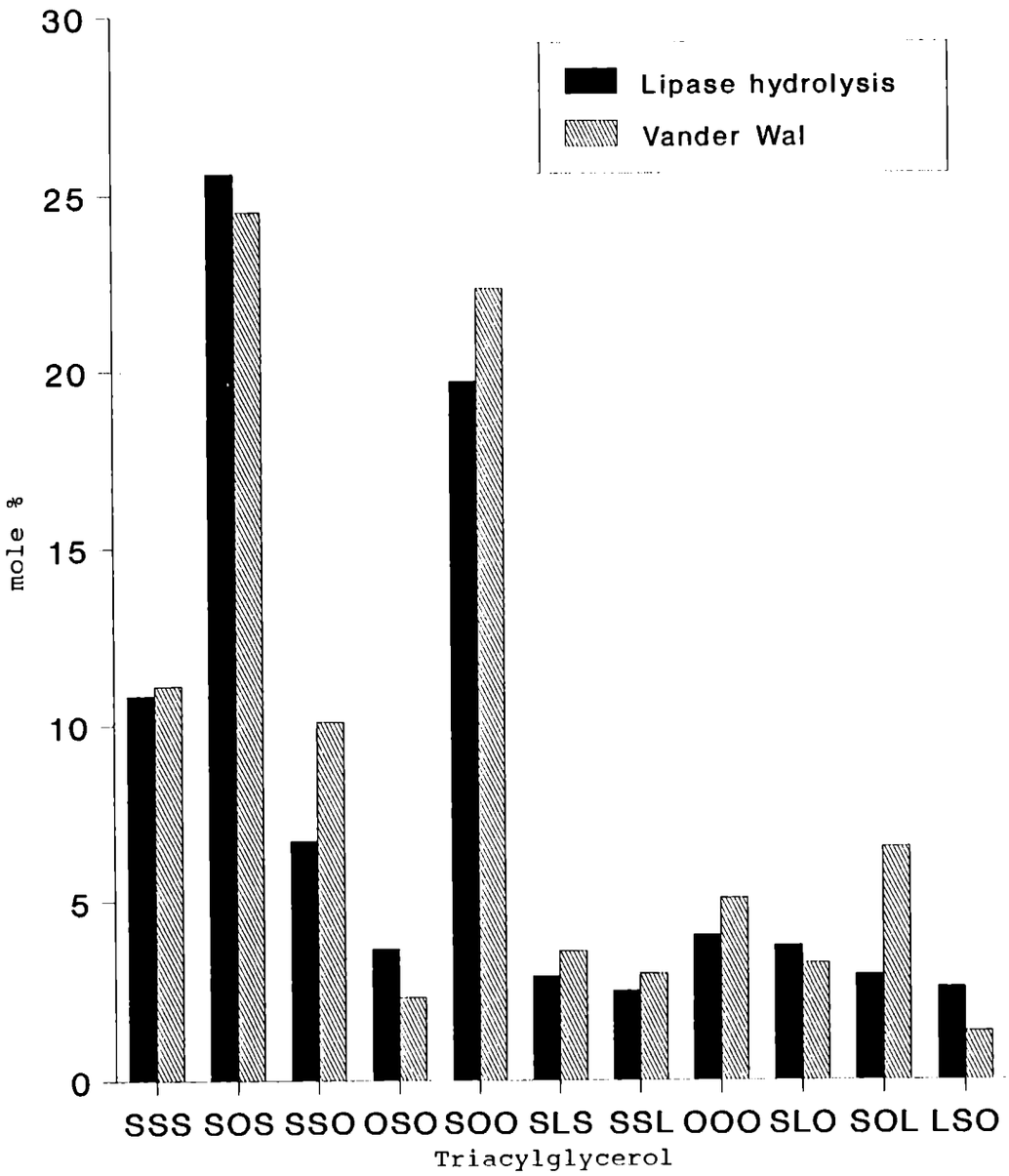


Figure 16. Triacylglycerol composition of oil palm fruit mesocarp calculated from Ag^{+}TLC and lipase hydrolysis data compared with values computed by Vander Wals Hypothesis.

i.e., the 1,3-random-2-random distribution theory which states that there are two pools of fatty acids available for biosynthesis of triacylglycerol; one for the 1,3-positions and the other for the sn-2-position. According to this distribution, the fatty acids for 1,3-positions are randomly esterified between these two positions and the fatty acid in the other pool to the sn-2- position. The theory is usually verified using the 1,3-specific pancreatic lipase.

The triacylglycerol composition of palm oil has been determined by several workers. Jurriens et al (1964), Jurriens and Kroesen (1965) and Tan et al (1981) have determined the triacylglycerol compositions of Congo, Sumatra and Malaysian palm oil respectively by argentation thin-layer chromatographic, gas chromatographic and lipase hydrolysis techniques and have compared the experimental values obtained with that calculated from Vander Wals theory (1960). Jacobsberg (1975) and Kifli (1975) have determined the composition of oil palm varieties and of Malaysian palm oil respectively from lipase hydrolysis data. Results of these investigations reveal that the triacylglycerol composition of palm oil does not differ very significantly with agro-climatic conditions or varieties. The major triacylglycerol species of palm oil were found to be POP and POO, minor amounts of PPP, PPO, OOO and POL and trace amounts of several others. Comparison of experimental values with those calculated according to Vander Wals theory (Jurriens et al 1964; Jurriens and Kroesen, 1965; Tan et al, 1981) indicates that the triacylglycerols of palm oil follow the 1,3-random-2-random distribution hypothesis (Vander Wal, 1960).

3.1.3. Distribution of Lipids in the Exocarp and Mesocarp of Three Varieties of Oil Palm Fruit

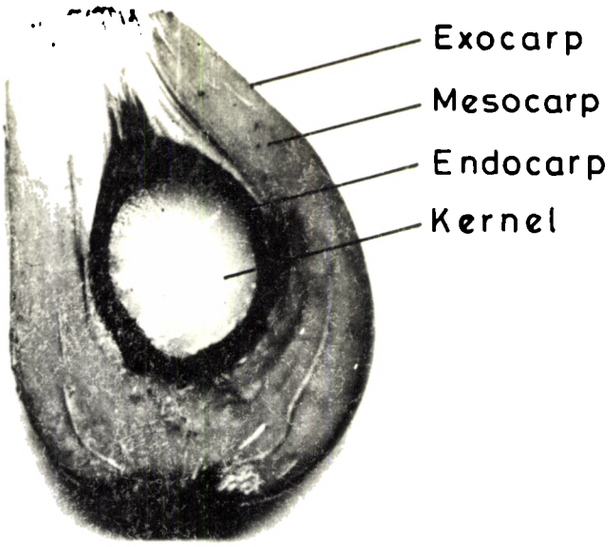
As part of the characterization of oil palm fruit lipids, the distribution of the lipids among the anatomically distinct regions of the fruit was investigated. As stated before, the oil palm fruit is a sessile drupe consisting normally of a single seed (kernel) surrounded by the pericarp (Hartley, 1977; Maycock, 1985; Wood, 1987). The latter includes three distinct regions, viz., the hard endocarp or shell, the fleshy, fibrous, oil bearing mesocarp and the thin, external, waxy skin or exocarp (Photograph VIII).

From the external morphology, the three varieties or fruit forms of E. guineensis are not distinguishable. The dura, pisifera and tenera varieties or fruit forms are identified based on the differences in shell thickness and mesocarp content, Photograph IX, (Hartley, 1977; Maycock, 1985). The nut of the dura fruit form has a thick shell (usually between 2 and 8 mm) with low to medium mesocarp content (35 to 55 percent). Pisifera is characterized by a shell-less fruit and a pea-like kernel inside. Often the kernel is absent and the fruit is composed entirely of fleshy oil bearing mesocarp. Tenera is a hybrid obtained by crossing dura (female) with pisifera (male). The nut of the tenera form has a thin shell (usually 0.5 to 4 mm thick) and medium to high mesocarp content (60-95 percent). When the fruit is cut transversely a prominent ring of fibres can be seen close to the shell and provides a way of identifying tenera fruit. Tenera is the widely cultivated type all over

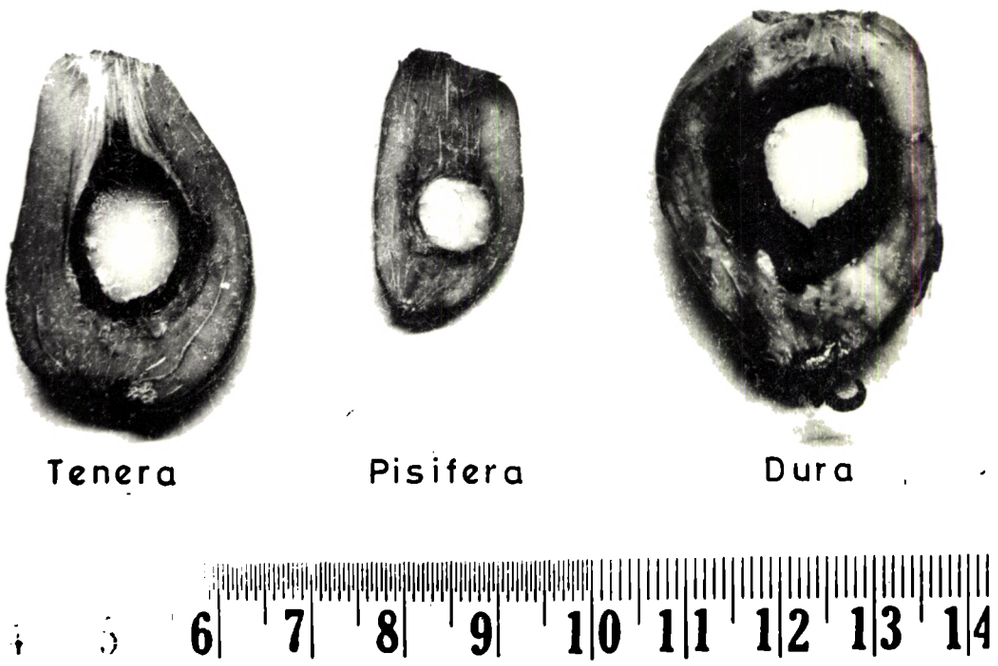
LEGENDS TO PHOTOGRAPHS VIII and IX

VIII : L.S of a mature oil palm fruit (tenera variety) showing anatomical features of the fruit: thin leathery skin or exocarp; fleshy, fibrous mesocarp containing palm oil; stony endocarp or shell enclosing a hard, oily endosperm or kernel.

IX : L.S. of mature fruits of the three varieties (fruit forms) of the oil palm: Dura with thick shell and thin mesocarp and pisifera with thick mesocarp and rudimentary endocarp and without viable seed; tenera, hybrid from the above two fruit forms having intermediate mesocarp and shell content. Tenera is the commercially grown variety for palm oil while the dura and pisifera are parental varieties for generating hybrids.



VIII. L.S. of mature oil palm fruit (tenera variety) showing anatomical features of the fruit.



IX. L.S. of mature fruits of the three varieties (fruit forms) of the oil palm

the world due to the higher mesocarp content and resultant higher yield of palm oil.

The mesocarp and the exocarp regions of the mature fruits of dura, pisifera and tenera varieties were separated. The total lipids were extracted from the fresh tissue and analyzed for the various lipid constituents and their fatty acids.

Distribution of polar lipids: The phospholipids and glycolipids of dura, pisifera and tenera varieties and their distribution within the fruit are summarized in Table 16 and Figure 17. The phospholipid and glycolipid contents varied between varieties. Phospholipid and glycolipid levels of the mesocarp and pericarp regions of tenera variety were found to be higher than the other two varieties. Exocarp of pisifera variety contained more phospholipids and glycolipids than the exocarp of the other two.

Within the fruit there was a distinct concentration difference of these lipids. Exocarps were found to have 6,3 and 5 fold increase in phospholipid content and 4,6, and 11 fold increase in glycolipid content than the corresponding mesocarps of dura, tenera and pisifera varieties respectively. Pericarp had intermediate levels.

Earlier reports on the polar lipids of palm oil have not specified varieties (Goh et al, 1982, 1985). However, the results presented here are consistent with the ranges of 1000-2000 ppm for phospholipids (Goh et al, 1982) and 1000-3000 ppm for glycolipids (Goh et al, 1985) for the pericarp regions. A differential distribution of

Table 16 Lipids of Dura, Pisifera and Tenera Varieties of Oil Palm and Their Distribution Within the Fruit

Variety	Region % (per fruit)		Lipid		
			Phospholipid (ppm)	Glycolipid (ppm)	Neutral lipid (g/100g)
Dura	Exocarp	1.7±0.1	11687±442	6242±179	98.20
	Mesocarp	56.6±1.8	2110±73	1774±94	99.61
	Pericarp		6198±210	2012±72	99.18
Pisifera	Exocarp	5.3±1.7	14856±344	11770±146	97.34
	Mesocarp	94.7±1.5	3084±73	1083±50	99.58
	Pericarp		5071±257	1847±108	99.31
Tenera	Exocarp	2.6±0.3	13148±892	10944±146	97.59
	Mesocarp	64.5±4.3	4420±231	1774±81	99.38
	Pericarp		6200±238	2147±73	99.17

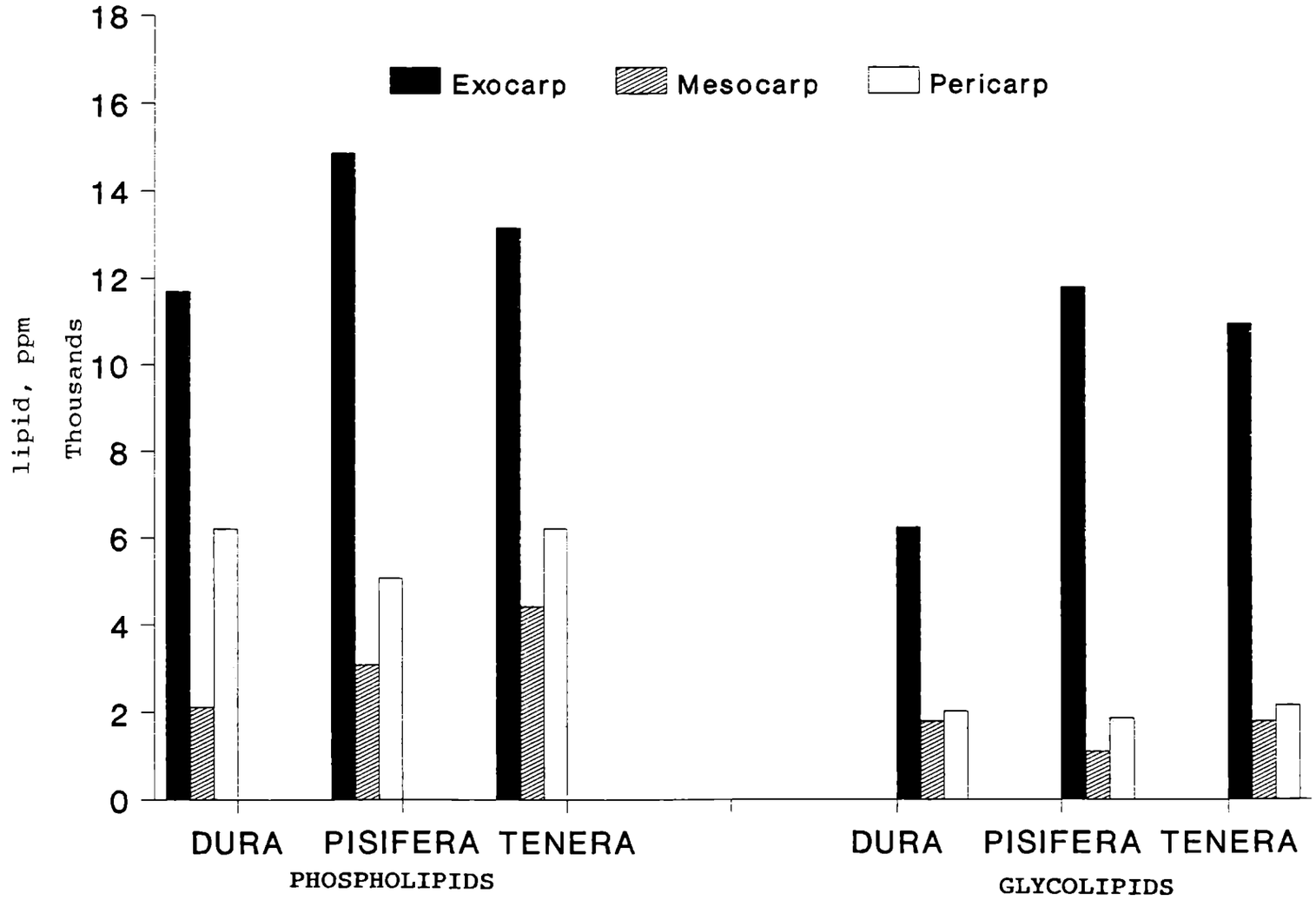


Figure 17. Polar lipids of dura, pisifera and tenera varieties of oil palm fruit mesocarp and their distribution within the fruit.

phospholipids and glycolipids within the fruit may be attributed to their functions as membrane lipids. The waxy skin or exocarp probably consists of more membrane lipids due to its protective function, while accumulation of the neutral lipids (storage lipids) occur chiefly in the mesocarp. Several workers have observed variations in the lipid and fatty acid compositions in the different anatomical parts of mature seeds or beans and fruits (Hitchock and Nicols, 1971; Appelqvist, 1975; Harwood, 1980; Christie, 1987). However there is no report regarding the distribution of lipid classes and fatty acids in the anatomically distinct regions of the oil palm fruit.

Distribution of fatty acids: Fatty acid composition of the total lipids and the lipid classes of dura, pisifera and tenera varieties are given in Tables 17 to 20. The fatty acid composition of the total lipids of the three varieties was found to be more or less similar (Table 17). Previous reports on the fatty acid composition of the total lipids of oil palm fruit varieties (Jacobsberg, 1975; Ng *et al*, 1976) indicate that fatty acid composition did not differ very significantly in terms of variety. These findings on the varieties grown in India are within the ranges reported. There was no significant difference in the total unsaturation of the neutral lipid fractions of the three regions of each variety. Phospholipids and glycolipids of the varieties reported here had similar fatty acid profiles and are fairly in agreement with report published (Goh *et al*, 1982). However, with the absence of the identity of the varieties of the earlier work, a direct comparison with values reported here would be inappropriate.

Table 17 Fatty Acid Composition of Total Lipids of Three Varieties of Oil Palm Fruit Mesocarp

Variety	Fatty acid (wt %)					
	12:0+14:0	16:0	18:0	18:1	18:2	18:3
Tenera	1.1	43.4	4.3	38.0	12.4	0.8
Dura	1.0	43.0	4.4	40.0	11.1	0.5
Pisifera	1.0	44.1	5.0	37.1	12.1	0.7

Table 18 Fatty Acid Composition of Neutral Lipids, Phospholipids and Glycolipids of Three Regions of the Fruit of Dura Variety

Region	Lipid Class	Fatty acid (wt %)					
		12:0+14:0	16:0	18:0	18:1	18:2	18:3
Exocarp	Neutral lipid	1.4	43.4	3.7	40.3	10.9	0.3
	Phospholipid	0.8	37.1	3.3	30.4	21.7	6.7
	Glycolipid	0.9	23.2	3.4	27.9	8.3	36.3
Mesocarp	Neutral lipid	0.9	43.6	4.8	39.8	10.6	0.3
	Phospholipid	0.2	31.7	3.5	43.0	20.5	1.1
	Glycolipid	1.2	25.3	3.1	36.5	17.0	16.9
Pericarp	Neutral lipid	0.9	43.5	4.8	40.3	10.3	0.2
	Phospholipid	2.1	32.2	2.9	40.5	20.2	2.1
	Glycolipid	2.2	32.3	3.7	36.9	11.3	13.6

Table 19 Fatty Acid Composition of Neutral Lipids, Phospholipids and Glycolipids of Three Regions of the Fruit of Pisifera Variety

Region	Lipid Class	Fatty acid (wt %)					
		12:0+14:0	16:0	18:0	18:1	18:2	18:3
Exocarp	Neutral lipid	1.2	43.6	3.8	38.4	12.3	0.7
	Phospholipid	0.7	29.2	3.2	34.2	23.2	9.5
	Glycolipid	0.9	23.2	3.4	27.9	8.3	36.3
Mesocarp	Neutral lipid	1.0	44.6	4.9	37.2	11.8	0.5
	Phospholipid	2.1	29.7	2.5	42.4	21.6	1.7
	Glycolipid	1.5	30.6	4.1	27.7	17.4	18.7
Pericarp	Neutral lipid	0.9	45.4	4.3	37.5	11.3	0.6
	Phospholipid	1.7	31.6	2.4	36.5	26.1	1.7
	Glycolipid	2.8	31.9	5.3	34.6	15.3	10.1

Table 20 Fatty Acid Composition of Neutral Lipids, Phospholipids and Glycolipids of Three Regions of the Fruit of Tenera Variety

Region	Lipid Class	Fatty acid (wt %)					
		12:0+14:0	16:0	18:0	18:1	18:2	18:3
Exocarp	Neutral lipid	1.3	44.4	3.9	38.6	11.4	0.4
	Phospholipid	0.5	31.1	2.5	34.5	27.1	4.3
	Glycolipid	1.4	29.4	4.6	25.6	8.8	30.2
Mesocarp	Neutral lipid	1.1	45.0	3.8	39.1	10.7	0.3
	Phospholipid	0.5	42.2	3.2	37.4	16.1	0.6
	Glycolipid	1.9	23.9	3.3	35.1	17.8	18.0
Pericarp	Neutral lipid	1.1	43.2	5.0	38.5	11.8	0.4
	Phospholipid	2.1	33.9	2.6	35.0	24.6	1.8
	Glycolipid	3.7	31.1	5.5	33.4	15.1	11.2

The fatty acid profiles of neutral lipids, phospholipids and glycolipids of the different regions of the fruit revealed appreciable variations in the distribution of major acids (16:0, 18:1, 18:2 and 18:3). Neutral lipid fractions from all the three regions had almost identical concentrations of 16:0, i.e., almost similar to that of palm oil. Phospholipids and glycolipids from exocarp and mesocarp in general contained significantly lower levels of 16:0 than the neutral lipids of corresponding regions. Phospholipids from exocarp and mesocarp had similar concentrations of 16:0. Exocarp and mesocarp glycolipids had comparable values for 16:0. Phospholipids from all the regions showed higher 16:0 content than the glycolipids from the respective regions.

There was no appreciable differences in levels of 18:1, though higher levels of 18:1 were present in neutral lipids than phospholipids of all regions while lowest levels of 18:1 were found in the glycolipids.

The most striking differences in distribution of fatty acids were noticed in 18:2 and 18:3. 18:2 and 18:3 were found to be more predominant in the polar lipids than in the non-polar lipids. Phospholipids of exocarp had greater abundance of 18:2 whereas glycolipids of all regions were characterized by the presence of 18:3 as a major acid. The relative percentage of 18:2 of exocarp phospholipids was almost as high as 16:0 and 18:1. On the other hand, glycolipids of mesocarp had exceptionally high levels of 18:2 and 18:3. Though 18:3 is insignificant in palm oil, it was found to be enriched in the glycolipid fractions, particularly of the exocarp. It appears that there is an

association of fatty acid types with lipid classes, viz., 16:0 with neutral lipids, 18:2 with phospholipids and 18:3 with glycolipids. There seems to be an inverse relation between 16:0, 18:2 and 18:3 regarding their distribution among the regions and their lipid classes. Similar association of 18:2 and 18:3 with phospholipids and glycolipids respectively have been reported for other oilseeds in the literature (Hitchcock and Nicols, 1971; Appelqvist, 1975; Harwood, 1980; Christie, 1987).

3.2. LIPID COMPOSITION OF DEVELOPING OIL PALM FRUIT

Lipids are the storage form of energy in the oilseeds. In exceptional cases, fleshy mesocarp of fruit tissues is the site of storage. The significant commercial oils from fruits are that of oil palm and olive. The lipid reserves in these tissues are not utilized for seed germination unlike the oilseeds and therefore is only of commercial significance.

Oil palm fruit lipids have been the subject of investigations mainly from the commercial point of view. Studies so far, therefore have been confined to the fatty acid composition of palm oil under various agro-climatic conditions (Jurriens et al, 1964; Jurriens and Kroesen, 1965; Jacobsberg, 1975; Ng et al, 1976; Tan et al, 1981, 1985; Rossell et al, 1983, 1985). However, there have been a few reports on the characterization of fruit lipids of the oil palm during its development (Wuidart, 1973; Crombie and Hardman, 1958; Thomas et al, 1971; Esechie, 1978; Oo et al, 1985, 1986; Bafor and Osagie, 1986, 1988a, 1988b, 1989). However a comprehensive study of the total characterization of the lipids of developing oil palm fruit has not been seriously considered. The oil palm is an important source of edible oil in the future for India and therefore a basic knowledge of the lipids vis-a-vis other geographical origins is important. The present investigation is an attempt in this direction.

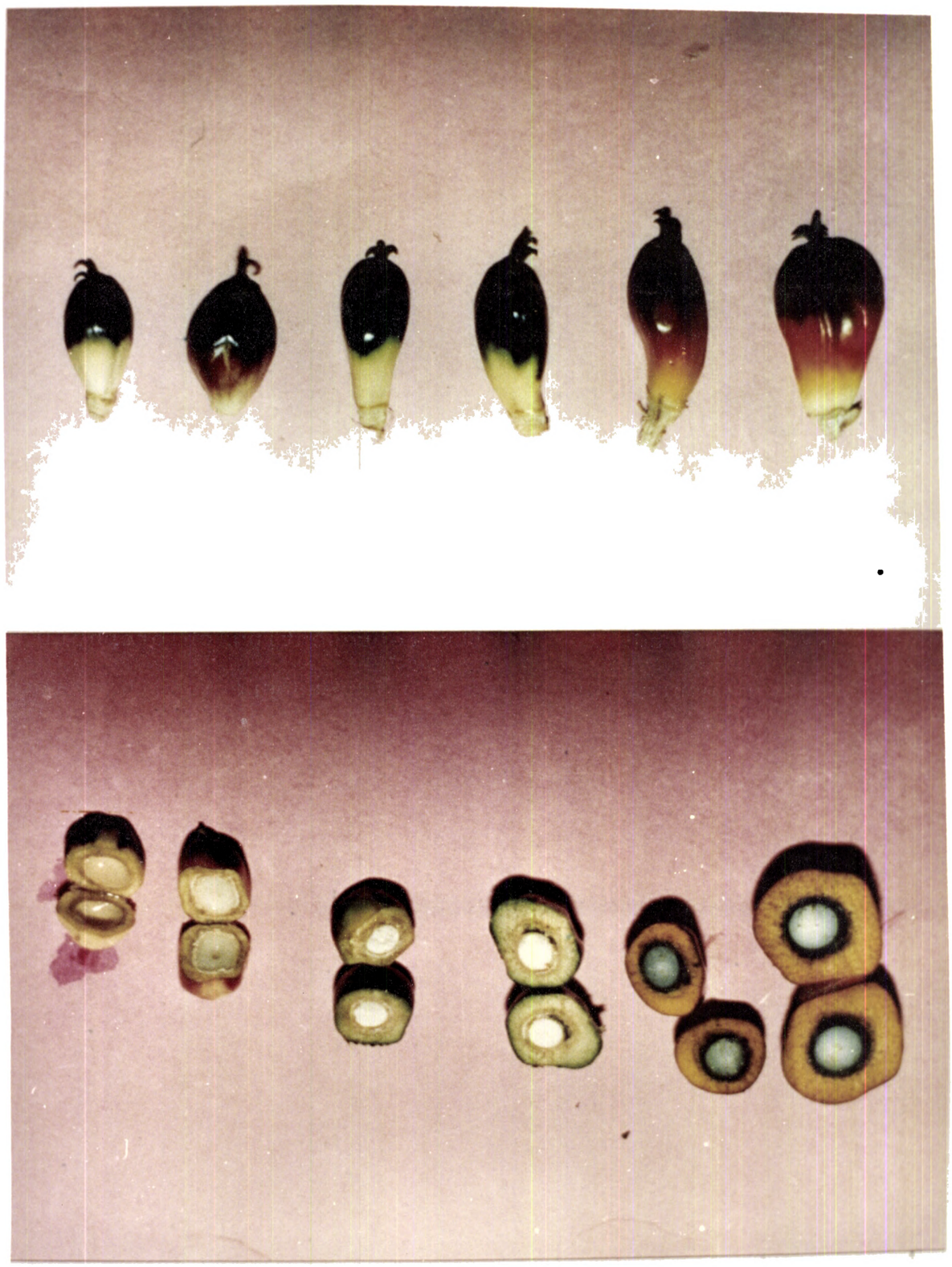
The oil palm fruit undergoes drastic changes during its development until it attains maturity. Under normal conditions it takes about 180 days from the date of anthesis for the complete development of the fruit (Hartley, 1977). During this period, changes occur in the fruit in terms of both physical and chemical parameters. Though the focus of this study was changes in the composition and structure of fruit lipids, other aspects such as physical characteristics of the fruit were also investigated to understand the overall process of oil palm fruit development.

Oil palms of the tenera variety were randomly selected, female inflorences were tagged when they became receptive and fruit samples were collected at different stages of development from 4 upto 24 weeks after anthesis (WAA). Physico-chemical parameters of the fruit were determined. Total lipids from the mesocarp of fruits of the various developmental stages were isolated. The lipid classes were separated by TLC and quantitated by GLC. Fatty acid composition of each lipid class was determined by GLC.

Morphological and anatomical changes of the palm fruits (tenera) at different stages of development are shown in Photograph X. Morphology of the fruit varied considerably during development. In the early stages (8 to 16 WAA), fruits were small and colorless or pale yellow at the base and deep violet to black at the apex and not oily. After 16 WAA, fruits gradually changed color and became orange and by 20 WAA fruits were large, bright, waxy and orange-red in color.

LEGEND TO PHOTOGRAPH X

X. Oil palm fruits (tenera variety) at different stages of development. Age of fruit in weeks after anthesis from L to R 4, 8, 12, 16 20 and 24. The color of the fruit changes from dark green to orange-red at maturity representing chlorophyll and carotenoid pigments. The large liquid endosperm within a thin mesocarp (at early stages) turns into a hard stony seed with a thick mesocarp. The mesocarp which is white and non-oily (upto 16 WAA) changes to orange colored oily tissue at maturity.



X. Oil palm fruits (tenera variety) at different stages of development
Age of fruit in weeks after anthesis from L toR 4,8,12,16,20 and 2

Physio-chemical characteristics: Tables 21 and 22 and Figure 18 summarize the changes in the physico-chemical characteristics of the oil palm fruit during development. There was an increase in bunch weight and fruit weight through the entire period of fruit maturation. In the early stages of development, moisture content was significantly high with very little oil in the kernel. Oil deposition in the kernel began from 10 WAA. By 16 WAA, oil deposition was found to be completed in the kernel when oil percent reached 36.9% and thereafter remained fairly constant. However, in the mesocarp, oil deposition commensed only by 14 WAA and reached 45.2% at maturity (24 WAA) with a corresponding decrease in moisture. Upto 14 WAA oil content was extremely low, followed by a short, extremely rapid rate of oil deposition between 16 and 20 WAA and a final phase when only minor amounts of lipid was accumulated.

The oil palm fruit is a drupe with a fleshy mesocarp enclosing a stony endocarp (Gurr, 1980) approximately in the ratio 80:20 for the commercial tenera variety (Photograph VIII). Palm oil is derived from the mesocarp while endosperm is the source of palm kernel oil (Hartley, 1977; Maycock, 1987). It is a unique feature that a fruit yields two distinct, commercially significant oils. The results presented in Tables 21 and 22 and Figure 18 demonstrate the interesting pattern of accumulation of these oils during fruit development. Usually the oil palm takes about 180 days for full development (Hartley, 1977). There was no appreciable formation of mesocarp constituents until 14 WAA. However, during this period, the development of the endocarp was completed. The subsequent changes begining from 16 WAA was largely in

Table 21 Physical Parameters of Developing Oil Palm Fruits (Tenera Variety)

Age of fruits in weeks after anthesis	Bunch weight (Kg)	Fruit weight (g)	Mesocarp %	Kernel %	Shell %
4	8.0+3.0	1.80+0.37	93.58+ 1.22	-	6.41+ 1.22
8	13.3+1.8	4.41+0.53	73.53+ 3.38	-	26.47+ 3.38
10	13.0+2.0	4.82+1.05	75.28+10.07	9.26+5.27	15.46+ 4.98
12	14.3+1.6	6.83+0.18	75.26+ 2.40	9.87+1.52	15.64+ 0.58
14	11.5+2.0	6.94+2.07	78.48+ 8.77	7.52+5.00	13.99+ 5.34
16	19.0+4.2	7.32+1.06	65.00+10.95	10.43+0.89	24.57+11.80
18	16.0+4.0	6.13+1.04	75.80+ 7.80	8.72+4.30	15.47+ 4.60
20	14.0+4.2	8.61+2.63	73.50+ 6.14	7.35+0.97	19.15+ 6.66
22	16.5+1.7	6.98+0.20	66.80+ 7.91	10.25+4.07	22.95+ 3.82
24	17.5+1.7	6.63+0.59	75.16+ 0.08	8.21+0.29	16.63+ 0.35

Table 22 Changes in Moisture and Oil Content During Development of Oil Palm Fruit

Age of fruit in weeks after anthesis	Mesocarp			Kernel		
	Moisture %	Oil		Moisture %	Oil	
		%dry weight	mg/fruit		%dry weight	mg/fruit
4	85.27+0.69	1.48+0.05	3.66	-	-	-
8	85.18+0.68	1.67+0.02	8.00	-	-	-
10	83.68+0.82	2.40+0.06	14.27	89.15+0.92	43.39+1.20	21.19
12	83.79+0.67	2.22+0.18	19.44	67.07+0.32	42.88+2.00	94.60
14	72.71+0.55	27.85+0.81	414.20	21.07+0.74	37.57+1.10	154.20
16	74.42+0.93	35.65+0.70	434.06	26.17+3.03	39.95+1.40	224.16
18	68.73+0.62	49.13+0.50	714.38	18.02+1.98	44.17+1.50	191.91
20	42.54+0.49	66.23+0.60	2408.94	15.34+0.55	44.89+0.72	239.94
22	42.17+0.51	73.22+0.50	1973.18	18.06+0.72	46.08+0.77	268.08
24	41.44+0.67	77.23+0.90	2252.25	14.87+1.60	46.30+0.95	212.84

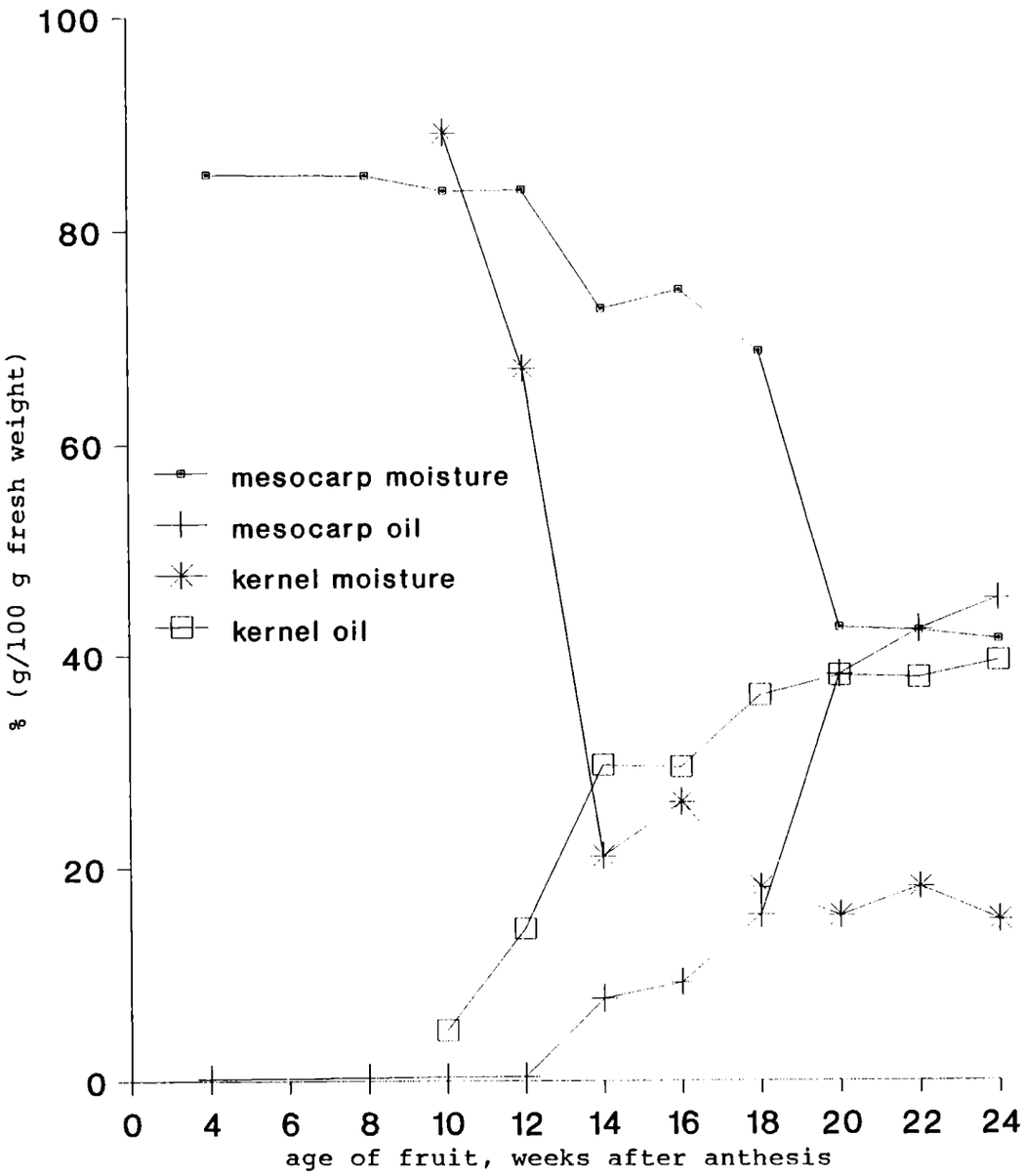


Figure 18. Changes in the oil and moisture content during development of oil palm fruit.

the mesocarp as seen in the rapid increase in the dry matter as well as total lipids. From the physical barrier it may be stated that these are two independent systems with sequential developmental stages with little scope for translocation of precursors between the endosperm and mesocarp. The duration of fruit development is subject to geographic and agro-climatic variations and therefore reports so far indicate a range of values from 150 to 180 days for full maturity (Crombie and Hardman, 1958; Rajaratnam and Williams, 1970; Thomas et al, 1971; Ng and Southworth, 1973; Hartley, 1977; Esechie, 1978). For the tenera variety studied here, it was observed that about 170 days were required for the full development of the fruit. Fat formation in the mesocarp occurred towards the end of fruit development. Crombie and Hardman (1958) reported that almost all the oil was deposited between 19 and 20 WAA in Nigerian palms, whereas Bafor and Osagie (1986) have determined the active period of oil accumulation to be between 18 and 22 weeks. Oil deposition occured more evenly in Malaysian palms (Thomas et al, 1971; Oo et al, 1985). The rapid phase of oil accumulation in the fruits observed here was between 16 and 20 weeks. Fixation of harvesting time is therefore very important for maximum oil recovery.

Lipid profile of developing oil palm fruit: The concentration of various lipid classes in the developing oil palm fruit mesocarp is presented in Tables 23 and 24 and Figure 19. It may be seen from the Tables and Figure that during the early states of fruit development, the lipids were predominently composed of polar lipids (phospholipids and glycolipids), partial glycerides (diacylglycerols and monoacylglycerols)

Table 23 Changes in the Phospholipid, Glycolipid and Neutral Lipid Content of Developing Oil Palm Fruit

Age of fruit in weeks after anthesis	Phospholipid		Glycolipid		Neutral lipid	
	g/100g total lipid	mg/fruit	g/100g total lipid	mg/fruit	g/100g total lipid	mg/fruit
4	21.68+1.4	0.79	30.19+1.2	1.1	48.13	1.76
8	37.78+1.1	3.02	15.51+1.1	1.24	46.71	3.74
10	34.41+0.7	4.91	10.91+0.17	1.56	54.68	7.80
12	45.44+1.4	8.83	21.37+1.7	4.15	33.19	6.45
14	3.02+0.5	12.51	1.02+0.03	4.22	95.96	397.47
16	1.86+0.8	8.07	0.78+1.7	3.39	97.36	422.60
18	1.64+0.27	11.72	0.60+0.03	4.29	97.76	698.38
20	0.86+0.08	20.72	0.55+0.13	13.25	98.59	2374.97
22	0.90+0.45	17.76	0.59+0.16	11.64	98.51	1943.78
24	0.62+0.24	13.96	0.21+0.73	4.73	99.17	2233.56

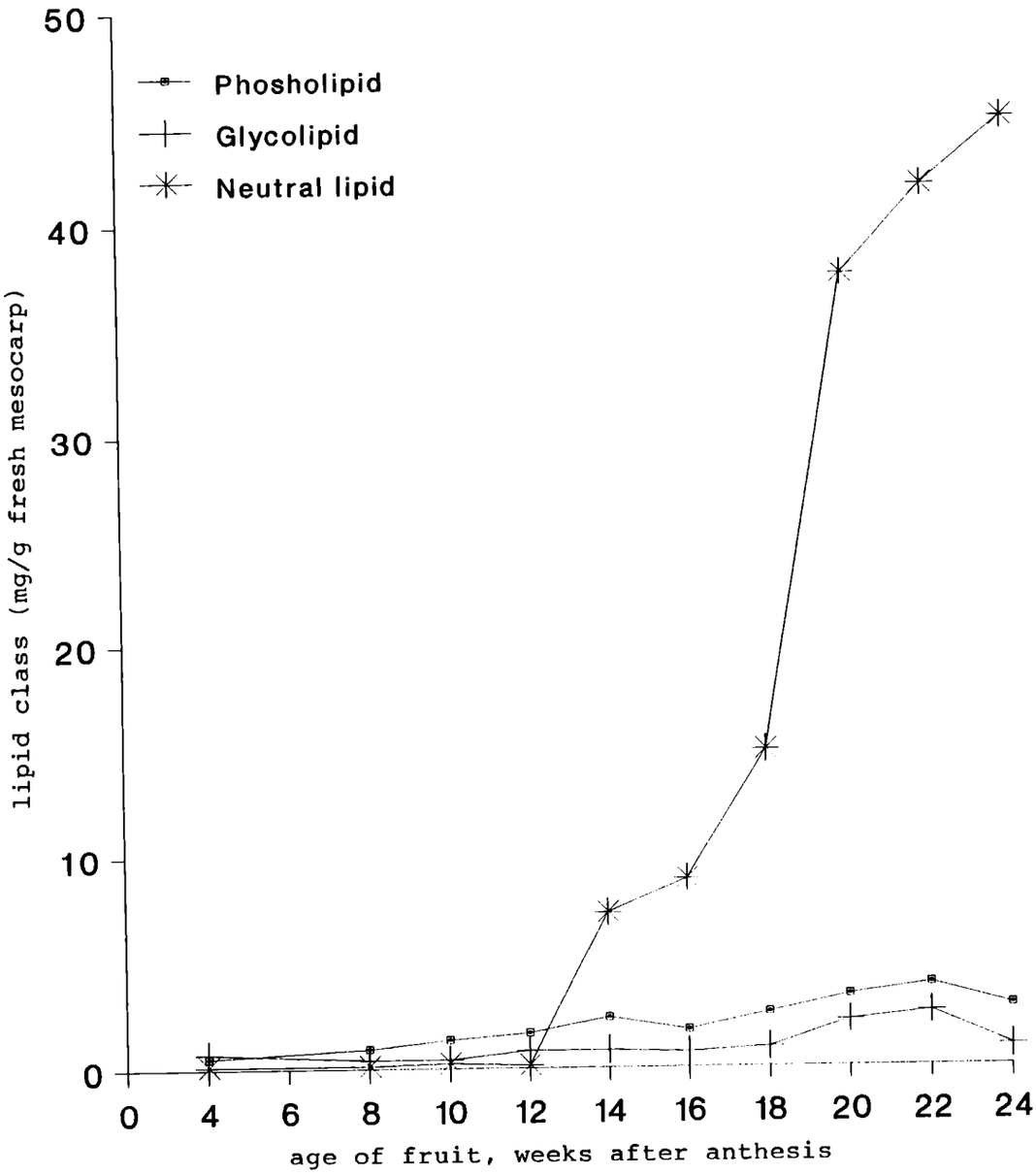


Figure 19. Accumulation of major lipid, classes of oil palm fruit during development (Values plotted for neutral lipids are x 10^{-1}).

Table 24 Changes in the Neutral Lipid Composition of Developing Oil Palm Fruit Mesocarp (g/100g of total lipid)

Age of fruit in weeks after anthesis	Triacylglycerol	Diacylglycerol	Monoacylglycerol	Fatty acid
4	11.09	18.98	6.92	9.04
8	11.39	18.52	4.83	9.47
12	11.61	8.84	4.00	5.83
14	54.48	17.71	6.23	15.71
16	87.79	4.17	1.41	1.78
18	87.38	5.48	1.47	1.52
20	88.91	4.29	1.77	1.75
22	89.94	4.05	1.70	1.26
24	95.27	1.91	0.50	0.64

and free fatty acids, comprising about 90% of the total lipids with triacylglycerol accounting for only about 10%. The lipid profile began to change drastically between the fourteenth and sixteenth week, synchronizing with the onset of the rapid phase of fat accumulation in the developing mesocarp. Subsequently, the shift of lipid biosynthesis was towards the formation of triacylglycerols accounting for 95% by 24 WAA. At this stage other lipid classes, viz., polar lipids and partial glycerides became insignificant in the overall lipid composition. Although there was a decrease in the relative percentages of polar lipids and partial glycerides, on an absolute basis, their levels remained almost constant throughout fruit development.

During the early stages, the total lipids were largely accounted for by the structural lipids (phospholipids and glycolipids) with very little in the storage form (triacylglycerols). The relative reduction in the non-triacylglycerol lipids is primarly due to the excessive synthesis of triacylglycerols and therefore due to the dilution effect. (Hitchock and Nichols, 1971; Appelqvist, 1975; Gurr, 1980; Harwood, 1980). The other explanation from the biosynthetic point of view is that triacylglycerols are formed either through the Kennedy pathway (α-glycerol phosphate) or via the phospholipid pathway (Hitchock and Nichols, 1971; Gurr, 1980). Both these routes involve phospholipids (phosphatidylcholine) and other partial glycerides (diacylglycerols) including fatty acids as intermediate products with a high turnover rate during the rapid phase of triacylglycerol formation, ie., from 16 WAA onwards. This would further explain the drastic reduction in the

phospholipids and partial glycerides towards fruit maturation. The reduction in the proportion of glycolipids could be due to the degradation of photosynthetic tissue in the oil palm fruit as it is known that glycolipids (particularly digalatosyldiacylglycerol) is a major constituent of chloroplast tissue (Harwood, 1980).

There are several reports on the lipid profile of developing oil seeds viz., castor (Canvin, 1963, 1965), cottonseed (Pandey and Subrahmanyan, 1988), corn (Weber, 1969, 1973), crambe (Sims, 1964; McKillican, 1966; Gurr et al, 1972); flax (Sims et al, 1961a, 1961b; McKillican and Sims, 1963, mustard (Karth and Narayanan, 1959; Dasgupta and Friend, 1973; Mukherjee and Kiewitt, 1984), peanut (Sanders, 1980), safflower (Sims et al, 1961a, 1961b; McKillican and Sims, 1963) and soybean (Simmons and Quackenbush, 1954; Hirayama and Hujii, 1965; Singh and Privett, 1970a, 1970b; Privett et al, 1973; Wilson and Rinne, 1978; Roehm and Privett, 1978) and for developing oleagineous fruits viz., olive (Cherif et al, 1979; Marzouk and Cherif, 1981a, 1981b), indicating similar trends. However very limited data are available for oil palm fruit. Bafor and Osagie (1986, 1988a, 1988b) and Oo et al, (1986) have reported the lipid profile for African and Malaysian oil palm fruits respectively and observed more or less similar pattern with quantitative variations.

Fatty acid composition of the lipid classes of developing oil palm fruit Table 25 indicates the fatty acid profile of triacylglycerols, diacylglycerols, monoacylglycerols and fatty acid fractions of the total lipids of developing oil palm fruit. The general

Table 25 Fatty Acid Composition of the Triacylglycerol, Diacylglycerol, Monoacylglycerol and Free Fatty Acid Classes of Developing Oil Palm Fruit Mesocarp

Lipid class	Age of fruit in weeks after anthesis	Fatty acid (wt %)						
		12:0	14:0	16:0	18:0	18:1	18:2	18:3
Triacylglycerol	4	1.69	1.61	19.60	3.63	17.35	47.72	8.39
	8	3.56	4.61	34.37	6.44	30.50	15.91	4.61
	12	2.56	1.43	32.69	9.03	22.59	25.09	6.60
	14	0.22	0.52	34.79	5.61	47.78	10.45	0.63
	16	0.63	0.94	46.69	6.06	36.32	8.85	0.50
	18	0.22	0.74	40.99	4.58	44.23	8.91	0.33
	20	0.17	1.17	39.95	4.92	45.50	7.75	0.54
	22	0.24	1.47	43.00	4.11	41.02	9.59	0.57
	24	0.59	1.75	42.56	4.08	41.71	8.96	0.35
Diacylglycerol	4	1.18	0.98	29.77	5.55	33.05	26.56	2.90
	8	8.15	4.81	41.50	5.95	11.92	23.64	4.02
	12	4.92	5.87	41.01	7.05	15.36	18.89	6.90
	14	4.30	2.51	26.62	8.22	47.48	10.13	0.73
	16	1.85	1.03	33.32	11.46	36.77	15.30	0.28
	18	1.75	8.16	24.87	8.45	47.97	8.73	0.07
	20	1.65	1.32	29.26	13.20	42.80	10.98	0.79
	22	1.38	1.56	27.59	9.75	47.41	11.57	0.72
	24	1.31	1.57	32.32	8.97	39.90	15.16	0.76
Monoacylglycerol	4	7.41	4.60	40.38	11.58	17.23	16.08	2.71
	8	0.86	11.44	41.03	4.91	28.38	8.97	4.40
	12	2.17	3.57	41.23	13.95	24.98	10.69	3.40
	14	3.18	3.80	34.11	5.14	48.74	2.29	2.74
	16	4.94	3.83	33.02	11.23	39.87	6.26	0.85
	18	3.68	3.40	33.11	9.47	43.74	6.61	-
	20	3.65	2.81	28.30	3.45	55.73	5.63	0.44
	22	3.01	2.84	30.34	6.73	51.57	5.04	0.47
	24	3.55	5.67	36.88	8.46	35.82	8.77	0.86
Fatty acid	4	3.72	3.88	32.05	11.86	18.21	23.36	6.91
	8	3.68	5.15	33.79	8.63	30.94	15.84	1.97
	12	5.79	1.37	33.62	7.38	26.33	19.87	5.63
	14	2.04	2.28	33.25	8.10	26.41	21.06	6.86
	16	4.63	4.51	37.29	9.81	31.74	9.29	2.74
	18	14.11	7.53	33.52	9.12	31.56	4.15	-
	20	2.50	2.67	38.95	14.68	33.62	7.11	0.47
	22	2.86	2.41	34.92	17.71	34.55	5.67	1.87
	24	4.79	3.77	39.85	9.60	34.32	7.01	0.65

pattern of the fatty acids was that in the early stages of fruit development, there was a predominance of unsaturated fatty acids with a corresponding lower content of saturated acids. This trend was slightly reversed towards the end of fruit maturation. It could be seen from the Table that the major fatty acids were 16:0, 18:1 and 18:2 in all the neutral lipid classes. However, 18:3 was present in significant quantities during the early stages among all the lipid classes.

With respect to the major acids, triacylglycerols exhibited a definite trend with 16:0 and 18:1 showing a gradual increase, and 18:2 and 18:3 registering the reverse. The transition was particularly noticeable at 12 and 16 WAA, coinciding with the begining of the rapid phase of fat synthesis. The other neutral lipid fractions did not exhibit such a remarkable change like the fatty acids of the triacylglycerols during fruit development.

The fatty acid composition of the diacylglycerols and monoacylglycerols generally indicated that they contained slightly lower proportions of 16:0 with higher levels of 18:1 in the respective stages of fruit maturation. The fatty acid pool also showed a lower content of 16:0 and 18:1 with corresponding higher levels of 12:0, 14:0 and 18:0 as compared to the fatty acid of the triacylglycerols. It may be therefore mentioned that the 16:0 and 18:1 are better utilized for triacylglycerol synthesis in the oil palm fruit. The partial glycerides and free fatty acids could be formed as intermediate products of triacylglycerol biosynthesis or as lipolytic products (Gurr, 1980). From the fatty acid composition of the monoacylglycerols and free fatty acids it may be

stated that they are intermediate products in triacylglycerol biosynthesis. Since lipase is specific to the primary positions (Galliard, 1980), it is expected that the fatty acids released will mostly be 16:0 leaving most of the 18:1 in the 2-position of the monoacylglycerols.

The fatty acid profiles of phospholipids and glycolipids of the developing oil palm fruit are shown in Table 26. The phospholipids had the major fatty acids as 16:0, 18:1 and 18:2; similar to the triacylglycerols. However, 16:0 was much lower than that of triacylglycerols and was mostly compensated by higher proportions of 18:2 with 18:1 remaining more or less the same. During fruit development, 16:0 remained almost unchanged, whereas, 18:1 registered a steep increase and 18:2 showed a rapid decrease. The glycolipid fraction had 16:0, 18:1, 18:2 and 18:3 as the major fatty acids. The 18:3 was present in substantial amount in glycolipid mostly at the cost of 18:2 and to some extent 18:1, as compared to phospholipid. In the case of glycolipid also the level of 16:0 remained almost the same during the course of fruit development, however 18:1 exhibited a steady increase while 18:2 and 18:3 showed a downward trend. A general pattern of fatty acid association with a particular lipid class is perceptible from the Tables 25 and 26, ie., 16:0 and 18:1 with the triacylglycerol, 18:2 with phospholipid and 18:3 with glycolipid. The biochemical mechanism to explain this is yet to be investigated.

Table 26 Fatty Acid Composition of Phospholipids and Glycolipids of Developing Oil Palm Fruit Mesocarp

Lipid class	Age of fruit in weeks after anthesis	Fatty acid (wt %)						
		12:0	14:0	16:0	18:0	18:1	18:2	18:3
Phospholipid	4	0.07	0.19	35.38	2.81	15.25	41.68	4.61
	8	0.06	0.30	33.94	3.55	10.48	44.19	7.48
	12	0.09	0.29	34.54	4.06	15.86	33.81	11.36
	14	0.51	0.70	30.24	3.01	37.23	23.33	4.97
	16	0.55	2.20	38.89	2.32	34.76	20.23	1.05
	18	0.54	0.95	28.75	0.83	42.94	20.55	5.44
	20	0.65	2.26	24.35	0.36	53.31	17.81	1.26
	22	0.35	1.09	33.55	3.70	41.64	19.42	0.25
	24	0.34	1.37	32.60	2.81	38.22	23.19	1.47
Glycolipid	4	0.63	0.77	26.27	3.36	8.01	25.98	34.97
	8	0.35	1.21	27.97	4.38	6.69	12.07	47.33
	12	0.27	1.08	24.12	4.96	9.08	8.50	51.98
	14	0.14	0.99	22.39	6.11	23.53	17.09	29.76
	16	0.42	1.62	33.34	3.08	17.07	13.65	30.82
	18	0.57	1.42	30.18	5.00	24.26	11.00	27.57
	20	0.64	2.00	32.52	5.45	23.99	15.43	19.97
	22	0.16	1.15	35.20	4.95	23.35	14.39	20.80
	29	2.40	4.05	27.63	8.36	37.65	10.67	9.14

Figure 20 indicates the changes in the total fatty acid content during development of oil palm fruit mesocarp. Although there was a decrease in the relative proportions of 18:2 and 18:3, total fatty acids showed a net increase in absolute quantity (with a rapid phase of increase between 16 and 20 WAA) during development. Appelqvist (1975) has reported that during the development of oilseeds, all fatty acids record an increase in absolute weight although their relative proportions may change. A similar trend has been indicated in Figure 20 for developing oil palm fruit.

Studies on the changes in the fatty acid composition during development of several oilseeds indicate that the levels of the unsaturated fatty acids, particularly 18:3, were higher during the early stages of development than towards the latter stages. (Hitchock and Nichols, 1971; Appelqvist, 1975; Gurr, 1980). The changes in the fatty acid composition of the total lipids and the lipid classes of developing oil palm fruit have been investigated by Crombie and Hardman, (1958) Bafor and Osagie (1986) and Oo *et al* (1986). The results reported by these authors are in agreement to the trends reported in this investigation, that more unsaturated fatty acids, particularly 18:3, were present in the early stages of fruit development and that higher levels of saturated fatty acids were present in the monoacylglycerol and free fatty acid classes when compared to the triacylglycerols of the corresponding stages of development.

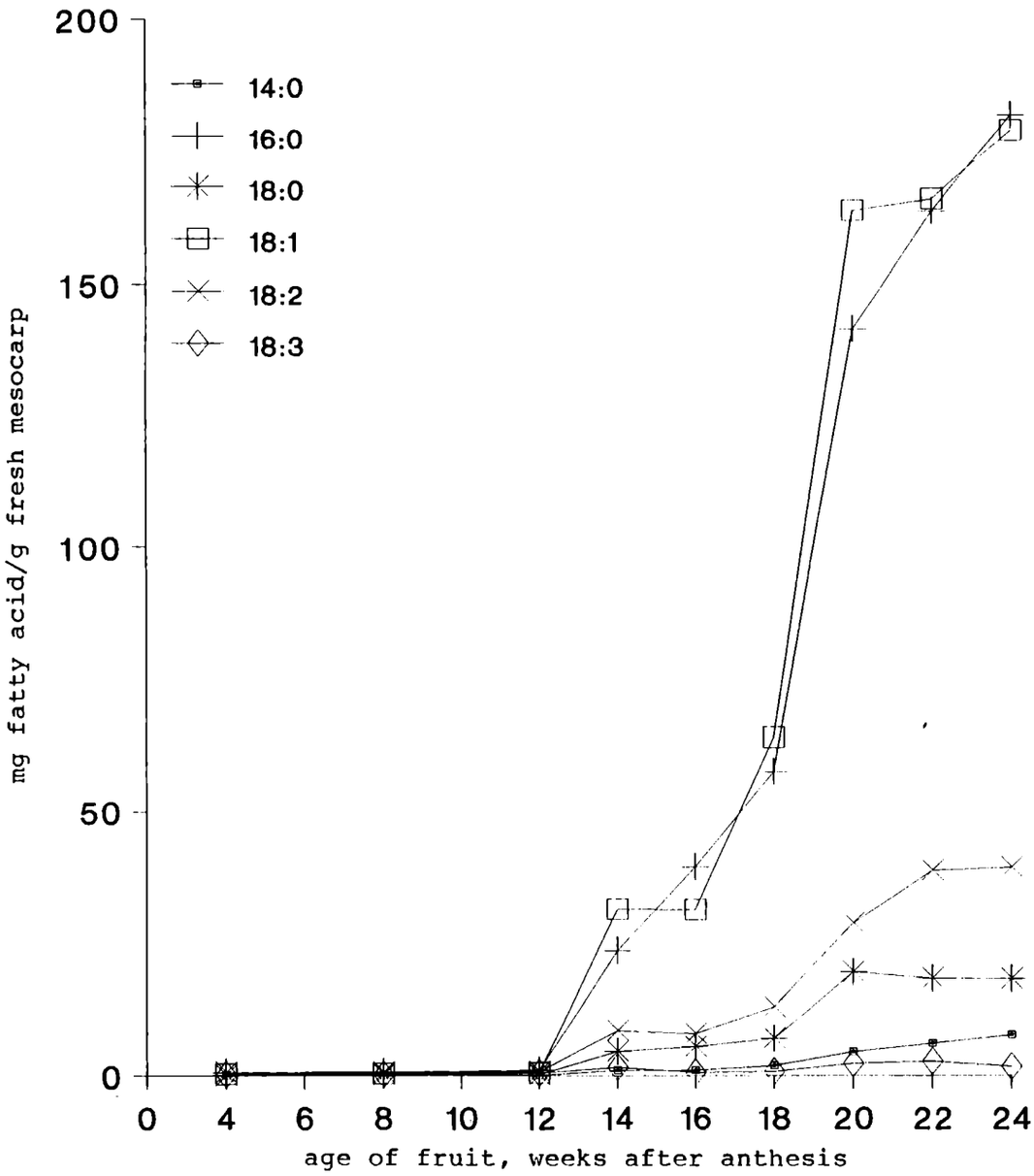


Figure 20. Changes in the fatty acid content of developing oil palm fruit mesocarp.

3.2.1. Structure and Composition of Triacylglycerols of Developing Oil Palm Fruit

The physico-chemical properties of a fat are not only the function of the overall fatty acid composition but are significantly influenced by the way the fatty acids are assembled in the triacylglycerol molecule during biosynthesis. It is known that both intramolecular as well as intermolecular distribution of fatty acids in the triacylglycerol determine the functional properties of a fat (Formo et al, 1979; Manganaro et al, 1981; Wada and Koizumi, 1983; Pease, 1985; Neff et al, 1992). The final composition of a fat in terms of the triacylglycerol species is ultimately the result of an abundance of individual fatty acids available for triacylglycerol biosynthesis (Roehm and Privett, 1970; Wilson and Rinne, 1978; Bafor and Osagie, 1989). Further, the concentration of fatty acids is known to undergo tremendous changes during development of the fat tissue (Hitchock and Nichols, 1971; Appelqvist, 1975). It is known from studies on the changes in triacylglycerol molecular species by several workers for crambe seeds (Gurr et al, 1972), corn kernel (Weber, 1973) and soybean (Roehm and Privett, 1970; Wilson and Rinne, 1978) during development that not only composition of the fatty acids change but also that of the triacylglycerols. Similar studies on developing oil palm fruit are confined to only fatty acid composition. (Bafor and Osagie, 1986; Oo et al, 1986). To understand the correlation between abundance of fatty acids and the triacylglycerol structure and composition, it is essential

to follow systematically various stages of development of the fat tissue.

3.2.1.1. Positional distribution of fatty acids in the triacylglycerols of developing oil palm fruit

Oil palm fruits of tenera variety of different stages of development (4 to 24 weeks after anthesis, WAA) were collected. The total lipids of fruit mesocarp of each stage of development were extracted. The triacylglycerol class was isolated from the total lipids by TLC and quantitated by GLC. Fatty acid compositions of the triacylglycerols were determined. The total triacylglycerol of each developmental stage were subjected to hydrolysis with porcine pancreatic lipase (Section 2.28). Fatty acid composition of the monoacylglycerols thus formed gave the composition of the sn-2-position of the original triacylglycerols (Figure 5). Fatty acid composition of the combined 1,3-positions of the triacylglycerols was computed following the method of Coleman (1964).

Formation of triacylglycerol in the developing oil palm fruit: The biosynthesis of lipids is shown in Table 27. The formation of lipids in the developing oil palm fruit has been discussed elsewhere but is also described here in the context of formation of fatty acids and triacylglycerol. The total fresh mesocarp content showed a gradual increase from 4 WAA to 20 WAA. Lower mesocarp content for 24 WAA fruit could be attributed to variations in the size of the fruit. In the case

Table 27 Accumulation of Total Lipid and Triacylglycerol in Developing Oil Palm Fruit Mesocarp

Age of fruit in weeks after anthesis	Fresh meoscarp (g/fruit)	Total lipid (g/100 g fresh mesocarp)	Triacylglycerol (g/100g lipid)	Triacylglycerol (g/100g fresh mesocarp)
4	1.68±0.9	0.22	11.09±0.38	0.03
8	3.24±0.2	0.25	11.39±0.92	0.03
12	5.14±0.1	0.36	11.61±1.52	0.05
16	4.76±0.7	9.12	87.79±1.68	8.19
20	6.33±1.9	38.06	88.91±0.39	34.49
24	4.98±0.4	45.23	95.27±1.45	43.45

of total lipids, there was very little accumulation upto 16 WAA. This lag phase was followed by a rapid phase, ie., between 16 and 20 WAA during which the maximum rate of biosynthesis of lipids occurred. Contribution of triacylglycerols to the total lipid was very low till 12 WAA which could probably be due to the predominance of structural lipids during this period. The actual formation of storage lipids occurred only from 16 WAA, with a rapid rise in the triacylglycerol content, and this was consistant with the rapid formation of total lipids. The results therefore indicate that the active lipid biosynthesis in oil palm fruit was between 16 and 20 WAA, which was primarly due to formation of the storage lipid, the triacylglycerols. Similar trend has been reported by few authors for developing oil palm fruit (Oo et al 1986; Bafor and Osagie, 1988a). A narrow rapid phase of lipid formation is characteristic of many other oilseeds. (Roehm and Privett, 1970; Hitchcock and Nichols, 1971; Gurr et al 1972; Privett et al, 1973; Weber, 1973; Appelqvist, 1975; Wilson and Rinne, 1978; Cherif et al, 1979; Gurr, 1980; Sanders, 1980; Pandey and Subrahmanyam, 1988).

Fatty acid profile of the triacylglycerols: The changes in the fatty acid composition of the triacylglycerol from progressive stages of fruit development is presented in Table 28. The major fatty acids were 16:0, 18:1 and 18:2. The relative abundance of these fatty acids exhibited significant changes during fruit development. 16:0 registered an increase from 20.8 mole % at 4 WAA to 44.5% at 24 WAA. Corresponding values for 18:1 were 16.8% and 39.8%. 18:2 showed a decrease from 46.5% to 8.6% for the corresponding stages. It is further evident from the

Table 28 Distribution of Fatty Acids in the Triacylglycerols, and at the sn-2- and combined 1,3-positions of the Triacylglycerols of Developing Oil Palm Fruit

Age of fruit in weeks after anthesis	Fatty acid position	Fatty acid (mole %)						
		12:0	14:0	16:0	18:0	18:1	18:2	18:3
4	Total	2.26	1.92	20.80	3.49	16.79	46.51	8.22
	sn-2-	0.40	1.24	11.37	0.31	31.38	50.19	5.09
	1,3-	3.19	2.26	25.52	5.08	9.50	44.67	9.79
8	Total	4.64	5.32	35.62	6.04	28.82	15.14	4.42
	sn-2-	6.49	3.47	24.02	2.96	43.83	17.08	2.15
	1,3-	3.72	6.25	41.42	7.58	21.32	14.17	5.55
12	Total	5.42	1.64	33.54	8.39	21.13	23.62	6.26
	sn-2-	1.55	1.86	23.90	1.81	32.16	36.17	2.55
	1,3-	7.36	1.53	38.36	11.68	15.62	17.34	8.12
16	Total	0.84	1.13	48.75	5.72	34.59	8.49	0.48
	sn-2-	1.74	1.29	19.41	1.76	58.93	16.03	0.84
	1,3-	0.39	1.05	63.42	7.70	22.42	4.72	0.30
20	Total	0.23	1.37	42.03	4.69	43.66	7.49	0.53
	sn-2-	0.41	1.35	16.34	0.87	69.40	10.81	0.81
	1,3-	0.14	1.38	54.88	6.60	30.79	5.83	0.39
24	Total	0.78	2.04	44.53	3.87	39.81	8.62	0.34
	sn-2-	1.20	1.98	22.38	2.59	62.27	9.16	0.42
	1,3-	0.57	2.07	55.61	4.51	28.58	8.35	0.30

data, that the fatty acid composition of triacylglycerols from early stages of fruit development (4 to 12 WAA) was more or less similar but differed appreciably from the subsequent stages. Similar trend has been reported for other oilseeds (Roehm and Privett, 1970; Gurr et al, 1972; Weber, 1973; Cherif et al, 1979). Most studies so far on the fatty acid composition of palm oil are related to total lipids of developing oil palm fruit (Crombie and Hardman, 1958; Bafor and Osagie, 1986). The fact that during early stages of fruit development (upto 16 WAA) total lipids are mostly contributed by the structural lipids of membranes consisting of polar lipids with very little triacylglycerol (Oo et al, 1986; Bafor and Osagie, 1986, 1988a), studies of this nature will not reflect the triacylglycerol fatty acid profile for the entire period of fruit development. However, Oo et al, (1986) reported the fatty acid composition of the triacylglycerols of developing oil palm fruit; the study indicating a similar compositional change as presented here. The differential rate of biosynthesis of fatty acids particularly after 12 WAA and the factors responsible for were not explained by these authors. It was observed here that the transition of fatty acid composition of the triacylglycerols towards that of the normal palm oil occurred at around 16 WAA, stabilizing by 20 weeks. Interestingly, this period was synchronizing with the rapid phase of triacylglycerol synthesis in the oil palm fruit. It may be also seen that even though there was a decrease in relative percent of 18:2 and 18:3 all acids registered an actual increase in terms of absolute quantity (Figure 21). The decrease in the relative concentration of 18:2 and 18:3 was due to the greater

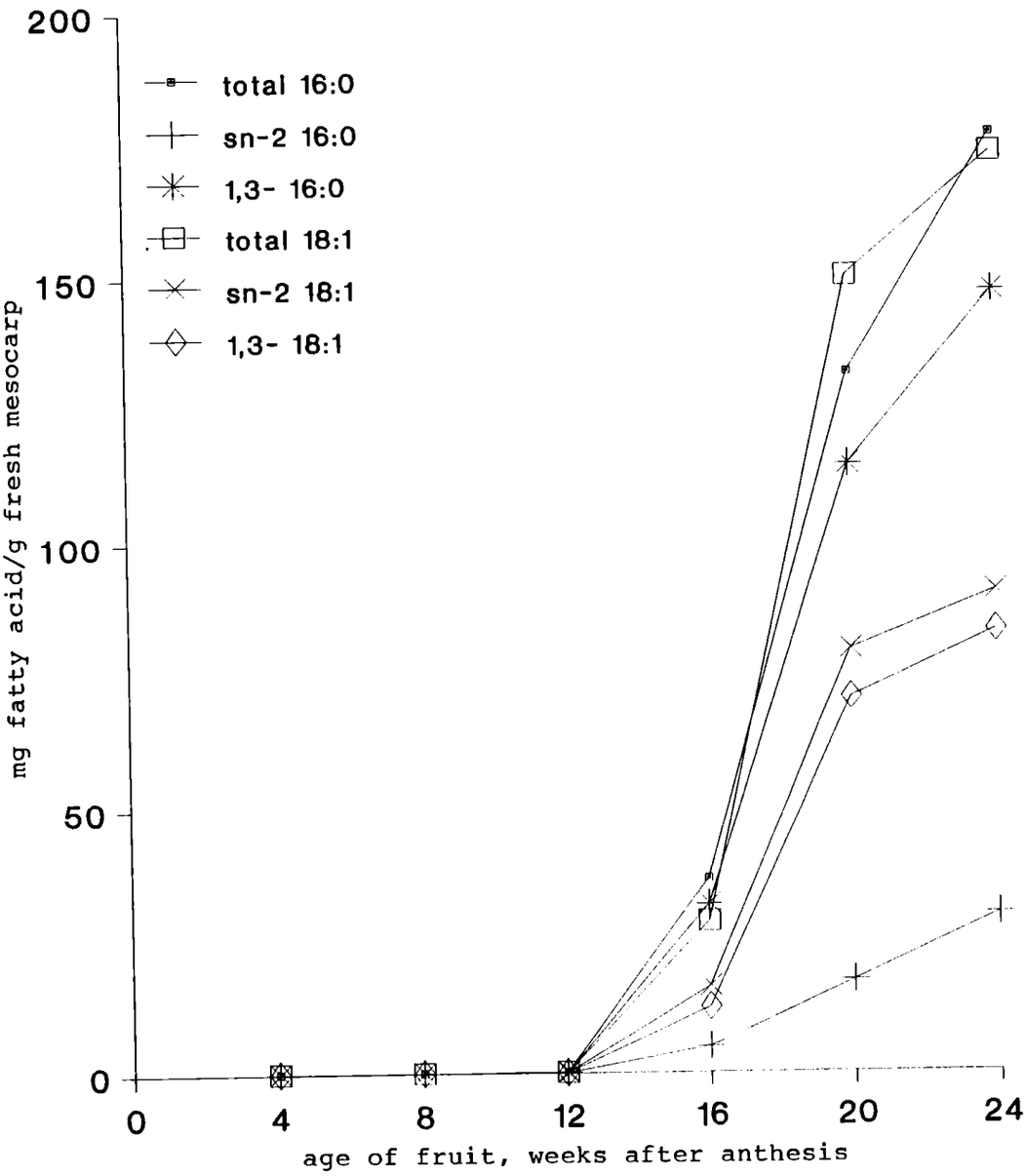


Figure 21. Changes in 16:0 and 18:1 fatty acid content of the triacylglycerols, and at the sn-2- position and combined 1,3-positions of the triacyl-glycerols of developing oil palm fruit.

rate of synthesis of 16:0 and 18:1, diluting the concentration of 18:2 and 18:3, formation of which were at lower rates. Hitchock and Nichols (1971), Appelqvist (1975) and Gurr (1980) have also indicated that there is no loss of any fatty acid during development of other oilseeds, but a difference in the rate of accumulation for the various fatty acids with stage of development. The higher turnover rate for 16:0 and 18:1 in the oil palm fruit during the latter half of development may be attributed to the activation of enzymes responsible during this period (Hitchock and Nichols, 1971).

Positional distribution of fatty acids: The distribution of fatty acids in the sn-2-position and, 1,3-positions of the triacylglycerols of developing oil palm fruit is also given in Table 28. The fatty acid profile for positions in the triacylglycerol molecules showed an overall pattern, i.e., saturated fatty acids preferring 1,3-positions with the unsaturated fatty acids showing affinity for sn-2-position, irrespective of the stage of development. However, the relative concentration of individual fatty acids in the respective positions was influenced by the abundance of the fatty acid for a given stage. As discussed elsewhere, there was a spurt in the total lipid biosynthesis around 16 WAA, with a rapid increase in all fatty acids particularly 16:0 and 18:1. From the point of high turn over rates, the newly formed 16:0 was found to be increasingly esterified to the 1,3-positions with a diminishing preference of this acid for sn-2-position (Figure 21). This higher rate of preference for the 1,3-positions by 16:0 was largely compensated for by 18:1 occupying the sn-2-position.

18:2 also exhibited a tendency to occupy sn-2-position, but not as exclusively as 18:1. The fatty acid composition of the positions also showed a clear distinction between early developmental stages and the latter stages (ie., 16 to 24 WAA) of triacylglycerol accumulation and was a consequence of and coincidental to the changes in fatty acid profile.

The positional distribution of the fatty acids in the sn-1, sn-2- and sn-3-positions of the triacylglycerol molecules influence the physical properties (Formo et al, 1979; Pease, 1985), nutritive value (Manganaro et al, 1981) and oxidative stability (Wada and Koitumi, 1983) of fats. Available studies that correlate positional distribution of fatty acids and properties of fat are (i) that the unique properties of cocoa butter, such as its sharp melting point, can be attributed to the symmetrical triacylglycerols, the mono-oleo-disaturates (Formo et al, 1979, Pease, 1985) (ii) that the atherogenicity of certain varieties of peanut oil is due to the predominance of unsaturated fatty acids in the sn-2-position of the triacylgycerol molecule (Manganaro et al, 1981) and (iii) that oxidative stability of certain fats is related to the positioning of polyunsaturated fatty acids in the sn-2-position (Wada and Koitumi 1983; Neff et al, 1982). Knowledge of the fatty acid distribution therefore assumes importance.

The distribution of fatty acids in normal palm oil has been carried out by a few authors with respect to geographic origin (Jurriens et al, 1964; Jurriens and Kroesen, 1965; Rossell et al, 1983, 1985) and variety (Jacobsberg, 1975). The results are comparable with those of the

present study for triacylglycerols of 20 and 24 WAA fruits. Positional distribution of fatty acids in the triacylglycerols from other oilseeds also demonstrate preference of the saturated acids for the 1,3-positions and unsaturated fatty acids for the 2-position (Hitchock and Nichols, 1971; Litchfield, 1972; Gurr, 1980). The consistency in fatty acid positional distribution irrespective of development stage has been observed by Roehm and Privett (1970), Privett et al, (1973) and Wilson and Rinne (1978) for soybean, Gurr et al (1972) for crambe seeds, and by Weber (1973) for maturing corn kernels.

Because of the similarity of palm oil, particularly palm mid fraction to cocoa butter (Pease, 1985) there have been attempts to use it as a cocoa butter equivalent and in this context comparisions have been made with respect to the positional distribution of fatty acids. The overall pattern is that palm mid fraction with predominantly POP compares well with POSt of cocoa butter, with comparative physical properties. Similarly, many other desirable properties of palm oil or its fractions could be attributed to the glyceride structure and therefore qualify them for formulations in shortenings, margarines, confectionery fats and so on (Pease, 1985).

3.2.1.2. Triacylglycerol composition of developing oil palm fruit

Though palm oil is one of the commercially important oils in the world, not many studies have been conducted to understand the triacylglycerols. The available reports are confined largely to overall

fatty acid composition under various conditions and only a few related to the composition and structure of the triacylglycerols of commercial palm oil (Jurriens et al, 1964; Jurriens and Kroesen, 1965; Jacobsberg, 1975; Kifli, 1975; Tan et al, 1981; Deffense, 1985; Petersson et al, 1981 Lago and Hartman, 1986). It is interesting that palm oil finds applications in both edible as well as industrial sectors primarily due to its triacylglycerol composition (Pease, 1985) which is due to the unique assembly of the fatty acids in the triacylglycerols. The present investigation has been an attempt to understand the relationship between the formation of fatty acids and triacylglycerols in the oil palm fruit during its development.

The total lipids of the mesocarp of oil palm fruits of various stages of development were extracted with chloroform-methanol solvent system. The triacylglycerols were separated from the total lipids by TLC and isolated. Total triacylglycerols were separated into various triacylglycerol classes by thin-layer chromatography on silica gel G adsorbent impregnated with 10% silver nitrate (Section 2.2.2.4). Total triacylglycerols and the triacylglycerol classes of each developmental stage were quantitated and their fatty acid compositions determined by GLC.

The proportion and fatty acid composition of the total triacylglycerol and the triacylglycerol classes obtained by argentation thin-layer chromatography of different stages of fruit development is presented in Table 29. The separation of the triacylglycerols by Ag^{+} TLC technique under the chromatographic conditions described here has been

Table 29 Argentation Thin-layer Chromatography of Triacylglycerols of Developing Oil Palm Fruit.
Proportion and fatty acid composition of the triacylglycerols and triacylglycerol classes.

Age of fruit in weeks after anthesis	Total triacyl-glycerol u mole/g mesocarp	Triacyl-glycerol class	Proportion of triacyl-glycerol class (mole %)	Fatty acid (mol %) 12:0	14:0	16:0	18:0	18:1	18:2	18:3	No. of double bonds per molecule
4	0.31			2.26	1.92	20.80	3.49	16.79	46.51	8.22	
		saturated	3.39	3.86	3.10	64.06	17.27	11.70			0.35
		monene	6.82	2.03	3.45	38.99	8.74	46.78		1.40	
		diene	3.13	3.73	3.15	33.60	9.29	42.61	7.63		1.74
		triene	29.59	1.37	2.64	40.80	5.75	21.61	27.83		2.32
		polyene	57.07	0.47	0.35	16.37	4.61	15.51	56.70	5.99	4.41
8	0.37			4.64	5.32	35.62	6.04	28.82	15.14	4.42	
		saturated	14.40	1.82	3.53	75.98	10.17	8.49			0.25
		monoene	26.37	1.35	4.14	55.87	10.22	28.42			0.85
		diene	24.27	0.97	1.75	32.27	5.49	58.12	1.40		1.83
		triene	15.44	4.11	5.54	22.87	7.24	31.96	28.27		2.67
		polyene	19.51	0.51	0.12	21.26	6.64	22.35	38.39	10.73	3.94
12	0.54			5.42	1.64	33.54	8.39	21.13	23.62	6.24	
		saturated	11.02	2.57	2.60	74.31	14.56	5.97			0.18
		monoene	11.12	3.04	2.87	48.90	10.10	35.09			1.05
		diene	7.42	4.29	2.79	32.61	9.36	47.51	3.44		1.63
		triene	21.08	1.93	2.01	43.12	9.72	18.78	24.43		2.03
		polyene	49.36	0.86	0.42	26.42	6.84	19.37	36.94	9.16	3.62
16	96.63			0.84	1.13	48.75	5.72	34.59	8.49	0.48	
		saturated	13.00	1.14	2.96	82.96	11.14	1.49			0.04
		monoene	37.98	0.61	0.60	61.80	5.33	31.65			0.95
		diene	25.95	0.47	0.37	29.51	6.40	61.81	1.45		1.94
		triene	11.28	0.45	0.64	33.12	6.61	37.64	21.54		2.42
		polyene	11.80	0.59	0.69	26.53	6.13	31.66	31.95	2.44	3.09
20	406.72			0.23	1.37	42.03	4.69	43.66	7.49	0.53	
		saturated	12.94	0.15	1.27	74.55	16.32	7.71			0.23
		monoene	32.56	0.51	1.27	57.46	6.71	34.04			1.02
		diene	29.75	0.35	0.99	29.07	1.29	63.42	4.89		2.20
		triene	12.44	0.70	1.81	23.57	4.26	44.42	25.25		2.85
		polyene	12.31	1.03	1.25	27.98	4.02	30.95	32.40	2.37	3.09
24	512.38			0.78	2.04	44.53	3.87	39.81	8.62	0.34	
		saturated	10.82	0.99	4.89	83.53	7.09	3.49			0.10
		monoene	32.32	0.48	2.73	58.99	3.56	34.24			1.03
		diene	30.84	0.96	1.42	33.23	1.24	57.36	5.79		2.07
		triene	11.19	0.48	2.53	22.19	5.49	42.80	26.51		2.87
		polyene	14.83	0.58	1.75	26.00	2.42	28.32	39.39	1.54	3.35

described elsewhere (section 3.1.2.2). The following five fractions or triacylglycerol classes were identified, 'saturated', 'monoene', 'diene', 'triene' and 'polyene' triacylglycerols based on their degree of total unsaturation. It is seen from the Table that the major triacylglycerol classes in mature oil palm fruit 24 WAA were the monoenes and dienes. Similar separations of palm oil triacylglycerols have been obtained by Jurriens et al (1964), Jurriens and Kroesen (1965) and Tan et al (1981) by argentation thin-layer chromatography of the triacylglycerols of palm oil. Higher levels of triene and polyene triacylglycerol classes were present in the earlier stages of development.

Fatty acid profile of the triacylglycerols classes: Fatty acid composition of the total triacylglycerols indicated significantly higher unsaturation at the early stages of development viz., 4,8 and 12 WAA as compared to the later stages, viz., 16, 20 and 24 WAA. It was also observed that there was a rapid phase of fatty acid biosynthesis from 16 WAA during which more than 80% of the fatty acids were accumulated. There was a remarkable increase in saturation largely due to 16:0 and also a corresponding reduction in unsaturation primarily attributed to the reduction in 18:2 and 18:3 with a concomitant increase in 18:1.

The fatty acid profile of the triacylglycerol classes has revealed an assocation of fatty acids with certain triacylglycerol classes in developing oil palm fruits. The salient observations with regard to the association of fatty acid and triacylglycerol class are summarized in Figures 22 and 23. 16:0 and 18:1 being the major fatty

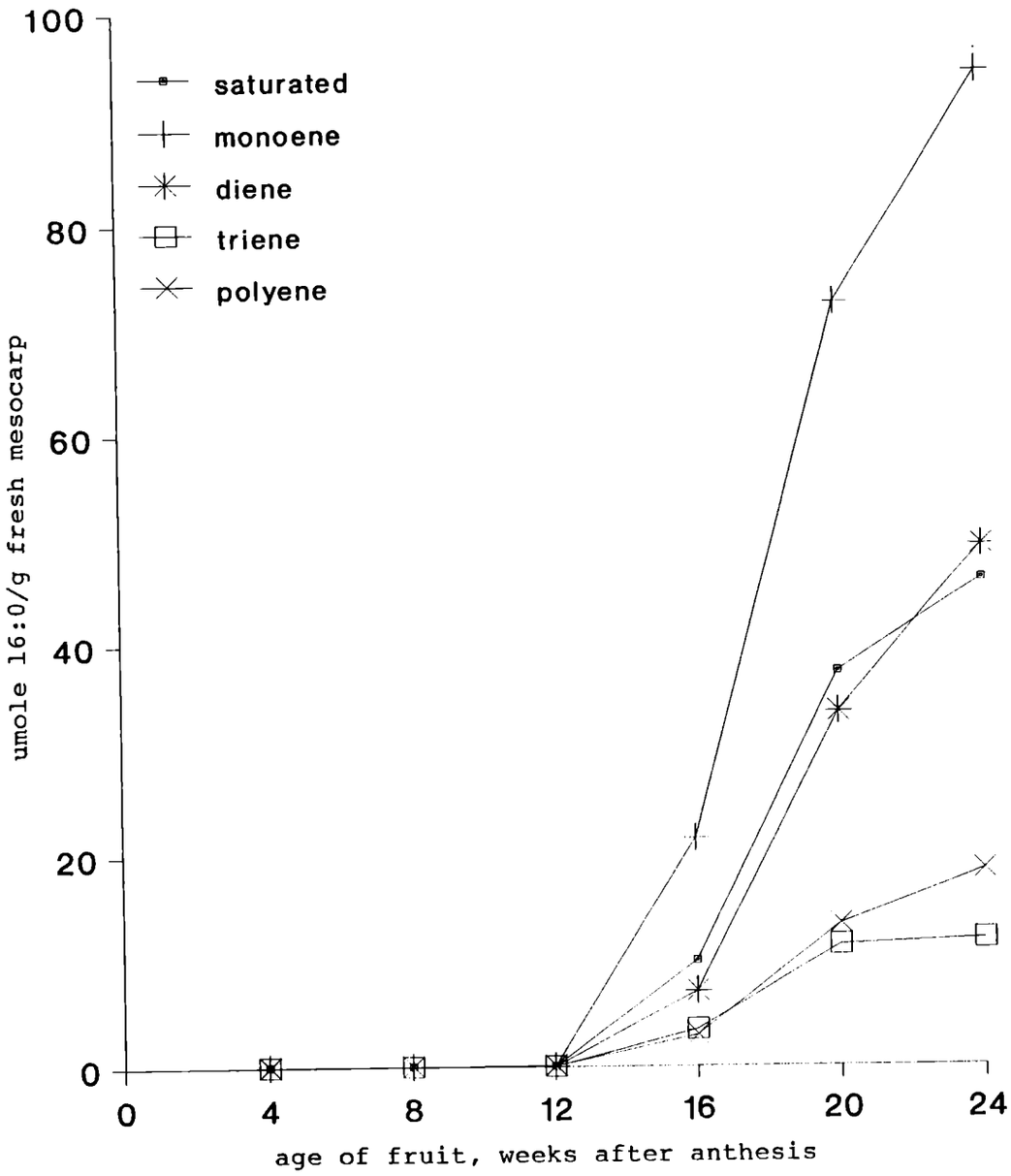


Figure 22. Incorporation of 16:0 in the triacylglycerol classes of developing oil palm fruit.

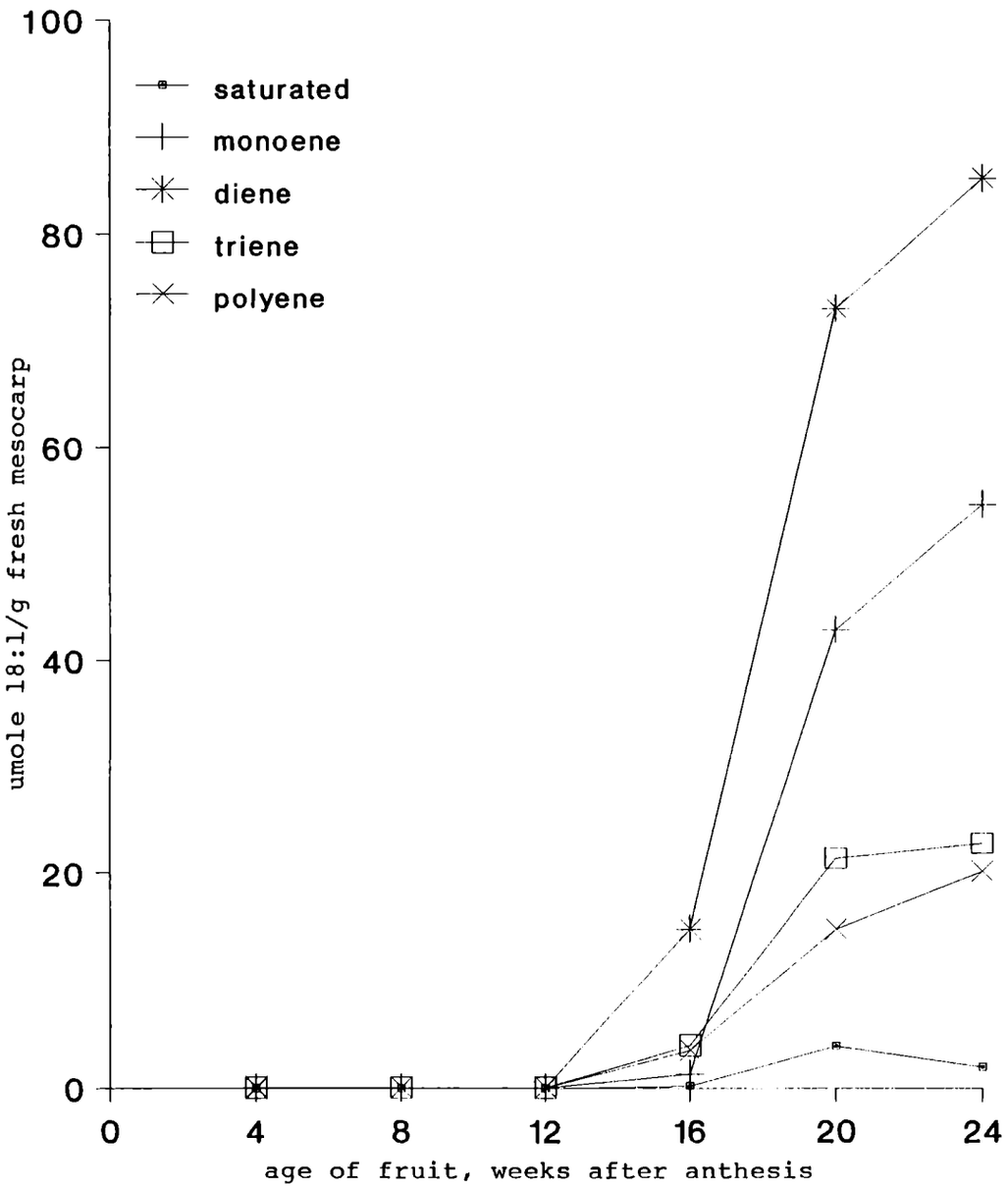


Figure 23. Incorporation of 18:1 in the triacylglycerol classes of developing oil palm fruit.

acids, only these fatty acids are represented in the Figures. It could be seen from Figure 22 that the rate at which 16:0 incorporated to different triacylglycerol classes is disproportionate during fruit development. For any given stage, more than 40 percent of the total 16:0 was associated with the monoene. Further, from 16 WAA the rate of incorporation of 16:0 to the monoene was found to be at a faster rate in consonance with the higher rate of accumulation of this fatty acid. Correspondingly, the other triacylglycerol classes received lower rate of incorporation of this acid.

Distribution of 18:1 in the triacylglycerol classes as shown in Figure 23 domonstrates that 18:1 was associated with the diene triacylglycerols. After 16 WAA, with the increasing synthesis of 18:1, a greater proportion of 18:1 was found to be incorporated into the diene triacylglycerols.

The composition of the triacylglycerol classes obtained by Ag^{+}TLC was calculated and given in Table 30. The component triacylglycerols in each triacylglycerol class were determined according to the procedures of Blank et al (1965) and Gunstone and Padley (1965). The following assumptions were considered - position of the band or fraction on the TLC plate, fatty acid composition of the fraction and the theoretical order of elution of triacylglycerols as predicted by Gunstone and Padley (1965). It was also assumed that each fraction or class did not contain triacylglycerols with the same number of double bonds since the number of double bonds calculated per molecule in a class was not exactly a whole number (Blank et al, 1965). Corrections

Table 30 Composition of the Triacylglycerol Classes Separated by Ag^+ TLC of Developing Oil Palm Fruits

Age of fruit in weeks after anthesis	Triacylglycerol class	Triacylglycerol (mol %)										
		SSS	SSO	SOO	SSL	OOO	SOL	LOO	SLL	OOL	SSLe	SOLe
4	saturated	64.9	35.1									
	monoene		59.66	40.34								
	diene		26.39	50.72	22.89							
	triene				68.19	16.51	15.3					
	polyene								82.03			17.97
8	saturated	74.53	25.47									
	monoene	14.74	85.26									
	diene		17.24	78.56	4.2							
	triene				34.44	15.16	50.40					
	polyene								67.81			32.19
12	saturated	82.09	17.91									
	monoene		94.73	5.27								
	diene		36.83	52.85	10.32							
	triene			23.76	73.29	2.95						
	polyene								72.52			27.48
16	saturated	95.53	4.47									
	monoene	5.05	94.95									
	diene		5.87	89.78	4.35							
	triene				57.84	35.38	6.78					
	polyene								92.68			7.32
20	saturated	76.87	23.13									
	monoene		97.87	2.13								
	diene			65.76	14.67	19.57						
	triene				15.27	24.25	60.48					
	polyene								92.89			7.11
24	saturated	89.53	10.47									
	monoene		97.28	2.72								
	diene			75.81	17.37	6.82						
	triene				12.54	20.47	66.99					
	polyene								95.38			4.62

for overlapping of bands was also made from a consideration of the fatty acid composition in relation to the adjoining bands on the TLC plate. However, in Section 3.1.2.3., for calculation of the triacylglycerol composition of mature oil palm fruits from Ag^{+}TLC data, appropriate corrections of fatty acid composition of the triacylglycerol classes were made to bring the average number of double bonds per class to a whole number according to the method followed by Jurriens et al (1964) and Jurriens and Kroesen (1965). For the triacylglycerols of immature oil palm fruits, presented here the various classes were not well resolved as seen from their fatty acid profiles and hence the method adopted earlier would not be appropriate (Blank et al, 1965).

The salient features of the findings are presented in Figure 24. The monene and diene triacylglycerols registered a faster rate of increase after 16 WAA consistent with the faster rate of biosynthesis of 16:0 and 18:1. The triacylglycerol composition in the early stages were different from that of the latter stages. The transition phase of triacylglycerol composition towards that of normal palm oil occurred concurrently with the phase of rapid triacylglycerol accumulation. In the positional analysis data (section 3.1.2.2) it was observed that more than 80 percent of the 16:0 favored 1,3-position of the triacylglycerol molecules. It could be derived that the monene triacylglycerols was predominantly SOS. Similarly, 18:1 having preference for sn-2-position, resulted in the greater formation of SOO. It could also be mentioned here that both 16:0 and 18:1, complementary in their positional preference, logically contributed to the formation of SOS and SOO. The

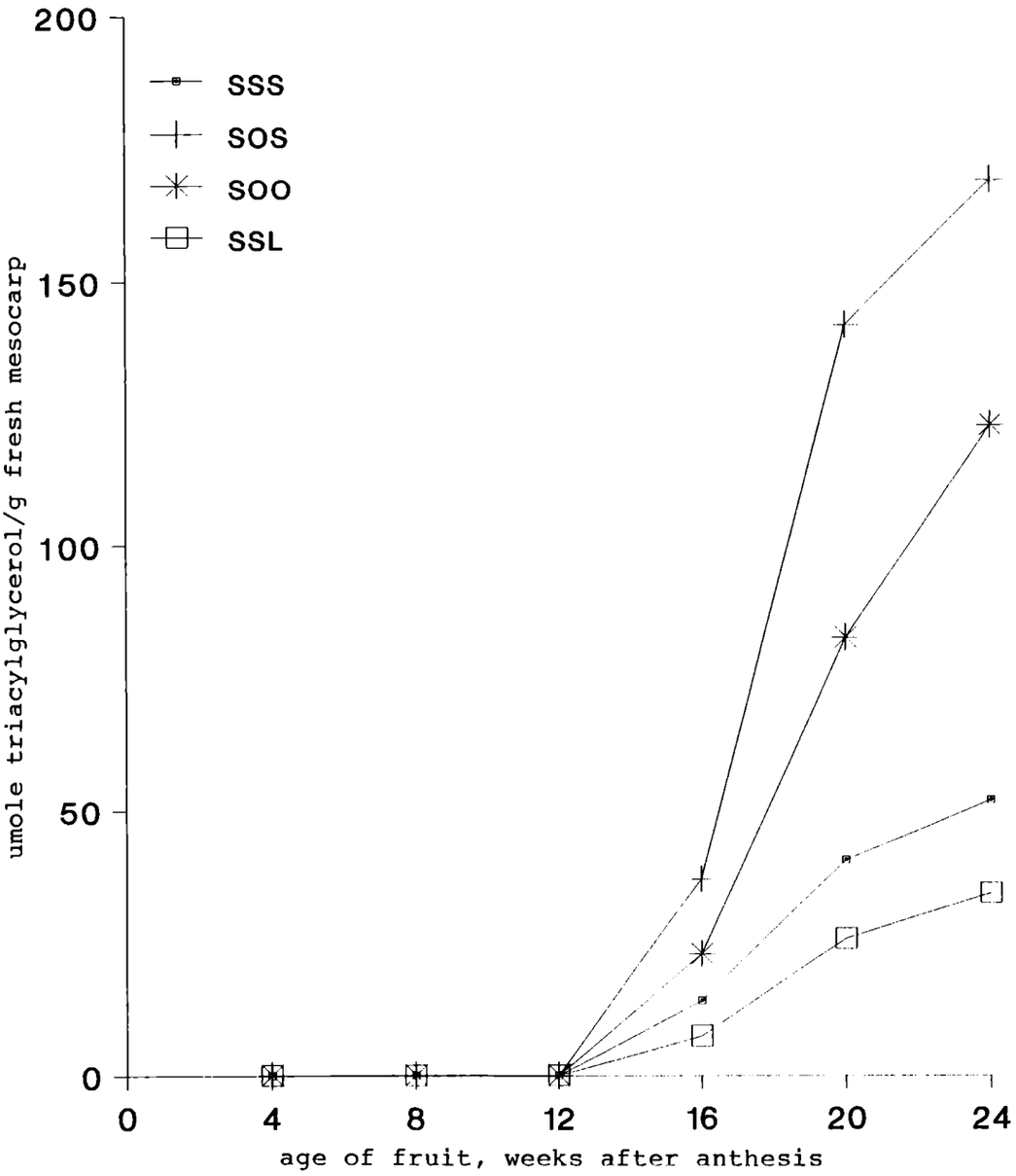


Figure 24. Changes in the saturated (SSS), monoene (SOS) and diene (SOO, SSL) triacylglycerols of developing oil palm fruit.

trisaturates (SSS) and linoleo-disaturates (SLS), the other major triacylglycerols formed in the developing oil palm fruit were mostly the products of 16:0 and 18:2 respectively.

Few authors have reported the triacylglycerol classes based on the degree of unsaturation of triacylglycerols from commerical palm oil (Jurriens et al, 1964, Jurriens and Kroesen, 1965; Tan et al, 1981) which are comparable with the results obtained in the present investigation for sample 24 WAA. With a view to obtain cocoa butter equivalents, palm oil was fractionated and the glyceride composition studied (Deffense, 1985; Pease, 1985). These results indicate that a fraction known as Palm Mid Fraction contains predominantly POP having properties similar to cocoa butter. The triacylglycerol analysis data presented here indicate the formation of POP during the course of fruit development. Comparable studies have not been reported except that of Bafor and Osagie (1989). These authors, based on Ag^{+}TLC data suggested the biosynthetic pathway for triacylglycerol formation. Available data on other oilseeds - soybean (Roehm and Privett, 1970; Wilson and Rinne, 1978) crambe seeds (Gurr et al, 1972) and corn kernel (Weber, 1973) demonstrate that the relative proportion of unsaturated triacylglycerols decrease progressively with seed development similar to the results obtained here for oil palm fruit.

3.3 LIPID PROFILE OF PROCESS STREAMS OF PALM OIL MILL

Commercially, palm oil is extracted from the mesocarp of the oil palm fruit following a wet rendering process. The essential steps consist of sterilization stripping, digestion, extraction, clarification and purification, Figure 25 (Arumughan et al, 1989, 1991; Sundaresan, et al , 1990). During these operations, the fruits and crude palm oil are subjected to varying degrees of thermal and mechanical stresses in order to obtain maximum yield of oil and at the same time preserving the quality of the end product. Nevertheless, 5 to 10% of the total oil present in the raw material is lost and the quality of the oil also suffers depending on the process and harvesting conditions (Eng and Tat, 1985). In the palm oil mill, oil loss occurs through the sterilizer condensate (sterilization), press fibre (pressing) and sludge effluent (clarification). These are generally known as the waste streams of palm oil processing (Berger, 1983). There is a tendency among millers to recycle the oil from these waste streams particularly from the sterilizer condensate and sludge in order to maximize the yield. This could affect overall quality of the end product.

Investigations on the composition and quality of the oil from waste streams and the product during progressive stages of milling are scanty and confined to a few parameters (Johansson and Persmark, 1971; Bek-Nielsen, 1972; Chin and Tan, 1977; Yeoh, 1977; Goh et al, 1982; Chong and Gapor, 1983; Jacobsberg, 1983; Let and Top, 1985; Choo, et al, 1990; Kuntom, 1991; Jideani, 1992). Reports are often limited to one

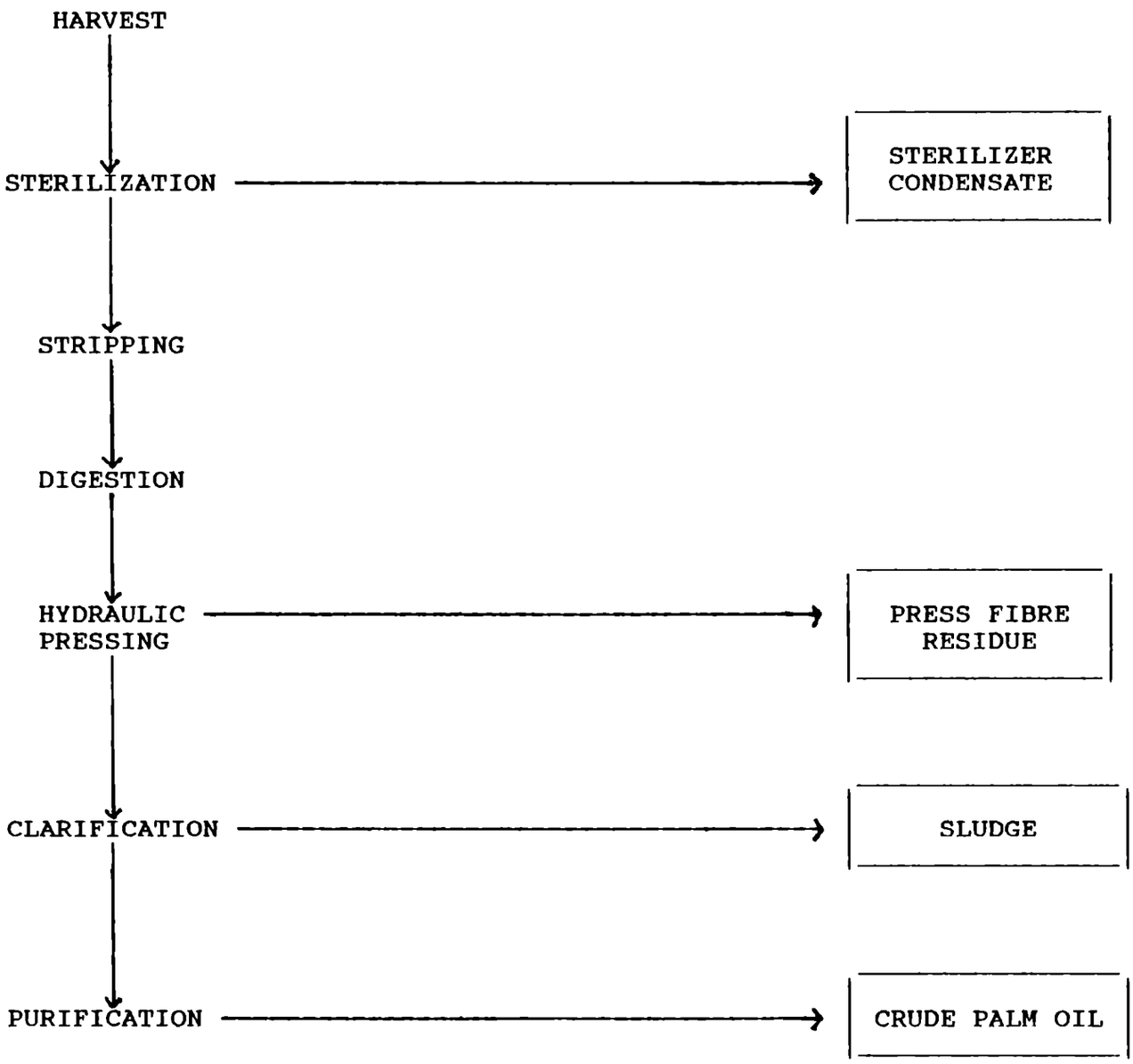


Figure 25 Flow chart indicating the various stages of extraction of palm oil and the waste streams from which samples were collected for detailed analysis

particular stage of operation (de Vries and Sue, 1985; Tan, 1985; Soon and Lan, 1985; Chow et al, 1987; Siew, 1992). So far, a comprehensive study regarding lipid composition of the waste streams has not been reported. This study attempts to follow the compositional variation of lipid classes and their constituent fatty acids as well as the quality of the oils of the various process streams as compared to the end product under actual commercial conditions of palm oil extraction.

The different process streams, viz., sterilizer condensate, sludge water, press fibre residue and crude palm oil were sampled by operating the Demonstration plant for Palm Oil established at C.P.C.R.I., Palode, Trivandrum. The process details have been reported earlier (Sundaresan et al, 1990). The collection of the various process streams from which the samples were taken for detailed analysis is described in Section 2.2.1.3 and indicated in Figure 25.

Total lipids were extracted from the fresh mesocarp of oil palm fruits, press fibre residue, sludge water and sterilizer condensate as described elsewhere. The total lipids of the various streams, viz., sterilizer condensate, press fibre residue, sludge, sterilizer condensate, crude palm oil and from fruits were separated into triacylglycerols, diacylglycerols, monoacylglycerols, free fatty acids, phospholipids and glycolipids by TLC.Individual lipid classes of all process streams were estimated (Section 2.2.7.1). Fatty acid composition of each lipid class was determined by GLC.

Lipid profile: The values presented in Table 31 for the composition of lipid classes were obtained for the various process steps as described in section 2.2.1.3. The results show an appreciable variation among the process streams. Sterilizer condensate contained the lowest levels of triacylglycerols (54.5%), whereas oil extracted with solvent from fresh mesocarp had the highest levels (97.0%). Corresponding values for free fatty acids were 24.0% and 0.7% respectively, for these samples. Partial glycerides also showed significant variations. Solvent extracted oil from fresh oil palm fruits had the lowest levels of diacylglycerols (2.0%) and monoacylglycerols (0.2%). Higher levels were present in the oils from the waste streams. The distribution of the polar lipids, phospholipids and glycolipids exhibited a much greater variation when compared to the neutral lipid classes. For instance, crude palm oil contained the lowest amounts of phospholipids and glycolipids whereas press fibre had nearly 20 to 50 times greater levels of these lipids respectively.

Total lipids from fresh mesocarp of unbruised fruits of correct maturity were extracted with solvent to determine the lipid composition actually present in oil palm fruits without being altered by process conditions. Values reported by other authors for triacylglycerol content of mature oil palm fruit mesocarp show great variation from 98% (Jacobsberg, 1983) to 78% (Oo *et al*, 1986). These differences can be attributed to maturity of the fruit and method of extraction of lipids. The high value for triacylglycerol of 97.1% with low values of 2.0% for diacylglycerol, 0.2% for monoacylglycerol and 0.7% for free fatty acids

Table 31 Lipid Composition of Oils From Various Process Streams of Palm Oil Mill

Lipid class	Sterilizer condensate	Press fibre	Sludge	Crude palm oil	Solvent extracted oil from fresh mesocarp
Neutral lipid (relative %)					
Triacylglycerol	54.9	65.4	72.8	93.0	97.1
Diacylglycerol	11.5	16.6	10.1	4.5	2.0
Monoacylglycerol	9.6	5.6	6.2	0.9	0.2
Free fatty acid	24.0	12.5	10.9	1.5	0.7
Polar lipid (ppm total lipid)					
Phospholipid	6721	25975	6636	1443	5633
Glycolipid	13925	20311	1139	438	2492

reported here indicate that fruits extracted under proper conditions will have very low levels of free fatty acid and partial glycerides with maximum triacylglycerol, as actually present in the fresh fruit. These values agree with that of Jacobsberg and Ho (1976) and Jacobsberg (1983). Any deviation from this composition can be attributed to post-harvest conditions in the field and in the mill.

In a typical palm oil mill, universally practiced process steps are- sterilization, stripping, digestion, pressing, clarification and purification. The major oil loss occurs through the sterilizer condensate, press fibre and sludge, with an approximate oil loss of 2%, 6% and 2%, respectively assuming 90% recovery as crude palm oil.

During milling operations, the palm fruits are subjected to varying degrees of thermal and mechanical abuse, resulting in chemical and quality alterations of the oil. Sterilization was conducted at steam pressure of 3 kg/cm^2 (equivalent to 130^oC) for 1 hour. During this process, about 50% of the total steam requirement for palm oil processing was consumed. The condensate obtained from this step carried about 1-2% of the total oil. Low levels of triacylglycerol (Table 31) could be due to accelerated hydrolysis at elevated temperature, which was further confirmed by the high levels of free fatty acid and partial glyceride, as reported here. High levels of polar lipids in condensate indicate that more structural lipids from the fruit exocarp (outer skin) were extracted. Therefore, the oil present in the condensate may also be derived from the exocarp. Bek-Nielsen (1972) and Eng and Tat (1985) have reported that oil from the condensate was heavily contaminated with

iron and was in a highly oxidized state. Bek-Nielsen (1977a,1977b, 1979) has recommended against the recycling and mixing of this recovered oil with production oil.

The loose fruits obtained after stripping of the sterilized bunches were converted into a mash in a digestor maintained at 95^{o}C with live steam (Sundaresan et al, 1990). This digested mash was then subjected to hot pressing to extract the crude oil-water mixture. Highest oil loss (6.0%) occurred at this stage because oil is entrained in the press fibre residue. The press fibre contained cellulosic fibre, fruit exocarp (skin) and calyx along with the seed. The oil content of the press fibre and the lipid composition of this oil as reported here showed exceptionally high levels of polar lipids and partial glycerides (Table 31). The study on the distribution of lipids within the fruit, viz., exocarp and mesocarp (Section 3.1.3) confirmed that exocarp contained markedly higher levels of polar lipids. These lipids are structural components of membranes and not being easily extractable by the method adopted here, are retained in the press fibre residue oil. Goh et al (1982) have reported high values for phospholipids from press fibre waste. According to Bek-Nielsen (1979) solvent extraction of residual oil from the fibre would extract a low- quality oil containing phosphatides and other nonglyceride impurities. High levels of partial glycerides in the press fibre (Table 31) could be attributed to an adsorptive property of the fibrous residues.

The oil-water mixture from the press was subjected to clarification at 95^{o}C to separate the crude palm oil from the watery

sludge (Figure 25). Oil recovered from the sludge had high contents of phospholipids (6636 ppm) and glycolipids (1139 ppm). Goh et al (1982) have shown that oil from sludge water has appreciable levels of these lipids since substantial amounts of hydratable polar lipids are removed along with the water phase during milling. The higher levels of partial glycerides obtained here could be due to their greater water solubility as compared to triacylglycerol.

In this experiment, about 90% of the oil present in the fresh fruit was obtained as crude palm oil, the final product stream. Composition of the different lipid classes of commercial palm oil has been reported by several authors in studies relating to crystallization (Jacobsberg and Ho, 1976; Okiy, 1978; Goh and Timms, 1985; van Putte and Bakker, 1987). The values obtained here (Table 31) fall within the range. However, when compared to the oil extracted with solvent from fresh mesocarp the lower content of triacylglycerol could be due to the hydrolysis of triacylglycerol resulting in relatively higher diacylglycerol, monoacylglycerol and free fatty acid fractions during milling. Solvent extraction removes the entire polar lipids present in the fruit which explains higher content of these lipids. Commercial crude palm oil is obtained by a wet extraction process during which the structural lipids are not extracted, thus explaining their lower levels in crude oil.

Fatty acid profile: Fatty acid compositions of the total lipids of the various streams of the palm oil mill are given in Table 32. Except for sterilizer condensate, other streams did not show

Table 32 Fatty Acid Composition of Total Lipids From Various Process Streams of Palm Oil Extraction

Fatty acid (wt. %)	Sterilizer condensate	Press fibre	Sludge	Crude palm oil	Solvent extracted oil from fresh mesocarp
12:0	0.1	0.1	0.1	0.1	0.1
14:0	1.8	1.2	1.3	1.4	1.3
16:0	49.2	42.6	43.3	44.5	40.2
18:0	4.6	4.5	4.6	4.7	4.9
18:1	36.7	38.9	39.5	38.9	40.6
18:2	7.3	11.6	10.8	10.0	12.3
18:3	0.3	1.1	0.4	0.4	0.6

appreciable variations in their fatty acid profiles. Sterilizer condensate oil contained greater proportion of saturated fatty acids and a corresponding lower unsaturated fatty acid content. Greater proportion of saturated fatty acids and correspondingly lower unsaturated acids in sterilizer condensate could be due to thermal oxidation of unsaturated fatty acids during sterilization. With respect to fatty acid composition of other streams, the values reported in this study are compatible with commercial crude palm oil (Chin, 1979; Chin et al, 1982; Maclellan, 1983; Rossell et al, 1983, 1985; Tan et al, 1983a; Chow et al, 1987).

Table 33 shows the fatty acid compositions of the phospholipid and glycolipid classes of various streams of the palm oil mill. Figure 26 indicates the proportion of the major fatty acids, 16:0, 18:1 and 18:2 present in the various lipid classes. It is interesting to note the association of 18:2 and 18:3 with the polar lipids. While 18:2 was mainly associated with phospholipids, 18:3 was primarily found to be in the glycolipid fractions. Furthermore, it can be stated that most of the 18:3 present in the fresh mesocarp lipids was concentrated in the glycolipid fraction, as the concentration of this acid is negligible in the total lipids. This association of 18:2 with phospholipids and of 18:3 with glycolipids has been observed by Goh et al (1982) for crude palm oil and by Bafor and Osagie (1986) and Oo et al (1986) in the developing oil palm fruit and elsewhere in this investigation. Except for sterilizer condensate other streams had more or less similar fatty acid profiles for polar lipids. In case of sterilizer condensate, unsaturated fatty acids were appreciably lower for reasons already stated.

Table 33 Fatty Acid Composition of Phospholipids and Glycolipids of Palm Oil Process Streams

Process stream	Lipid class	Fatty acid (wt. %)						
		12:0	14:0	16:0	18:0	18:1	18:2	18:3
Sterilizer condensate	Phospholipid	0.4	2.3	45.4	5.0	37.8	8.9	2.2
	Glycolipid	0.8	1.5	53.6	5.0	31.9	5.0	0.2
Press fibre	Phospholipid	0.1	0.4	31.1	1.5	40.6	24.2	2.1
	Glycolipid	0.2	0.6	23.8	2.6	30.8	17.7	24.3
Sludge	Phospholipid	0.5	0.7	37.5	3.1	39.2	16.3	2.7
	Glycolipid	0.6	1.4	35.9	3.6	30.3	11.7	16.5
Crude palm oil	Phospholipid	-	2.1	38.4	4.6	42.6	11.7	0.6
	Glycolipid	0.2	1.2	40.2	4.8	36.0	11.4	6.2
Solvent-extracted oil	Phospholipid	-	2.0	35.1	2.6	35.1	24.6	0.6
	Glycolipid	2.0	3.6	30.5	5.4	32.7	14.9	10.9

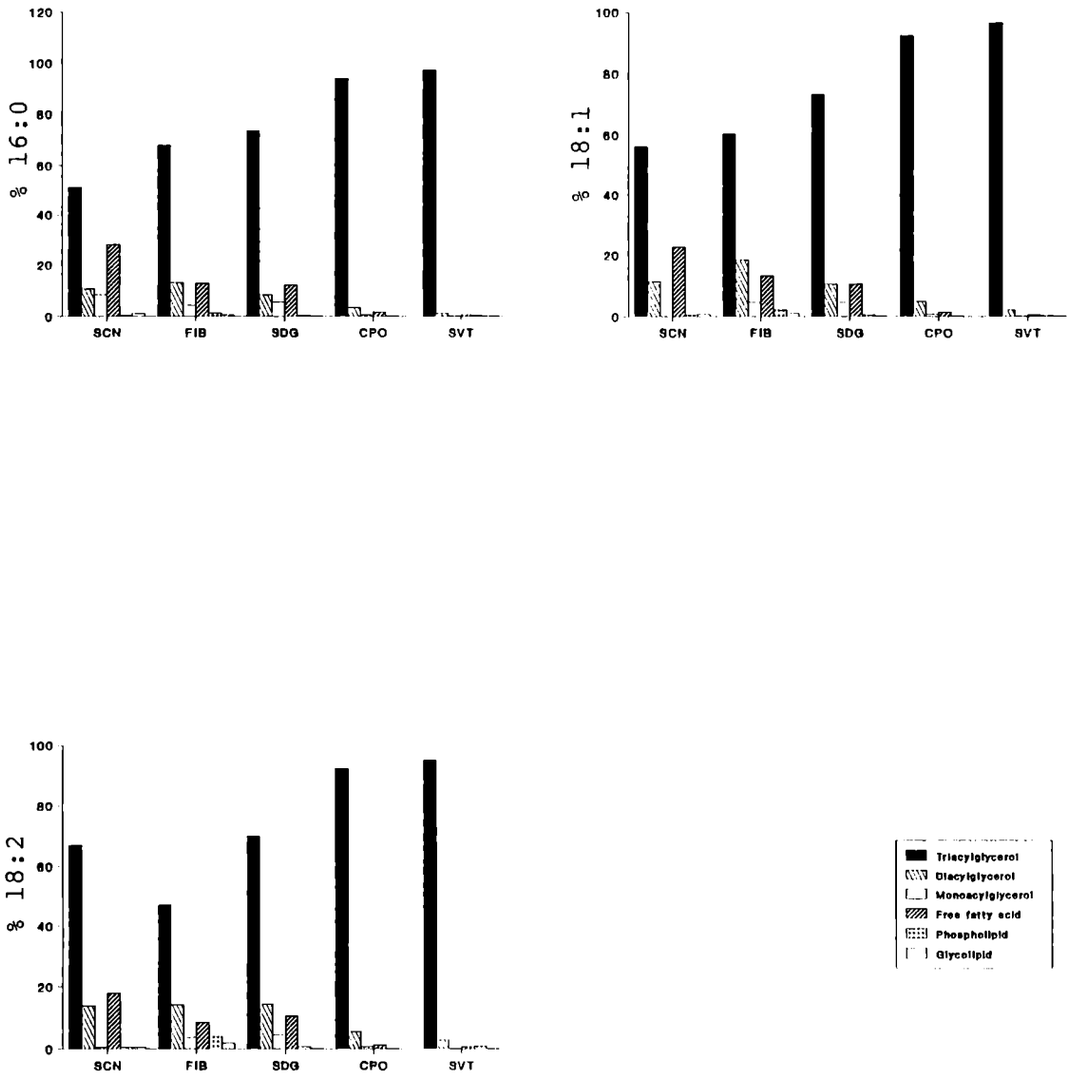


Figure 26. Proportion of 16:0, 18:1 and 18:2 in the lipid classes of the various process streams of palm oil mill. SCN - sterilizer condensate; FIB - press fibre residue; SDG - sludge; CPO - crude palm oil; SVT - solvent extracted oil.

The fatty acid composition of the various neutral lipid classes, viz. triacylglycerol, diacylglycerol, monoacylglycerol and free fatty acid, are presented in Table 34 and the proportion of 16:0, 18:1 and 18:2 in these lipid classes is given in Figure 26. Perusal of this Table shows no marked deviation in fatty acid distribution among the neutral lipid classes from the various process streams. This suggests that although there was significant differences in the distribution of the neutral lipid classes, the relative percentage of the component fatty acids were not subject to great variations due to selective hydrolysis or to process conditions. However, there was a slight reduction in the total unsaturation in the end product (Crude Palm Oil). Earlier reports for fatty acid composition of neutral lipid classes extracted from mature fruits with respect to development studies agree with those reported here (Bafor and Osagie, 1986; Oo et al, 1986).

The above findings demonstrate the drastic difference in oils from the various process streams in terms of lipid composition and quality. There is a tendency among the palm oil processors to recycle waste stream oils to obtain higher oil yield. The high levels of partial glycerides, free fatty acids and polar lipids in the oil from sludge and sterilizer condensate, when mixed with the end product, would impair oil quality on storage and subsequent refining processes (Maclellan, 1983; Goh et al, 1985; Jacobsberg, 1988).

Table 34 Fatty Acid Composition of Triacylglycerol, Diacylglycerol, Monoacylglycerol and Fatty Acid Classes of Process Streams of Palm Oil Extraction

Lipid class	Process stream	Fatty acid (wt. %)						
		12:0	14:0	16:0	18:0	18:1	18:2	18:3
Triacylglycerol	Sterilizer condensate	0.1	1.4	44.9	5.2	37.9	9.6	0.9
	Press fibre	0.7	2.2	47.6	3.6	36.5	9.2	0.2
	Sludge	0.1	1.4	43.7	4.2	40.5	10.0	0.1
	Crude palm oil	0.1	1.3	45.0	4.8	38.5	9.8	0.5
	Solvent-extracted oil	-	1.4	41.2	4.9	41.0	11.2	0.3
Diacylglycerol	Sterilizer condensate	0.2	1.4	45.2	5.3	37.3	9.7	0.9
	Press fibre	0.5	1.4	37.0	4.0	45.1	11.2	0.8
	Sludge	0.2	1.2	36.5	2.8	43.9	15.2	0.2
	Crude palm oil	0.5	1.3	35.3	3.4	45.7	12.4	1.4
	Solvent-extracted oil	1.2	1.4	30.7	3.6	45.4	16.9	0.8
Monoacylglycerol	Sterilizer condensate	1.0	2.3	46.3	8.0	36.4	5.7	0.3
	Press fibre	4.7	3.2	39.5	5.9	36.5	9.1	1.1
	Sludge	3.2	2.6	44.5	7.4	34.1	8.2	-
	Crude palm oil	6.3	3.8	38.9	5.8	35.7	9.2	0.3
	Solvent-extracted oil	1.4	7.0	38.0	6.3	35.5	10.8	0.9
Free fatty acid	Sterilizer condensate	0.2	1.8	54.0	4.9	33.3	5.6	0.2
	Press fibre	0.4	1.4	44.9	4.5	39.7	8.2	0.9
	Sludge	0.2	1.5	45.8	4.8	37.5	9.4	0.8
	Crude palm oil	1.3	2.1	46.8	4.2	37.4	7.9	0.3
	Solvent-extracted oil	6.8	4.2	37.5	6.2	34.5	10.3	0.4

CHAPTER 4

CONCLUSION AND FUTURE PERSPECTIVE

In the present study, lipids of oil palm fruit with particular reference to the composition and structure of the glycerides of palm oil have been investigated comprehensively. The salient features of the study are the following:

i. Oil palm is a new crop to India and oil palm plantation development is poised for massive growth to become a major source of edible oil for India in the years to come.

ii. The objectives of the study were framed to gather information on the oil palm fruit mesocarp lipids, commonly known as palm oil, derived under the agro-climatic conditions prevailing in India specifically in Kerala State. This would further provide the basic input in the quality of palm oil produced in the country.

iii. The physico-chemical characteristics of fresh oil palm fruit bunches of tenera variety under the rainfed conditions of Kerala State are comparable with those of Malayasia and elsewhere.

iv. The lipids which constitute 45% of the fresh mesocarp of the oil palm fruit comprised of lipid classes in the proportion: triacylglycerol 95%, diacylglycerol 2%, monoacylglycerol 0.5%, fatty acid 0.6%, phospholipid 0.6% and glycolipid 0.2%.

v. The fatty acid profile of the lipid classes indicated an association of fatty acid type with lipid class, viz., 16:0 with neutral lipid, 18:2 with phospholipid and 18:3 with glycolipid. This observation was not highlighted in any of the earlier reports.

vi. Characterization of the fruit lipids of the three varieties of the oil palm showed that they do not differ in their lipid composition qualitatively. However, the distribution of the lipid classes in the different anatomical regions of the fruit (mesocarp, exocarp and pericarp) exhibited remarkable differences that could be attributed to the physiological functions of the respective anatomical regions, ie., the exceptionally high levels of polar lipids in the exocarp provide the structural integrity and protective function of the exocarp. The mesocarp, being the reserve tissue contained almost exclusively of triacylglycerol with minor proportions of polar lipids and partial glycerides.

vii. The early part of fruit development was characterized by the presence of high levels of polar lipids and unsaturated fatty acids which were replaced by triacylglycerols and saturated fatty acids in the later stages.

viii. The positional distribution of fatty acids demonstrated that, of the major fatty acids (16:0, 18:1, 18:2), 16:0 was preferentially esterified to the combined 1,3-positions to the extend of 80%, whereas, 18:1 was found associated with sn-2-position to the tune of 52% with 18:2 being distributed almost evenly among the sn-1, 2, and 3-positions. During fruit development,the rate of incorporation of 16:0 and 18:1 was enhanced to the combined 1,3-positions and to the sn-2-position respectively. This is the reflection of the higher rate of biosynthesis of 16:0 and 18:1.

ix. The composition of the triacylglycerols as estimated by Ag^{+}TLC and lipase hydrolysis (Vander Wal, 1964) showed close similarity suggesting that palm oil triacylglycerol synthesis followed 1,3-random-2-random distribution.

x. Palm oil triacylglycerols were largely composed of a few triacylglycerols (POP, POO) similar to cocoa butter. (This structural similarity has been exploited to make cocoa butter equivalents from plam oil particularly from palm mid fraction). Palm oil triacylglycerols also showed a compositional shift from the higher unsaturated species (triene and polyene) to mixed types in consonance with the changes in the fatty acid profile during fruit development.

xi. Palm oil is essentially derived by wet rendering process of the fresh oil palm fruit mesocarp and hence it is distinct from other oilseeds processing. In this process, oil palm fruits are subjected to mechanical and thermal stresses that ultimately have

bearing on the quality of the oil. The characteristics of lipids from the product stream and waste streams were significantly different due to these factors, for instance, the lipids from press fibre and sterilizer condensate were enriched ten fold in polar lipids as compared to the end product, crude palm oil, which could be attributed to these lipids being released from the pericarp. The characterization of lipids of process streams threw light on the undesirability of mixing these streams to maximize oil yield at the cost of oil quality.

xii. Though data on the composition of palm oil and its fatty acids have been reported from the commercial angle, a comphrehensive study of this nature, particularly with reference to the biosynthesis of various lipid classes, positional distribution of fatty acids in the triacylglycerols, and triacylglycerol species during the course of fruit development have not been reported earlier.

xiii. The present study has also opened new directions to substantiate these analytical values - eg. following the biosynthetic route of formation of various lipid classes during development of oil palm fruit using labelled precursors. A rapid phase of lipid synthesis (16 WAA to 20 WAA) during which 90% of the lipids are synthesised suggests that there is an activation of enzymes involved in lipid synthesis, particularly with a shift from the polar lipids to the neutral ones which warrants for detailed biochemical investigations.

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APPENDIX

APPENDIX-I

LIST OF PUBLICATIONS

1. **George, S.**, and Arumughan, C., Distribution of Lipids in the Exocarp and Mesocarp of Three Varieties of Oil Palm Fruit (Elaeis Guineensis), **J. Sci. Food Agric. 56:219 (1991)**
2. Prasad, K.V.S.V., **George, S.**, and Arumughan, C., Oil Characteristics and Composition of Palm Oil Process Streams, **Indian Oil Palm Journal 1:23 (1991)**
3. **George, S.**, Arumughan, C., Lipid Profile of Process Streams of Palm Oil Mill, **J. Am. Oil Chem. Soc. 69:3 (1992)**
4. **George, S.**, and Arumughan, C., Positional Distribution of Fatty Acids in the Triacylglycerols of Developing Oil Palm Fruit, **J. Am. Oil Chem. Soc. 70:1 (1993)**
5. **George, S.**, and Arumughan, C., Triacylglycerol composition of Developing Oil Palm Fruit (Communicated)
6. **George, S.**, and Arumughan, C., Lipid Profile of Developing Oil Palm Fruit (Communicated)

APPENDIX II

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Distribution of Lipids in the Exocarp and Mesocarp of Three Varieties of Oil Palm Fruit (*Elaeis guineensis*)

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Key words: Dura, tenera, pisifera, pericarp, exocarp, mesocarp, phospholipid, glycolipid, fatty acid composition.

Distribution of lipids in the oil palm (Elaeis guineensis *Jacq*) *fruit from three varieties* (*dura, tenera, pisifera*) *were studied. Extremely high contents of phospholipids and glycolipids were noticed in the exocarp* (*outer skin*) *of the fruit compared with the fleshy mesocarp. Significant differences were also observed in the composition of the lipid classes of oil palm fruits from three varieties. High concentrations of unsaturated fatty acids, particularly 18:2 and 18:3, were present in the polar lipids and correspondingly lower proportions in the neutral fractions.*

The oil palm (*Elaeis guineensis* Jacq) is the second largest source of edible oil in the world and the bulk of it is derived from the hybrid variety tenera. There are two parental varieties, dura and pisifera, distinguished by the relative thickness of shell and ratio of mesocarp to fruit (Hartley 1977). Dura is the thick-shelled variety (2–8 mm shell thickness) with low to medium mesocarp content (35–55%). Pisifera is the shell-less variety. Tenera has a thin shell (0·5–4·0 mm) and medium to high (60–96%) mesocarp content. Botanically each fruit is a sessile drupe consisting of a single seed (kernel) surrounded by the pericarp. The latter includes three distinct regions, viz the inner, hard endocarp or shell, the fleshy, fibrous, oil-bearing mesocarp, and the thin, external, waxy skin or exocarp (Fig 1).

There are a few reports on the polar lipids of palm oil (Khor *et al* 1980; Goh *et al* 1982, 1985). Changes in the polar lipids and fatty acid composition of lipid classes during development of fruit have been analysed (Bafor and Osagie 1986, 1988; Oo *et al* 1986). A preliminary study of the polar lipids of palms grown in India has also been reported (Kulkarni *et al* 1987). The fatty acid profiles of the total lipids of tenera, dura and pisifera varieties have been the subject of a

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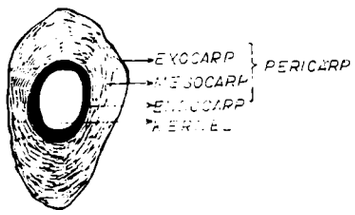

Fig 1. Section through a mature oil palm fruit (tenera variety).

comparative investigation by Jacobsberg (1975). There have been no reports on the phospholipid and glycolypid contents of different varieties and their distribution in the anatomically distinct regions of the fruit.

Mature fruits (dura, pisifera and tenera varieties) of oil palm were collected from the Central Plantation Crops Research Institute (ICAR), Trivandrum. Exocarp and mesocarp regions (and pericarp from other whole fruits) were identified, peeled and separated. Tissues from the respective zones were obtained from a number of whole fruits taken from different regions of a single bunch and mixed to obtain a homogeneous sample. Three bunches were analysed for each variety. Samples were analysed in duplicate and the results were subjected to statistical analysis.

Total lipids were extracted using chloroform/methanol (2:1 v) by the procedure of Goh *et al* (1982) for oil palm mesocarp. Total lipids were fractionated into various lipid classes by thin layer chromatography on silica gel G (1 mm) adsorbent (TLC) with petroleum ether diethyl ether/formic acid (60:40:1·6 v; Ong *et al* 1981). Neutral lipids were eluted from the adsorbent with chloroform, glycolipids with acetone and phospholipids with methanol (Bafor and Osagie 1988). Recovery of lipids was complete. Phospholipids were quantified by elemental determination of phosphorus (Fiske and Subba Row 1925). Total phosphorus content was converted into phospholipid by multiplying by the factor appropriate for palm oil (Goh *et al* 1982). Glycolipids were determined from the hexose content using anthrone/thiourea reagent (Southgate 1976). The quantity of glycolipid was calculated as digalactosyldiglyceride from the hexose content. For both phospholipid and glycolipid determinations, correction for background absorption due to silica gel was made using silica gel from the same TLC plate in the preparation of the blank. Methyl esters were prepared by saponification with methanolic KOH followed by esterification with methanolic H_2SO_4 according to the IUPAC procedure (1987). Fatty acid composition was determined by GC of the methyl esters using a Hewlett-Packard 5840A model gas chromatograph. Methyl esters were separated using a 10% EGSS-X on Chromosorb W(100) column (2 m × 2 mm ID) maintained isothermally at 180°C; injection port and detector temperatures were 250°C and 350°C, respectively; nitrogen carrier gas flow rate was 20 ml min^{-1}. Methyl esters were identified with known standards (Sigma, St Louis, MO, USA), and peaks were quantified by digital integration.

The percentages of exocarp and mesocarp per fruit of the varieties used in this study were respectively: dura 1·7 ± 0·1, 56·6 ± 1·8; tenera 2·6 ± 0·3, 64·5 ± 4·3; and pisifera 5·3 ± 1·7, 94·7 ± 1·5. The phospholipid and glycolipid contents of tenera, dura and pisifera and their distribution within the fruit are summarised in Table 1. Within the fruit there were distinct differences in these lipid concentrations. Exocarp

TABLE 1
Polar lipids of dura, tenera and pisifera varieties of oil palm and their distribution within the fruit (g kg^{-1})

Variety	*Region*	*Phospholipids*	*Glycolipid*
Dura	Exocarp	11·687 ± 0·442 (15·72 ± 0·58)	6·242 ± 0·179
	Mesocarp	2·110 ± 0·073 (2·84 ± 0·10)	1·774 ± 0·094
	Pericarp	6·198 ± 0·210 (8·34 ± 0·28)	2·012 ± 0·072
Tenera	Exocarp	13·148 ± 0·892 (17·69 ± 1·20)	10·944 ± 0·146
	Mesocarp	4·420 ± 0·231 (5·95 ± 0·31)	1·774 ± 0·081
	Pericarp	6·200 ± 0·238 (8·34 ± 0·32)	2·147 ± 0·073
Pisifera	Exocarp	14·856 ± 0·344 (19·62 ± 0·73)	11·770 ± 0·146
	Mesocarp	3·084 ± 0·073 (4·15 ± 0·10)	1·083 ± 0·050
	Pericarp	5·071 ± 0·257 (6·82 ± 0·34)	1·847 ± 0·108

Values are the means of six determinations per sample ± SE(M).
Values in parenthesis are μmol P g^{-1} lipid.

was found to contain significantly higher levels of phospholipids and glycolipids than the mesocarp regions. This differential distribution of polar lipids in the fruit may be attributed to their function as membrane lipids. The waxy skin (exocarp) probably consists of more membrane lipids on account of its protective function, while accumulation of neutral lipids (storage lipids) occurs chiefly in the mesocarp.

Earlier reports on the polar lipids of palm oil have not specified varieties. Results presented in this communication are consistent with the values reported (Khor *et al* 1980; Goh *et al* 1982, 1985). Phospholipid and glycolipid levels of mesocarp and pericarp of tenera were higher than those of the other two varieties. Pisifera exocarp contained the highest levels of polar lipids.

The fatty acid composition of the total lipids of dura and pisifera did not show appreciable differences from that of tenera, which was found to be 14:0–1·1%, 16:0–43·4%, 18:0–4·3%, 18:1–38·0%, 18:2–12·4% and 18:3–0·8% total fatty acids. Our findings on the varieties grown in India are within the ranges reported by Jacobsberg (1975) for Malaysian varieties. The fatty acid profiles of the phospholipids and glycolipids determined are in fair agreement with those published for an unspecified variety (Goh *et al* 1982). All three varieties investigated had comparable fatty acid compositions for all three lipid fractions. The fatty acid composition of the lipid classes of tenera is given in Table 2. The fatty acid profiles of the various lipid classes studied in the three regions of the fruit revealed appreciable variations in the distribution of the major acids (16:0, 18:1, 18:2 and 18:3). Neutral lipids from all regions had almost identical concentrations of 16:0. Phospholipids and glycolipids from exocarp and mesocarp had similar 16:0 levels significantly lower than that in the neutral lipids for a given region. In each region phospholipids showed higher 16:0 content than the glycolipids. There were no appreciable differences in levels of 18:1, though higher levels were present in neutral lipids than in phospholipids of all regions; lowest 18:1 levels were found in glycolipids. The most striking differences were in the distributions of 18:2 and 18:3 acids which were predominant in the polar lipids. Phospholipids of exocarp had a greater abundance of 18:2 (as high as 16:0 and 18:1) whereas glycolipids of all regions were characterised by the presence of 18:3 as a major fatty acid. It

TABLE 2
Fatty acid composition of neutral lipids, phospholipids and glycolipids of three regions of the fruit of tenera variety (% total fatty acids)

Region	*Lipid class*	*Fatty acid*					
		14:0	*16:0*	*18:0*	*18:1*	*18:2*	*18:3*
Exocarp	Neutral lipid	1·3	44·4	3·9	38·6	11·4	0·4
	Phospholipid	0·5	31·1	2·5	34·5	27·1	4·3
	Glycolipid	1·4	29·4	4·6	25·6	8·8	30·2
Mesocarp	Neutral lipid	1·1	45·0	3·8	39·1	10·7	0·3
	Phospholipid	0·5	42·2	3·2	37·4	16·1	0·6
	Glycolipid	1·9	23·9	3·3	35·1	17·8	18·0
Pericarp	Neutral lipid	1·1	43·2	5·0	38·5	11·8	0·4
	Phospholipid	2·1	33·9	2·6	35·0	24·6	1·8
	Glycolipid	3·7	31·1	5·5	33·4	15·1	11·2

appears that there is an association of fatty acid types with lipid classes, viz 16:0 with neutral lipids, 18:2 with phospholipids and 18:3 with glycolipids. There seems to be an inverse relation between 16:0, 18:2 and 18:3 regarding their distribution among the regions and their lipid classes.

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Lipid Profile of Process Streams of Palm Oil Mill

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During palm oil extraction, oil loss occurs mainly at three stages of processing, namely sterilization, pressing and clarification. Samples from a semi-commercial palm oil mill were analyzed for their lipid composition (triacylglycerol, diacylglycerol, monoacylglycerol, free fatty acid, phospholipid and glycolipid contents and fatty acid compositions of these lipid classes) and compared with the end product, *viz.*, raw palm oil. The results indicate significant variations between the samples with respect to oil quality and lipid profile. Data relating to the lipid classes showed that sterilizer condensate had the highest levels of free fatty acids (24%), followed by press fiber (12.5%) and sludge effluent (10.9%), as compared to raw oil (1.5%). Diacylglycerol and monoacylglycerol contents were also markedly higher for these streams. Press fiber was characterized by extremely high proportions of phospholipids and glycolipids. Distribution of fatty acids (16:0, 18:1, 18:2 and 18:3) also varied among lipid classes of the process streams, particularly between polar lipids. This paper discusses the compositional aspects of lipids relating to quality of oils of the palm oil mill streams.

KEY WORDS: Condensate, lipid profile, press fiber, process streams, sludge.

Commercially, palm oil is extracted from the mesocarp of the oil palm fruit by following a wet rendering process. The essential steps consist of sterilization, stripping, digestion, extraction, clarification and purification (1,2). During these operations, the fruits and crude palm oil are subjected to varying degrees of thermal and mechanical stresses to obtain maximum yield of oil while preserving the quality of the end product. Nevertheless, 5–10% of the total oil present in the raw material is lost and the quality of the oil also suffers, depending on the process and harvesting conditions (3). In the palm oil mill, oil loss occurs through the sterilizer condensate (sterilization), press fiber (pressing) and sludge effluent (clarification). These are generally known as the waste streams of palm oil processing (4). There is a tendency among millers to recycle the oil from these waste streams, particularly from the sterilizer condensate and sludge, in order to maximize the yield. This could affect overall quality of the end product.

Investigations on the composition and quality of the oil from waste streams and the product during progressive stages of milling are scanty and confined to a few parameters (5–7). Reports are often limited to one particular stage of operation (8). Bek-Nielsen (5) has studied the formation of peroxides at various stages of palm oil milling. To study the feasibility of solvent extraction for oil palm, Chin and Tan (6) have reported the characteristics of oils extracted by solvent from materials taken at different stages of processing. They investigated carotene, tocopherol, free fatty acid, anisidine value, peroxide value, $E^{1\%}_{c233}$, $E^{1\%}_{c269}$ and bleachability of the various oils. Phospholipid levels of various grades of palm oil and its fractions, and from oil recovered from sludge and press fiber was investigated by Goh *et al.* (7). The nature of the oil present in the sludge effluent was reported by Chow *et al.* (8), who examined iron, phosphorus, free fatty acid content and fatty acid composition of the oil. So far, no comprehensive studies regarding lipid composition of the waste streams have been reported. This paper attempts to follow the compositional variations of lipid classes and their constituent fatty acids as well as the quality of the oils of the various process streams as compared to the end product under actual commercial conditions of palm oil extraction.

EXPERIMENTAL PROCEDURES

Collection of samples. Fresh fruits (Tenera variety) were obtained from the Central Plantation Crops Research Institute (C.P.C.R.I., Palode, Trivandrum, India). The different process streams, *viz.*, sterilizer condensate, sludge water, press fiber residue and crude palm oil, were sampled by operating the demonstration plant for palm oil established at C.P.C.R.I., which has a capacity of 1 ton fresh fruit bunches (FFB) per hour. The process and the various process streams are indicated in Figure 1. The process details were reported earlier by Sundaresan *et al.* (1). The plant was operated specifically to collect samples at various stages of operation. Condensate of approximately 300 kg from the sterilizer was collected from an entire batch of 1000 kg FFB. Representative samples were taken from the bulk. The method of extraction of oil was hydraulic pressing, and samples were collected from the press fiber after it was subjected to 75 kg/cm^2 pressure as optimized for the demonstration plant (1). The oil-water mixture (400 kg) from the press with an oil-water ratio of 1:2 (v/v) was clarified. The sludge from the bottom was

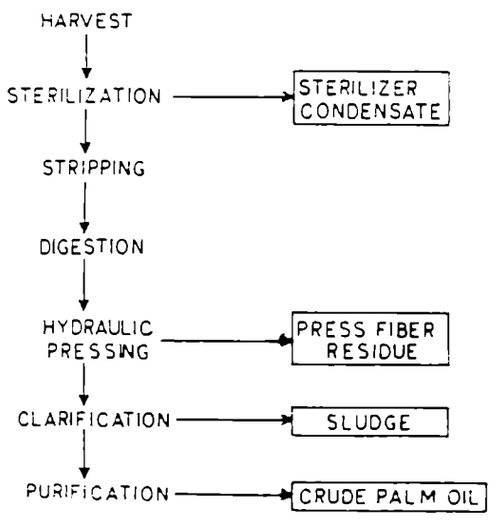

FIG. 1. Flow sheet indicating the various stages of extraction of palm oil and the waste streams from which samples were collected for detailed analysis.

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removed for sampling. Oil from the clarifier was further purified by a high-speed centrifuge for the crude palm oil sample. Two trials were conducted for collection of samples as described, and they were analyzed (in duplicate) separately.

Solvent extraction of mesocarp lipids. Total lipids were extracted with chloroform/methanol (2:1, v/v) solvent mixture from fresh mesocarp as described by Goh *et al.* (7) for oil palm fruits.

Extraction of lipids from process streams. Total lipids of fiber residue was extracted with chloroform/methanol (2:1, v/v), and sludge water and sterilizer condensate lipids were extracted with chloroform (7). The solvents were evaporated under vacuum in a rotary evaporator. The total lipids obtained were dissolved in a minimum quantity of chloroform and stored for further analyses.

Separation of lipid classes. About 20 mg of total lipids were spotted and separated into triacylglycerol, diacylglycerol, monoacylglycerol, free fatty acids and polar lipid classes by thin-layer chromatography on 1-mm thick silica gel G adsorbent with a petroleum ether:diethyl ether:formic acid (60:40:1.6, v/v/v) solvent system (9). Plates were prewashed and equilibrated prior to development, as in standard chromatographic procedures. Lipid bands were detected by exposure to iodine vapor. Triacylglycerol, diacylglycerol, monoacylglycerol and fatty acid fractions were eluted from the gel with chloroform. The polar lipid band was first eluted with acetone to obtain glycolipids, followed by methanol to extract the phospholipids (10). Individual lipid classes were quantitated and fatty acid compositions were determined.

Quantitation of neutral lipid classes. Triacylglycerol, diacylglycerol, monoacylglycerol and free fatty acid classes were quantitated by the oxidative dichromate method of Bragdon (11).

Estimation of phospholipids. Phospholipids were quantitated by elemental determination of phosphorus (12). The phosphorus content was converted to phospholipid by multiplying with the phospholipid factor for palm oil (7).

Estimation of glycolipids. Glycolipids were estimated from the hexose content by using anthrone-thiourea reagent (13). The quantity of glycolipid was calculated as digalactosyldiglyceride from the hexose content.

Determination of fatty acid composition. Methyl esters of the fatty acids were prepared by saponification with alcoholic potassium hydroxide, followed by esterification with alcoholic sulfuric acid reagent according to IUPA procedure (14). A Hewlett-Packard 5840 A model g chromatograph equipped with a flame ionization dete tor (FID) (Hewlett-Packard, Palo Alto, CA) was used f gas liquid chromatography (GLC) analysis of the meth esters. Methyl esters were analyzed on a 2 m × 2 mm i 10% EGSS-X on Chromosorb W 100 metal column. I jector and detector temperatures were 250°C and 300° respectively. Column temperature was maintained isothe mally at 180°C. Carrier gas was nitrogen at a flow ra of 20 mL/min. Methyl esters were identified with referen to standards (Sigma Chemical Co., St. Louis, MO), ar the peaks were quantitated by digital integration.

RESULTS AND DISCUSSION

Details of the process and collection of the samples a given in the experimental section. The values presente in Table 1 for the composition of lipid classes were obtai ed for the various process steps as described. The resul show an appreciable variation among the process stream Sterilizer condensate contained the lowest levels triacylglycerol (54.5%), whereas oil extracted with solve from fresh mesocarp had the highest levels (97.0%). Co responding values for free fatty acids were 24.0% ar 0.7%, respectively, for these samples. Partial glycerid also showed significant variations. The distribution of t polar lipids, phospholipids and glycolipids exhibited much greater variation when compared to the neutral lip classes. For instance, crude palm oil contained the lowe amounts of phospholipids and glycolipids, whereas pre fiber had nearly 20–50 times greater levels of these lipi

Total lipids from fresh mesocarp of unbruised fruits correct maturity were extracted with solvent to determi the lipid composition actually present in oil palm fru without being altered by process conditions. Valu reported by other authors for triacylglycerol content mature oil palm fruit mesocarp show great variation, fr 98% (15) to 78% (16). These differences can be attribut to maturity of the fruit and method of extraction of lipi The high value for triacylglycerol of 97.1% with low valu of 2.0% for diacylglycerol, 0.2% for monoacylglycerol a 0.7% for free fatty acids reported here indicate that fru extracted under proper conditions will have low levels free fatty acid and partial glycerides with maximu triacylglycerol, as are actually present in the fresh fr These values agree with those of Jacobsberg (15). A

TABLE 1

Lipid Composition of Oils from Various Process Streams of Palm Oil Mill

Lipid class	Sterilizer condensate	Press fiber	Sludge	Crude palm oil	Solvent-extracted oil from fresh mesocarp
Neutral lipid (relative %)					
Triacylglycerol	54.9	65.4	72.8	93.0	97.1
Diacylglycerol	11.5	16.6	10.1	4.5	2.0
Monoacylglycerol	9.6	5.6	6.2	0.9	0.2
Free fatty acid	24.0	12.5	10.9	1.5	0.7
Polar lipid (ppm total lipid)					
Phospholipid	6721	25975	6636	1443	5633
Glycolipid	13925	20311	1139	438	2492

deviation from this composition can be attributed to post-harvest conditions in the field and in the mill.

In a typical palm oil mill, universally practiced process steps are sterilization, stripping, digestion, pressing, clarification and purification. The major oil loss occurs through the sterilizer condensate, press fiber and sludge, with an approximate oil loss of 2%, 6% and 2%, respectively.

During milling operations, the palm fruits are subjected to varying degrees of thermal and mechanical abuse, resulting in chemical and quality alterations of the oil. Sterilization was conducted at steam pressure of 3 kg/cm^2 (equivalent to 130°C) for 1 hr. During this process, about 50% of the total steam requirement for palm oil processing was consumed. The condensate obtained from this step carried about 1–2% of the total oil. Low levels of triacylglycerol (Table 1) could be due to accelerated hydrolysis at elevated temperature, which was further confirmed by the high levels of free fatty acid and partial glyceride, as reported here. High levels of polar lipids in condensate indicate that more structural lipids from the fruit exocarp (outer skin) were extracted. Therefore, the oil present in the condensate may also be derived from the exocarp. Eng *et al.* (3) and Bek-Nielsen (5) have reported that oil from the condensate was heavily contaminated with iron and was in a highly oxidized state. Bek-Nielsen (17) has recommended against the recycling and mixing of this recovered oil with production oil.

The loose fruits obtained after stripping of the sterilized bunches were converted into a mash in a digester maintained at 95°C with live steam (1). This digested mash was then subjected to hot pressing to extract the crude oil-water mixture. Highest oil loss (6.0%) occurred at this stage because oil is entrained in the press fiber residue. The press fiber contained cellulosic fiber, fruit exocarp (skin) and calyx, along with the seed. The oil content of the press fiber and the lipid composition of this oil showed exceptionally high levels of polar lipids and partial glycerides (Table 1). A previous report from this laboratory (18) on the distribution of lipids within the fruit, *viz.*, exocarp and mesocarp, confirmed that exocarp contained markedly higher levels of polar lipids. These lipids are structural components of membranes and are not easily extractable by the method adopted here; thus, they are retained in the press fiber residue oil. Goh *et al.* (7) have reported high values for phospholipids from press fiber waste. According to Bek-Nielsen (17), solvent extraction of residual oil from the fiber would extract a low-quality oil, containing phosphatides and other nonglyceride impurities. High levels of partial glycerides in the press fiber (Table 1) could be attributed to an adsorptive property of the fibrous residues.

The oil-water mixture from the press was subjected to clarification at 95°C to separate the crude palm oil from the watery sludge (Fig. 1). Oil recovered from the sludge had high contents of phospholipids (6636 ppm) and glycolipids (1139 ppm). Goh *et al.* (7) have shown that oil from sludge water has appreciable levels of these lipids because substantial amounts of hydratable polar lipids are removed along with the water phase during milling. The higher levels of partial glycerides obtained here could be due to their greater water solubility as compared to triacylglycerol.

In this experiment, about 90% of the oil present in the fresh fruit was obtained as crude palm oil, the final product stream. Composition of the different lipid classes of commercial palm oil has been reported by several authors in studies related to crystallization (19–21). The values obtained here (Table 1) fall within that range. However, when compared to the oil extracted with solvent from fresh mesocarp, the lower content of triacylglycerol is likely due to the hydrolysis of triacylglycerol, resulting in relatively higher diacylglycerol, monoacylglycerol and free fatty acid fractions during milling. Solvent extraction removes the entire polar lipids present in the fruit, which explains the higher content of these lipids. Commercial crude palm oil is obtained by a wet extraction process during which the structural lipids are not extracted, thus explaining their lower levels in crude oil.

Fatty acid compositions of the total lipids of the various streams of the palm oil mill are given in Table 2. Except for sterilizer condensate, other streams did not show appreciable variations in their fatty acid profiles. Greater proportion of saturated fatty acids and correspondingly lower unsaturated acids in sterilizer condensate could be due to thermal oxidation of unsaturated fatty acids during sterilization. With respect to fatty acid composition of other streams, the values reported in this study are compatible with commercial crude palm oil (8,15,22,23).

Table 3 shows the fatty acid compositions of phospholipid and glycolipid classes of various streams of the palm oil mill. It is interesting to note the association of 18:2 and 18:3 with the polar lipids. While 18:2 was mainly associated with phospholipids, 18:3 was primarily found to be in the glycolipid fractions. Furthermore, it can be stated that most of the 18:3 present in the fresh mesocarp lipids was concentrated in the glycolipid fraction, as the concentration of this acid is negligible in the total lipids. This association of 18:2 with phospholipids and of 18:3 with glycolipids has been observed by Goh *et al.* (7) for crude palm oil samples and by Oo *et al.* (16) and Bafor and Osagie (24) in the developing oil palm fruit. Except for sterilizer condensate, other streams had more or less similar fatty acid profiles for polar lipids. In case of sterilizer condensate, unsaturated fatty acids were appreciably lower for reasons already stated.

The fatty acid composition of various neutral lipid classes are presented in Table 4. Perusal of this Table shows no marked deviation in fatty acid distribution among the neutral lipid classes from the various process streams. This suggests that although there was significant difference in the distribution of neutral lipid classes (Table 1), the relative percentage of the component fatty acids were not subjected to great variations due to selective hydrolysis or to process conditions. However, there was a slight reduction in the total unsaturation in the end product (crude palm oil). Earlier reports for fatty acid composition of neutral lipid classes extracted from mature fruits with respect to development studies agree with those reported in Table 4 (16,24).

The above findings demonstrate the drastic differences in oils from the various process streams in terms of lipid composition and quality. There is a tendency among the palm oil processors to recycle waste stream oils to obtain higher oil yield. The high levels of partial glycerides, free fatty acids and polar lipids in the oils from sludge and sterilizer condensate, when mixed with the end product,

TABLE 2

Fatty Acid Composition of Total Lipids from Various Process Streams of Palm Oil Extraction

Fatty acid (wt. %)	Sterilizer condensate	Press fiber	Sludge	Crude palm oil	Solvent-extracted oil from fresh mesocarp
12:0	0.1	0.1	0.1	0.1	0.1
14:0	1.8	1.2	1.3	1.4	1.3
16:0	49.2	42.6	43.3	44.5	40.2
18:0	4.6	4.5	4.6	4.7	4.9
18:1	36.7	38.9	39.5	38.9	40.6
18:2	7.3	11.6	10.8	10.0	12.3
18:3	0.3	1.1	0.4	0.4	0.6

TABLE 3

Fatty Acid Composition of Phospholipids and Glycolipids of Palm Oil Process Streams

		Fatty acid (wt. %)						
Process stream	Lipid class	12:0	14:0	16:0	18:0	18:1	18:2	18:3
Sterilizer condensate	Phospholipid	0.4	2.3	45.4	5.0	37.8	8.9	2.2
	Glycolipid	0.8	1.5	53.6	5.0	31.9	5.0	0.2
Press fiber	Phospholipid	0.1	0.4	31.1	1.5	40.6	24.2	2.1
	Glycolipid	0.2	0.6	23.8	2.6	30.8	17.7	24.3
Sludge	Phospholipid	0.5	0.7	37.5	3.1	39.2	16.3	2.7
	Glycolipid	0.6	1.4	35.9	3.6	30.3	11.7	16.5
Crude palm oil	Phospholipid	—	2.1	38.4	4.6	42.6	11.7	0.6
	Glycolipid	0.2	1.2	40.2	4.8	36.0	11.4	6.2
Solvent-extracted oil	Phospholipid	—	2.0	35.1	2.6	35.1	24.6	0.6
	Glycolipid	2.0	3.6	30.5	5.4	32.7	14.9	10.9

TABLE 4

Fatty Acid Composition of Triacylglycerol, Diacylglycerol, Monoacylglycerol and Fatty Acid Fractions of Process Streams of Palm Oil Extraction

		Fatty acid (wt. %)						
Lipid class	Process stream	12:0	14:0	16:0	18:0	18:1	18:2	18:3
Triacylglycerol	Sterilizer condensate	0.1	1.4	44.9	5.2	37.9	9.6	0.9
	Press fiber	0.7	2.2	47.6	3.6	36.5	9.2	0.2
	Sludge	0.1	1.4	43.7	4.2	40.5	10.0	0.1
	Crude palm oil	0.1	1.3	45.0	4.8	38.5	9.8	0.5
	Solvent-extracted oil	—	1.4	41.2	4.9	41.0	11.2	0.3
Diacylglycerol	Sterilizer condensate	0.2	1.4	45.2	5.3	37.3	9.7	0.9
	Press fiber	0.5	1.4	37.0	4.0	45.1	11.2	0.8
	Sludge	0.2	1.2	36.5	2.8	43.9	15.2	0.2
	Crude palm oil	0.5	1.3	35.3	3.4	45.7	12.4	1.4
	Solvent-extracted oil	1.2	1.4	30.7	3.6	45.4	16.9	0.8
Monoacylglycerol	Sterilizer condensate	1.0	2.3	46.3	8.0	36.4	5.7	0.3
	Press fiber	4.7	3.2	39.5	5.9	36.5	9.1	1.1
	Sludge	3.2	2.6	44.5	7.4	34.1	8.2	—
	Crude palm oil	6.3	3.8	38.9	5.8	35.7	9.2	0.3
	Solvent-extracted oil	1.4	7.0	38.0	6.3	35.5	10.8	0.9
Free fatty acid	Sterilizer condensate	0.2	1.8	54.0	4.9	33.3	5.6	0.2
	Press fiber	0.4	1.4	44.9	4.5	39.7	8.2	0.9
	Sludge	0.2	1.5	45.8	4.8	37.5	9.4	0.8
	Crude palm oil	1.3	2.1	46.8	4.2	37.4	7.9	0.3
	Solvent-extracted oil	6.8	4.2	37.5	6.2	34.5	10.3	0.4

would impair oil quality for storage and subsequent refining processes (23,25,26).

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Oil Characteristics and Composition of Palm Oil process streams

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Introduction

Palm oil ranks second in world production of edible oils. Palm oil is extracted through wet extraction consisting of unit operations viz. Sterilization, Stripping, Digestion, Extraction, Clarification and Purification. The aim of a processor is to obtain maximum quantity of high quality palm oil. However five to ten per cent of the total oil present in the raw material is lost during processing through the various process streams (Waste Streams) viz. Sterilizer condensate (Sterilization), Fibre residue (Extraction) and Sludge (Clarification) and the quality undergoes various changes during extraction depending on the process and harvesting conditions.

The literature on the composition and quality of the oil from the various process streams of palm oil production is scanty and work has been done on a few parameters only. The formation of peroxides at various stages of palm oil milling have been studied by Bek-Neilsen[1]. The solvent extracted oils of samples taken at various stages of processing were characterised by Chin and Tan[2]. Goh *et al*[3] investigated phospholipid levels of various grades of palm oil and its fractions and of sludge oil and press fibre. Iron content, phosphorus, free fatty acid content and fatty acid composition of the oil from the sludge was examined by Chow *et al*[4]. Crude palm oil contains a number of minor constituents such as carotenoids, tocopherols, phenolics and contaminants like iron and copper that play a major role in the oxidative stability of the oil on storage and processing[5]. A comprehensive study on the lipid composition and physiochemical properties of oils obtained from process streams of palm oil mill has not been reported. It is important to monitor the quality of the oil and also the nature of the contaminants at various stages of milling since there is a tendency among the processors to mix the oils from waste streams to maximise the yield hampering quality of crude palm oil. The chemical characteristics of the oils of the various process streams as compared with crude palm oil under actual commercial conditions and oil extracted with solvent from fresh mesocarp are presented in this paper.

Materials and Methods

Fresh Fruit Bunches (FFB) of *Elaeis guineensis* jacq. var. *tenera* were harvested from the plantations of Central Plantation Crops Research Institute (CPCRI), Research Centre at Palode, Trivandrum, Kerala.

The essential unit operations in the extraction of palm oil and the major process streams are indicated in Fig. 1. Process details were reported in an earlier publication[6].

Condensate of approximately 300 kgs. from the sterilizer of one tonne FFB capacity was collected. Samples were drwan from the total condensate for analysis.

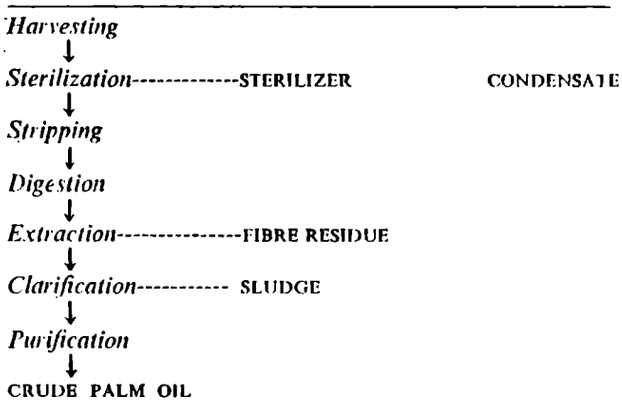

Fig. 1. *Flow Chart for Extraction of Palm Oil*

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Hydraulic pressing at 75 kg./sq. cm is the method of expression in the unit and the representative press fibre samples were collected from this step.

The oil-water mixture (400 kg.) from the press was subjected to clarification with an oil-water ratio of (1:2, v/v). The sludge from the bottom was taken for the sampling purpose.

The crude palm oil from the clarifier was further purified in a high speed centrifuge. Representative samples from three trials were collected and analysis was done in duplicate.

Total lipids were extracted with chloroform-methanol (2:1, v/v) solvent mixture from fresh mesocarp as described by Goh *et al*[3] for oil palm fruits. Sterilizer condensate and sludge lipids were extracted with chloroform. Total lipids of fibre residue were extracted with chloroform-methanol (2:1, v/v).

Sterilizer condensate oil, fibre residue oil, sludge oil, crude palm oil and solvent extracted oil were analysed for free fatty acids (FFA), Peroxide Value (PV), Anisidine Value (AV), Ec_{233} 1 per cent, Ec_{269} 1 per cent, carotene, iron content, copper content and Unsaponifiable Matter (USM) as per Palm Oil Research Institute of Malaysia (PORIM) test methods[7]. For the estimation of total tocopherols, the procedure of Wong *et al*[8] was followed. Total phenolics and flavonols were extracted and quantified using the method of Swain and Hillis[9].

The total lipids of the various process streams were separated into different lipid classes (TAG, DAG, MAG, FFA and polar lipids) by Thin Layer Chromatography on 1 mm. thick silica gel G adsorbent using petroleum ether-diethyl ether-formic acid (60:40:1.6, v/v/v) solvent system. Choloroform was used for the elution of Triacylglycerol, Diacylglycerol, Monoacylglycerol and fatty acid fractions from the gel. The polar lipid band from the plate was eluted with acetone to obtain glycolipids followed by methanol to extract the phospholipids. Triacylglycerol, Diacylglycerol, Monoacylglycerol and free fatty acid classes were quantified by the oxidative dichromate method of Bragdon[10]. Phospholipids were quantified by the elemental determination of phosphorous[11]. The phosphorous content was converted to phospholipid by multiplying with the factor for palm oil[3]. Glycolipids were estimated from the hexose content using anthrone-thiourea reagent[12]. The quantity of glycolipid was calculated as Digalactosyl diglyceride from the hexose content.

Methyl esters of the fatty acids were prepared by saponification with alchoholic potassium hydroxide followed by esterification with alcoholic sulphuric acid reagent according to IUPAC procedure[13]. A Hewlett packard 5840 A model gas chromatograph equipped with a FID was used for Gas Liquid Chromatography (GLC) analysis of the methyl esters. Methyl esters were analysed on a 2 m. * 2mm. i.d. column of 10 per cent EGSS-X on chromosorb W. Injector and detector temperatures were 250°C and 300°C respectively. Column temperature was maintained isothermally at 180°C. Carrier gas Nitrogen had a flow rate of 20 ml./min. Methyl esters were identified with reference to standards (Sigma chemical Co. USA) and the peaks were quantified by digital integration.

Results

Quality characteristics of oils obtained from various process streams showed variation as observed in Table 1. Iron and copper concentration was highest in sterilizer condensate oil. No variation was noticed between oil samples in the case of iodine value excepting fibre residue oil which had higher value. The sterilizer condensate oil, fibre residue oil and sludge oil had significantly different saponification values than the normal range.

Crude palm oil and solvent extracted oil were least oxidised and sterilizer condensate and fibre residue oils showed maximum values for oxidative indices as shown in Table 2.

The minor constituents influencing the oxidative stability of oil samples are presented in Table 3 Sterilizer condensate oil showed the lowest concentration of carotenes, tocopherols and highest amounts of phenolics and flavonols.

The carotene content was highest in fibre residue oil. Crude palm oil and sludge oil had comparable contents of carotenes and tocopherols. The polar lipids, phospholipids and glycolipids exhibited greater variation. Lowest levels were present in crude palm oil whereas the fibre residue oil had nearly 20 to 50 fold greater levels.

Table 1: Quality characteristics of oils from different process streams of palm oil extraction.

Oil	Iron (ppm)	Copper (ppm)	Saponification Value	Iodine Value	Unsaponifiable Matter
Solvent extracted oil	—	—	199.16	51.88	0.54
Sterilizer condensate oil	24.27	2.00	164.65	52.47	0.61
Fibre residue oil	3.95	0.38	176.88	56.19	0.55
Sludge oil	9.50	0.87	178.02	53.04	0.69
Crude palm oil	4.85	0.48	194.91	50.09	0.40

Table 2: Oxidation of oils from different process streams of palm oil mill.

Oil	Peroxide value	Anisidine value	Totox	$Ec_{233}1\%$	Ec_{269} 1%
Solvent extracted oil	0.52	12.99	14.03	2.167	0.488
Sterilizer condensate oil	5.11	47.16	57.18	7.837	5.976
Fibre residue oil	3.98	146.42	154.38	5.361	3.675
Sludge oil	2.59	36.08	41.26	2.235	0.414
Crude palm oil	1.08	26.40	28.56	2.133	0.754

Table 3: Minor constituents in palm oil process srtreams (ppm total lipids)

Oil	Carotenes	Tocopherols	Total phenols	Flavonols	Phospho lipids	Glyco-lipids
Solvent extracted oil	945	622	399	519	5633	2492
Sterilizer condensate oil	413	413	10359	3885	6721	13925
Fibre residue oil	1884	580	6403	2749	25975	20311
Sludge oil	645	558	767	595	6636	1139
Crude palm oil	672	569	87	87	1443	438

The neutral lipid classes and their composition varied among the process streams of palm oil mill (Table 4.) Solvent extracted oil from fresh mesocarp contained the highest TAG content. Triacylglycerol content was significantly low in sterilizer condensate oil. Partial glycerides also showed significant variations.

Fatty acid compositions of the total lipids of the various streams in the extraction of palm oil are shown in Table 5. Except for sterilizer condensate, remaining oil samples showed only very slight variations in their fatty acid profiles.

Table 4: Distribution of neutral lipid classes (relative per cent) in oils of palm oil process streams.

Oil	Tri acyl glycerol	Di acyl glycerol	Mono acyl glycerol	Free Fatty Acid
Solvent extracted oil	97.1	2.0	0.2	0.7
Sterilizer condensate oil	54.9	11.5	9.6	24.0
Fibre residue oil	65.4	16.6	5.6	12.5
Sludge oil	72.8	10.1	6.2	10.9
Crude palm oil	93.0	4.5	0.9	1.5

Table 5: Fatty acid composition of oils from different process streams.

Fatty Acid (per cent)	Sterilizer condensate oil	Fibre residue oil	Sludge oil	Crude palm oil	Solvent extracted oil
12:0	0.1	0.1	0.1	0.1	0.1
14:0	1.8	1.2	1.3	1.4	1.3
16:0	49.2	42.6	43.3	44.5	40.2
18:0	4.6	4.5	4.6	4.7	4.9
18:1	36.7	38.9	39.5	38.9	40.6
18:2	7.3	11.6	10.8	10.0	12.3
18:3	0.3	1.1 .	0.4	00.4	0.6

Discussion

Oil obtained from the scund unbruised fruits of correct maturity by extraction with solvent (2:1 choloroform-methanol) represents the total lipid which is not altered by process conditions. High quantity of triacylglycerol with low quantities of partial glycerides, trace amounts of the free fatty acid, absence of trace metals, presence of moderate amounts of phenols and flavonols and fatty acid composition are consistent with the earlier values reported for palm oil[14].

Sterilization is the first unit operation in which fresh fruit bunches are subjected to highest temperature and steam for the inactivation of endogenous lipase and to facilitate removal of fruits. The oil loss through cordensate amounts to approximately 2 per cent. High levels of polar lipids suggest that structural lipids from the fruit skin were extracted with the condensate[15]. Low level of triacylglycerol could be due to accelerated hydrolysis at elevated temperatures which was further confirmed by highest free fatty acid content, trace metal

content (iron and copper), reduced content of carotene and tocopherol increasing the oxidation of the oil as evidenced by totox value, peroxide value and diene content. A strong linear correlation has been observed between inorganic phosphate, free fatty acid and iron[16, 17]. The oxidative characteristics of this oil corroborates the earlier work of Bek-Neilsen[1]. Fatty acid composition of sterilizer condensate oil indicate a higher level of saturated fatty acids and a corresponding lower unsaturated fatty acid content which may be due to the thermal oxidation during sterilization.

Highest oil loss (approximately 6 per cent) occurred after the extraction of the digested mash in the hydraulic press in the palm oil mill. The oil extracted with solvent from the press fibre is probably the bound oil comprising of predominantly polar lipids which could not be leached by the extraction process[2]. The oil obtained from fibre residue was observed to be highly oxidised as shown by totox value, anisidine value, diene content and triene content. Highest carotene content could possibly be due to the extraction of carotenes from the accompanying floral parts, fruit skin and materials of the bunch that comprises the residue which were not extracted into normal production oil. High levels of phospholipids were also observed by Goh *et al*[3] in press fibre. The occurrence of high levels of partial glycerides could be attributed to adsorptive nature of fibre residues.

The oil obtained from the sludge has slightly higher amounts of trace metals (iron and copper), peroxides, diene, triene, carotenes and tocopherols than the crude palm oil. The phospholipid and glycolipid levels were appreciably greater which might be due to their hydratable nature and which are lost through centrifugation in crude palm oil when the last traces of water are removed. The oil loss through the sludge (approximately 2 per cent) is dependent on the clarification technique employed, the similarity of the sludge to the crude palm oil could be attributed to the fact that sludge is separated from an oil-water mixture that has not been further purified. Fatty acid composition of sludge oil was similar to that of crude oil confirming the earlier work of Chow *et al*[4].

Harvesting at correct maturity and ideal process conditions ensures the production of crude palm oil with free fatty acids less than 2 per cent. The concentration of desirable minor constitutents (carotenes and tocopherols) indicates their high recovery in the process of palm oil extraction. The chemical characteristics and fatty acid composition of this crude palm oil obtained in these trials are in the range of standard Malaysian palm oil reported[14, 17].

Results reveal that differences exist in the composition and quality attributes of the various palm oil process streams. The presence of high level of partial glycerides, polar lipids in the sludge oil and sterilizer condensate oil when mixed with the crude palm oil has a deleterious effect hampering oil quality on storage and subsequent processing[5, 14, 17].

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