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)

<table>
<thead>
<tr>
<th>Component</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bunch weight, kg</td>
<td>17.5±1.7</td>
</tr>
<tr>
<td>Fruit weight, g</td>
<td>6.63±0.59</td>
</tr>
<tr>
<td>Mesocarp, %</td>
<td>75.16±0.08</td>
</tr>
<tr>
<td>Kernel, %</td>
<td>8.21±0.29</td>
</tr>
<tr>
<td>Shell, %</td>
<td>16.63±0.35</td>
</tr>
<tr>
<td>Mesocarp, moisture %</td>
<td>41.44±0.67</td>
</tr>
<tr>
<td>Mesocarp, fat, % (dry weight)</td>
<td>77.23±0.90</td>
</tr>
<tr>
<td></td>
<td>(fresh weight) 45.23</td>
</tr>
<tr>
<td>Kernel, moisture %</td>
<td>14.87±1.60</td>
</tr>
<tr>
<td>Kernel, fat, % (dry weight)</td>
<td>46.30±0.95</td>
</tr>
<tr>
<td></td>
<td>(fresh weight) 39.42</td>
</tr>
</tbody>
</table>
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 constituent. 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

<table>
<thead>
<tr>
<th>Lipid</th>
<th>g/100g total lipid</th>
<th>g/100g fresh mesocarp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutral lipid</td>
<td>99.17</td>
<td>44.85</td>
</tr>
<tr>
<td>Triacylglycerol</td>
<td>95.27</td>
<td>48.45</td>
</tr>
<tr>
<td>Diacylglycerol</td>
<td>1.91</td>
<td>0.87</td>
</tr>
<tr>
<td>Monoacylglycerol</td>
<td>0.50</td>
<td>0.23</td>
</tr>
<tr>
<td>Free fatty acid</td>
<td>0.64</td>
<td>0.29</td>
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<tr>
<td>Polar lipid</td>
<td>0.83</td>
<td>0.37</td>
</tr>
<tr>
<td>Glycolipid</td>
<td>0.21</td>
<td>0.09</td>
</tr>
<tr>
<td>Phospholipid</td>
<td>0.62</td>
<td>0.28</td>
</tr>
</tbody>
</table>

Phospholipid (mole %)

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>Phosphatidic acid</td>
<td>2.38</td>
</tr>
<tr>
<td>Phosphatidylcholine</td>
<td>25.40</td>
</tr>
<tr>
<td>Phosphatidylethanolamine</td>
<td>24.60</td>
</tr>
<tr>
<td>Phosphatidylglycerol</td>
<td>12.70</td>
</tr>
<tr>
<td>Diphosphatidylglycerol</td>
<td>3.97</td>
</tr>
<tr>
<td>Phosphatidylinositol</td>
<td>22.22</td>
</tr>
<tr>
<td>Phosphatidylserine/</td>
<td>8.73</td>
</tr>
<tr>
<td>Lysophosphatidylethanolamine</td>
<td></td>
</tr>
</tbody>
</table>

121
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), phosphatidylglycerol (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 (6.8% (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>
<thead>
<tr>
<th>Lipid class</th>
<th>Fatty acid (wt %)</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>S/U</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>12:0</td>
<td>14:0</td>
<td>16:0</td>
<td>18:0</td>
<td>18:1</td>
<td>18:2</td>
<td>18:3</td>
<td></td>
</tr>
<tr>
<td>Neutral lipid</td>
<td>0.10</td>
<td>1.00</td>
<td>43.20</td>
<td>5.00</td>
<td>38.50</td>
<td>11.80</td>
<td>0.40</td>
<td>49/51</td>
</tr>
<tr>
<td>Triacylglycerol</td>
<td>0.59</td>
<td>1.75</td>
<td>42.56</td>
<td>4.08</td>
<td>41.71</td>
<td>8.96</td>
<td>0.35</td>
<td>49/51</td>
</tr>
<tr>
<td>Diacylglycerol</td>
<td>1.31</td>
<td>1.57</td>
<td>32.32</td>
<td>8.97</td>
<td>39.90</td>
<td>15.16</td>
<td>0.76</td>
<td>44/56</td>
</tr>
<tr>
<td>Monoacylglycerol</td>
<td>3.55</td>
<td>5.67</td>
<td>36.88</td>
<td>8.46</td>
<td>35.82</td>
<td>8.77</td>
<td>0.86</td>
<td>55/45</td>
</tr>
<tr>
<td>Free fatty acid</td>
<td>4.79</td>
<td>3.77</td>
<td>39.85</td>
<td>9.60</td>
<td>34.32</td>
<td>7.01</td>
<td>0.65</td>
<td>58/42</td>
</tr>
<tr>
<td>Phospholipid</td>
<td>0.34</td>
<td>1.37</td>
<td>32.60</td>
<td>2.81</td>
<td>38.22</td>
<td>23.19</td>
<td>1.47</td>
<td>37/63</td>
</tr>
<tr>
<td>Glycolipid</td>
<td>2.40</td>
<td>4.05</td>
<td>27.63</td>
<td>8.36</td>
<td>37.65</td>
<td>10.67</td>
<td>9.14</td>
<td>43/57</td>
</tr>
<tr>
<td>Total lipid</td>
<td>-</td>
<td>1.1</td>
<td>43.40</td>
<td>4.30</td>
<td>38.00</td>
<td>12.40</td>
<td>0.80</td>
<td>49/51</td>
</tr>
</tbody>
</table>

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 abundant 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

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

** calculated from the method of Colman (1964)

* Proportion at 2-position = w/3T x 100, 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 Rf 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 i.e., 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>
<thead>
<tr>
<th>Triacylglycerol class</th>
<th>Triacylglycerol Position of fatty acid</th>
<th>Fatty acid (mole %)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>12:0</td>
<td>14:0</td>
</tr>
<tr>
<td>Saturated</td>
<td></td>
<td></td>
</tr>
<tr>
<td>total</td>
<td>0.99</td>
<td>4.89</td>
</tr>
<tr>
<td>sn-2</td>
<td>1.90</td>
<td>3.67</td>
</tr>
<tr>
<td></td>
<td>(64.0)</td>
<td>(25.0)</td>
</tr>
<tr>
<td>1,3-</td>
<td>0.54</td>
<td>5.5</td>
</tr>
<tr>
<td>Monoene</td>
<td></td>
<td></td>
</tr>
<tr>
<td>total</td>
<td>0.48</td>
<td>2.73</td>
</tr>
<tr>
<td>sn-2</td>
<td>0.81</td>
<td>1.41</td>
</tr>
<tr>
<td></td>
<td>(56.3)</td>
<td>(17.2)</td>
</tr>
<tr>
<td>1,3-</td>
<td>0.32</td>
<td>3.39</td>
</tr>
<tr>
<td>Diene</td>
<td></td>
<td></td>
</tr>
<tr>
<td>total</td>
<td>0.96</td>
<td>1.42</td>
</tr>
<tr>
<td>sn-2</td>
<td>1.88</td>
<td>1.28</td>
</tr>
<tr>
<td></td>
<td>(65.3)</td>
<td>(30.0)</td>
</tr>
<tr>
<td>1,3-</td>
<td>(0.50)</td>
<td>(1.49)</td>
</tr>
<tr>
<td>Triene</td>
<td></td>
<td></td>
</tr>
<tr>
<td>total</td>
<td>0.48</td>
<td>2.53</td>
</tr>
<tr>
<td>sn-2</td>
<td>0.73</td>
<td>1.27</td>
</tr>
<tr>
<td></td>
<td>(50.69)</td>
<td>(16.73)</td>
</tr>
<tr>
<td>1,3-</td>
<td>0.36</td>
<td>3.16</td>
</tr>
<tr>
<td>Polyene</td>
<td></td>
<td></td>
</tr>
<tr>
<td>total</td>
<td>0.58</td>
<td>1.75</td>
</tr>
<tr>
<td>sn-2</td>
<td>1.05</td>
<td>2.05</td>
</tr>
<tr>
<td></td>
<td>(60.3)</td>
<td>(39.0)</td>
</tr>
<tr>
<td>1,3-</td>
<td>0.35</td>
<td>1.60</td>
</tr>
</tbody>
</table>

Figures in parenthesis represent relative abundance of a fatty acid at the sn-2 position. The 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 primarily 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).

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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 demonstrated the positive correlation of atherogenicity 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 is 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-palmitoylglycerol (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)

<table>
<thead>
<tr>
<th>Saturated</th>
<th>Monoene</th>
<th>Diene</th>
<th>Triene</th>
<th>Polyene</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPP</td>
<td>6.92</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PMSt</td>
<td>0.16</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PPSt</td>
<td>1.12</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PPM</td>
<td>0.66</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PSTSt</td>
<td>0.13</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PSTP</td>
<td>0.80</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PMP</td>
<td>0.98</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>others</td>
<td>0.30</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>others</td>
<td>0.33</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>11.07</td>
<td>34.68</td>
<td>31.25</td>
<td>16.55</td>
</tr>
</tbody>
</table>

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

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

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

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

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

<table>
<thead>
<tr>
<th>Variety</th>
<th>Fatty acid (wt %)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>12:0+14:0</td>
</tr>
<tr>
<td>Tenera</td>
<td>1.1</td>
</tr>
<tr>
<td>Dura</td>
<td>1.0</td>
</tr>
<tr>
<td>Pisifera</td>
<td>1.0</td>
</tr>
<tr>
<td>Region</td>
<td>Lipid Class</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>Exocarp</td>
<td>Neutral lipid</td>
</tr>
<tr>
<td></td>
<td>Phospholipid</td>
</tr>
<tr>
<td></td>
<td>Glycolipid</td>
</tr>
<tr>
<td>Mesocarp</td>
<td>Neutral lipid</td>
</tr>
<tr>
<td></td>
<td>Phospholipid</td>
</tr>
<tr>
<td></td>
<td>Glycolipid</td>
</tr>
<tr>
<td>Pericarp</td>
<td>Neutral lipid</td>
</tr>
<tr>
<td></td>
<td>Phospholipid</td>
</tr>
<tr>
<td></td>
<td>Glycolipid</td>
</tr>
</tbody>
</table>
Table 19  Fatty Acid Composition of Neutral Lipids, Phospholipids and Glycolipids of Three Regions of the Fruit of Pisifera Variety

<table>
<thead>
<tr>
<th>Region</th>
<th>Lipid Class</th>
<th>12:0+14:0</th>
<th>16:0</th>
<th>18:0</th>
<th>18:1</th>
<th>18:2</th>
<th>18:3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exocarp</td>
<td>Neutral lipid</td>
<td>0.7</td>
<td>29.2</td>
<td>3.2</td>
<td>18.2</td>
<td>23.2</td>
<td>8.3</td>
</tr>
<tr>
<td></td>
<td>Phospholipid</td>
<td>43.6</td>
<td>3.8</td>
<td>38.4</td>
<td>12.3</td>
<td>0.7</td>
<td>16.0</td>
</tr>
<tr>
<td></td>
<td>Glycolipid</td>
<td>0.9</td>
<td>23.2</td>
<td>3.4</td>
<td>27.9</td>
<td>8.3</td>
<td>36.3</td>
</tr>
<tr>
<td>Mesocarp</td>
<td>Neutral lipid</td>
<td>1.0</td>
<td>44.6</td>
<td>4.9</td>
<td>37.2</td>
<td>11.8</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>Phospholipid</td>
<td>2.1</td>
<td>29.7</td>
<td>2.5</td>
<td>42.4</td>
<td>21.6</td>
<td>1.7</td>
</tr>
<tr>
<td></td>
<td>Glycolipid</td>
<td>1.5</td>
<td>30.6</td>
<td>4.1</td>
<td>27.7</td>
<td>17.4</td>
<td>18.7</td>
</tr>
<tr>
<td>Pericarp</td>
<td>Neutral lipid</td>
<td>0.9</td>
<td>45.4</td>
<td>4.3</td>
<td>37.5</td>
<td>11.3</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td>Phospholipid</td>
<td>2.8</td>
<td>31.9</td>
<td>5.3</td>
<td>34.6</td>
<td>15.3</td>
<td>10.1</td>
</tr>
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</table>

151
<table>
<thead>
<tr>
<th>Region</th>
<th>Lipid Class</th>
<th>12:0+14:0</th>
<th>16:0</th>
<th>18:0</th>
<th>18:1</th>
<th>18:2</th>
<th>18:3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exocarp</td>
<td>Neutral lipid</td>
<td>1.3</td>
<td>44.4</td>
<td>3.9</td>
<td>38.6</td>
<td>11.4</td>
<td>0.4</td>
</tr>
<tr>
<td></td>
<td>Phospholipid</td>
<td>0.5</td>
<td>31.1</td>
<td>2.5</td>
<td>34.5</td>
<td>27.1</td>
<td>4.3</td>
</tr>
<tr>
<td></td>
<td>Glycolipid</td>
<td>1.4</td>
<td>29.4</td>
<td>4.6</td>
<td>25.6</td>
<td>8.8</td>
<td>30.2</td>
</tr>
<tr>
<td>Mesocarp</td>
<td>Neutral lipid</td>
<td>1.1</td>
<td>45.0</td>
<td>3.8</td>
<td>39.1</td>
<td>10.7</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>Phospholipid</td>
<td>0.5</td>
<td>42.2</td>
<td>3.2</td>
<td>37.4</td>
<td>16.1</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td>Glycolipid</td>
<td>1.9</td>
<td>23.9</td>
<td>3.3</td>
<td>35.1</td>
<td>17.8</td>
<td>18.0</td>
</tr>
<tr>
<td>Pericarp</td>
<td>Neutral lipid</td>
<td>1.1</td>
<td>43.2</td>
<td>5.0</td>
<td>38.5</td>
<td>11.8</td>
<td>0.4</td>
</tr>
<tr>
<td></td>
<td>Phospholipid</td>
<td>2.1</td>
<td>33.9</td>
<td>2.6</td>
<td>35.0</td>
<td>24.6</td>
<td>1.8</td>
</tr>
<tr>
<td></td>
<td>Glycolipid</td>
<td>3.7</td>
<td>31.1</td>
<td>5.5</td>
<td>33.4</td>
<td>15.1</td>
<td>11.2</td>
</tr>
</tbody>
</table>
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 inflorescences 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.
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 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 commenced 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 beginning from 16 WAA was largely in
Table 21: Physical Parameters of Developing Oil Palm Fruits (Tenera Variety)

<table>
<thead>
<tr>
<th>Age of fruits in weeks after anthesis</th>
<th>Bunch weight (Kg)</th>
<th>Fruit weight (g)</th>
<th>Mesocarp %</th>
<th>Kernel %</th>
<th>Shell %</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>8.0+3.0</td>
<td>1.80+0.37</td>
<td>93.58+ 1.22</td>
<td>-</td>
<td>6.41+ 1.22</td>
</tr>
<tr>
<td>8</td>
<td>13.3+1.8</td>
<td>4.41+0.53</td>
<td>73.53+ 3.38</td>
<td>-</td>
<td>26.47+ 3.38</td>
</tr>
<tr>
<td>10</td>
<td>13.0+2.0</td>
<td>4.82+1.05</td>
<td>75.28+10.07</td>
<td>9.26+5.27</td>
<td>15.46+ 4.98</td>
</tr>
<tr>
<td>12</td>
<td>14.3+1.6</td>
<td>6.83+0.18</td>
<td>75.26+ 2.40</td>
<td>9.87+1.52</td>
<td>15.64+ 0.58</td>
</tr>
<tr>
<td>14</td>
<td>11.5+2.0</td>
<td>6.94+2.07</td>
<td>78.48+ 8.77</td>
<td>7.52+5.00</td>
<td>13.99+ 5.34</td>
</tr>
<tr>
<td>16</td>
<td>19.0+4.2</td>
<td>7.32+1.06</td>
<td>65.00+10.95</td>
<td>10.43+0.89</td>
<td>24.57+11.80</td>
</tr>
<tr>
<td>18</td>
<td>16.0+4.0</td>
<td>6.13+1.04</td>
<td>75.80+ 7.80</td>
<td>8.72+4.30</td>
<td>15.47+ 4.60</td>
</tr>
<tr>
<td>20</td>
<td>14.0+4.2</td>
<td>8.61+2.63</td>
<td>73.50+ 6.14</td>
<td>7.35+0.97</td>
<td>19.15+ 6.66</td>
</tr>
<tr>
<td>22</td>
<td>16.5+1.7</td>
<td>6.98+0.20</td>
<td>66.80+ 7.91</td>
<td>10.25+4.07</td>
<td>22.95+ 3.82</td>
</tr>
<tr>
<td>24</td>
<td>17.5+1.7</td>
<td>6.63+0.59</td>
<td>75.16+ 0.08</td>
<td>8.21+0.29</td>
<td>16.63+ 0.35</td>
</tr>
<tr>
<td>Age of fruit in weeks after anthesis</td>
<td>Mesocarp</td>
<td></td>
<td>Kernel</td>
<td></td>
<td></td>
</tr>
<tr>
<td>------------------------------------</td>
<td>----------</td>
<td>-------</td>
<td>----------</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Moisture %</td>
<td>Oil %dry weight</td>
<td>mg/fruit</td>
<td>Moisture %</td>
<td>Oil %dry weight</td>
</tr>
<tr>
<td>4</td>
<td>85.27+0.69</td>
<td>1.48+0.05</td>
<td>3.66</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>85.18+0.68</td>
<td>1.67+0.02</td>
<td>8.00</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>83.68+0.82</td>
<td>2.40+0.06</td>
<td>14.27</td>
<td>89.15+0.92</td>
<td>43.39+1.20</td>
</tr>
<tr>
<td>12</td>
<td>83.79+0.67</td>
<td>2.22+0.18</td>
<td>19.44</td>
<td>67.07+0.32</td>
<td>42.88+2.00</td>
</tr>
<tr>
<td>14</td>
<td>72.71+0.55</td>
<td>27.85+0.81</td>
<td>414.20</td>
<td>21.07+0.74</td>
<td>37.57+1.10</td>
</tr>
<tr>
<td>16</td>
<td>74.42+0.93</td>
<td>35.65+0.70</td>
<td>434.06</td>
<td>26.17+3.03</td>
<td>39.95+1.40</td>
</tr>
<tr>
<td>18</td>
<td>68.73+0.62</td>
<td>49.13+0.50</td>
<td>714.38</td>
<td>18.02+1.98</td>
<td>44.17+1.50</td>
</tr>
<tr>
<td>20</td>
<td>42.54+0.49</td>
<td>66.23+0.60</td>
<td>2408.94</td>
<td>15.34+0.55</td>
<td>44.89+0.72</td>
</tr>
<tr>
<td>22</td>
<td>42.17+0.51</td>
<td>73.22+0.50</td>
<td>1973.18</td>
<td>18.06+0.72</td>
<td>46.08+0.77</td>
</tr>
<tr>
<td>24</td>
<td>41.44+0.67</td>
<td>77.23+0.90</td>
<td>2252.25</td>
<td>14.87+1.60</td>
<td>46.30+0.95</td>
</tr>
</tbody>
</table>
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 occurred 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 predominantly 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

<table>
<thead>
<tr>
<th>Age of fruit in weeks after anthesis</th>
<th>Phospholipid</th>
<th>Glycolipid</th>
<th>Neutral lipid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g/100g total lipid</td>
<td>mg/fruit</td>
<td>g/100g total lipid</td>
</tr>
<tr>
<td>4</td>
<td>21.68±1.4</td>
<td>0.79</td>
<td>30.19±1.2</td>
</tr>
<tr>
<td>8</td>
<td>37.78±1.1</td>
<td>3.02</td>
<td>15.51±1.1</td>
</tr>
<tr>
<td>10</td>
<td>34.41±0.7</td>
<td>4.91</td>
<td>10.91±0.17</td>
</tr>
<tr>
<td>12</td>
<td>45.44±1.4</td>
<td>8.83</td>
<td>21.37±1.7</td>
</tr>
<tr>
<td>14</td>
<td>3.02±0.5</td>
<td>12.51</td>
<td>1.02±0.03</td>
</tr>
<tr>
<td>16</td>
<td>1.86±0.8</td>
<td>8.07</td>
<td>0.78±1.7</td>
</tr>
<tr>
<td>18</td>
<td>1.64±0.27</td>
<td>11.72</td>
<td>0.60±0.03</td>
</tr>
<tr>
<td>20</td>
<td>0.86±0.08</td>
<td>20.72</td>
<td>0.55±0.13</td>
</tr>
<tr>
<td>22</td>
<td>0.90±0.45</td>
<td>17.76</td>
<td>0.59±0.16</td>
</tr>
<tr>
<td>24</td>
<td>0.62±0.24</td>
<td>13.96</td>
<td>0.21±0.73</td>
</tr>
</tbody>
</table>
Figure 19. Accumulation of major lipid classes of oil palm fruit during development (Values plotted for neutral lipids are x 10^-1).
<table>
<thead>
<tr>
<th>Age of fruit in weeks after anthesis</th>
<th>Triacylglycerol</th>
<th>Diacylglycerol</th>
<th>Monoacylglycerol</th>
<th>Fatty acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>11.09</td>
<td>18.98</td>
<td>6.92</td>
<td>9.04</td>
</tr>
<tr>
<td>8</td>
<td>11.39</td>
<td>18.52</td>
<td>4.83</td>
<td>9.47</td>
</tr>
<tr>
<td>12</td>
<td>11.61</td>
<td>8.84</td>
<td>4.00</td>
<td>5.83</td>
</tr>
<tr>
<td>14</td>
<td>54.48</td>
<td>17.71</td>
<td>6.23</td>
<td>15.71</td>
</tr>
<tr>
<td>16</td>
<td>87.79</td>
<td>4.17</td>
<td>1.41</td>
<td>1.78</td>
</tr>
<tr>
<td>18</td>
<td>87.38</td>
<td>5.48</td>
<td>1.47</td>
<td>1.52</td>
</tr>
<tr>
<td>20</td>
<td>88.91</td>
<td>4.29</td>
<td>1.77</td>
<td>1.75</td>
</tr>
<tr>
<td>22</td>
<td>89.94</td>
<td>4.05</td>
<td>1.70</td>
<td>1.26</td>
</tr>
<tr>
<td>24</td>
<td>95.27</td>
<td>1.91</td>
<td>0.50</td>
<td>0.64</td>
</tr>
</tbody>
</table>
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 primarily due to the excessive synthesis of triacylglycerols and therefore due to the dilution effect. (Hitchcock 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 (ω-glycerol phosphate) or via the phospholipid pathway (Hitchcock 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; McKillop, 1966; Gurr et al, 1972); flax (Sims et al, 1961a, 1961b; McKillop 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; McKillop 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 oleaginous 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 Trineylglycerol, Diacylglycerol, Ionoacylglycerol and Free Fatty Acid Classes of Developing Oil Palm Fruit Memocarp

<table>
<thead>
<tr>
<th>Lipid class</th>
<th>Age of fruit</th>
<th>Fatty acid (wt %)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>in weeks</td>
<td>12:0 14:0 16:0 18:0 18:1 18:2 18:3</td>
</tr>
<tr>
<td>after</td>
<td>After anthesis</td>
<td></td>
</tr>
<tr>
<td>Trineylglycerol</td>
<td>4</td>
<td>1.69 1.61 19.60 3.63 17.35 47.72 0.39</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>3.56 4.61 34.37 6.44 30.50 15.91 4.61</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>2.56 1.43 32.69 9.03 23.59 25.09 6.60</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>0.22 0.52 34.79 5.61 47.78 10.45 0.63</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>0.63 0.84 46.69 6.06 36.32 8.85 0.50</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>0.22 0.74 40.99 4.58 44.23 8.91 0.33</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>0.17 1.17 39.95 4.92 45.50 7.75 0.54</td>
</tr>
<tr>
<td></td>
<td>22</td>
<td>0.24 1.47 43.00 4.11 41.02 9.59 0.57</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>0.59 1.75 42.56 4.08 41.71 8.96 0.35</td>
</tr>
<tr>
<td>Diacylglycerol</td>
<td>4</td>
<td>1.18 0.98 29.77 5.95 33.05 26.56 2.00</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>0.15 4.81 41.50 5.95 11.92 23.64 4.03</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>4.92 5.87 41.01 7.05 15.36 18.89 6.90</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>4.70 2.51 26.62 8.32 47.48 10.13 0.73</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>1.85 1.03 33.32 11.46 36.77 15.30 0.38</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>1.75 2.16 24.87 8.46 47.97 8.73 0.07</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>1.65 1.32 29.26 13.20 42.80 10.98 0.79</td>
</tr>
<tr>
<td></td>
<td>22</td>
<td>1.38 1.56 27.59 9.75 47.41 11.57 0.72</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>1.31 1.57 32.32 8.97 39.90 15.16 0.76</td>
</tr>
<tr>
<td>Monoacylglycerol</td>
<td>4</td>
<td>7.41 4.60 40.38 11.58 17.33 16.08 2.71</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>0.86 11.44 41.03 4.91 28.38 8.97 4.40</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>2.17 3.57 41.23 13.95 24.98 10.69 3.40</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>3.18 3.80 34.11 5.14 48.74 2.29 2.74</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>4.94 3.83 33.02 11.23 39.87 6.26 0.85</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>3.68 3.40 33.11 9.47 43.74 6.61</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>3.65 2.81 28.30 3.45 55.73 5.63 0.44</td>
</tr>
<tr>
<td></td>
<td>22</td>
<td>3.01 2.84 30.34 6.73 51.97 5.94 0.47</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>3.55 5.67 36.88 8.46 35.82 8.77 0.86</td>
</tr>
<tr>
<td>Fatty acid</td>
<td>4</td>
<td>3.72 3.88 32.05 11.86 18.21 23.56 6.91</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>3.60 5.15 33.79 8.63 30.94 15.84 1.97</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>5.79 1.37 33.62 7.38 26.33 19.87 5.63</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>2.04 2.38 33.25 8.20 26.41 21.06 6.86</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>4.63 4.51 37.29 9.81 31.74 9.29 2.74</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>14.11 7.93 33.52 9.12 31.56 4.15</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>2.50 2.67 38.95 14.68 33.62 7.11 0.47</td>
</tr>
<tr>
<td></td>
<td>22</td>
<td>2.68 3.41 34.92 17.71 34.55 9.67 1.87</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>4.79 3.77 39.85 9.60 34.32 7.01 0.65</td>
</tr>
</tbody>
</table>
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 beginning 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, i.e., 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>
<thead>
<tr>
<th>Lipid class</th>
<th>Age of fruit in weeks after anthesis</th>
<th>Fatty acid (wt %)</th>
<th>12:0</th>
<th>14:0</th>
<th>16:0</th>
<th>18:0</th>
<th>18:1</th>
<th>18:2</th>
<th>18:3</th>
</tr>
</thead>
<tbody>
<tr>
<td>phospholipid</td>
<td>4</td>
<td>0.07</td>
<td>0.19</td>
<td>35.38</td>
<td>2.81</td>
<td>15.25</td>
<td>41.68</td>
<td>4.61</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>0.06</td>
<td>0.30</td>
<td>33.94</td>
<td>3.55</td>
<td>10.48</td>
<td>44.19</td>
<td>7.48</td>
<td></td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>0.09</td>
<td>0.29</td>
<td>34.54</td>
<td>4.06</td>
<td>15.86</td>
<td>33.81</td>
<td>11.36</td>
<td></td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>0.51</td>
<td>0.70</td>
<td>30.24</td>
<td>3.01</td>
<td>37.23</td>
<td>23.33</td>
<td>4.97</td>
<td></td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>0.55</td>
<td>2.20</td>
<td>38.89</td>
<td>2.32</td>
<td>34.76</td>
<td>20.23</td>
<td>1.05</td>
<td></td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>0.54</td>
<td>0.95</td>
<td>28.75</td>
<td>0.83</td>
<td>42.94</td>
<td>20.55</td>
<td>5.44</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>0.65</td>
<td>2.26</td>
<td>24.35</td>
<td>0.36</td>
<td>53.31</td>
<td>17.81</td>
<td>1.26</td>
<td></td>
</tr>
<tr>
<td></td>
<td>22</td>
<td>0.35</td>
<td>1.09</td>
<td>33.55</td>
<td>3.70</td>
<td>41.64</td>
<td>19.42</td>
<td>0.25</td>
<td></td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>0.34</td>
<td>1.37</td>
<td>32.60</td>
<td>2.81</td>
<td>38.22</td>
<td>23.19</td>
<td>1.47</td>
<td></td>
</tr>
</tbody>
</table>

| 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

<table>
<thead>
<tr>
<th>Age of fruit in weeks after anthesis</th>
<th>Fresh mesocarp (g/fruit)</th>
<th>Total lipid (g/100 g fresh mesocarp)</th>
<th>Triacylglycerol (g/100 g lipid) (g/100 g fresh mesocarp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>1.68±0.9</td>
<td>0.22</td>
<td>11.09±0.38</td>
</tr>
<tr>
<td>8</td>
<td>3.24±0.2</td>
<td>0.25</td>
<td>11.39±0.92</td>
</tr>
<tr>
<td>12</td>
<td>5.14±0.1</td>
<td>0.36</td>
<td>11.61±1.52</td>
</tr>
<tr>
<td>16</td>
<td>4.76±0.7</td>
<td>9.12</td>
<td>87.79±1.68</td>
</tr>
<tr>
<td>20</td>
<td>6.33±1.9</td>
<td>38.06</td>
<td>88.91±0.39</td>
</tr>
<tr>
<td>24</td>
<td>4.98±0.4</td>
<td>45.23</td>
<td>95.27±1.45</td>
</tr>
</tbody>
</table>
of total lipids, there was very little accumulation up to 16 WAA. This lag phase was followed by a rapid phase, i.e., 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 consistent 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 primarily 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; Baf or 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

177
<table>
<thead>
<tr>
<th>Age of fruit in weeks after anthesis</th>
<th>Fatty acid position</th>
<th>12:0</th>
<th>14:0</th>
<th>16:0</th>
<th>18:0</th>
<th>18:1</th>
<th>18:2</th>
<th>18:3</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 Total</td>
<td></td>
<td>2.26</td>
<td>1.92</td>
<td>20.80</td>
<td>3.49</td>
<td>16.79</td>
<td>46.51</td>
<td>8.22</td>
</tr>
<tr>
<td>sn-2-</td>
<td></td>
<td>0.40</td>
<td>1.24</td>
<td>11.37</td>
<td>0.31</td>
<td>31.38</td>
<td>50.19</td>
<td>5.09</td>
</tr>
<tr>
<td>1,3-</td>
<td></td>
<td>3.19</td>
<td>2.26</td>
<td>25.52</td>
<td>5.08</td>
<td>9.50</td>
<td>44.67</td>
<td>9.79</td>
</tr>
<tr>
<td>8 Total</td>
<td></td>
<td>4.64</td>
<td>5.32</td>
<td>35.62</td>
<td>6.04</td>
<td>28.82</td>
<td>15.14</td>
<td>4.42</td>
</tr>
<tr>
<td>sn-2-</td>
<td></td>
<td>6.49</td>
<td>3.47</td>
<td>24.02</td>
<td>2.96</td>
<td>43.83</td>
<td>17.08</td>
<td>2.15</td>
</tr>
<tr>
<td>1,3-</td>
<td></td>
<td>3.72</td>
<td>6.25</td>
<td>41.42</td>
<td>7.58</td>
<td>21.32</td>
<td>14.17</td>
<td>5.55</td>
</tr>
<tr>
<td>12 Total</td>
<td></td>
<td>5.42</td>
<td>1.64</td>
<td>33.54</td>
<td>8.39</td>
<td>21.13</td>
<td>23.62</td>
<td>6.26</td>
</tr>
<tr>
<td>sn-2-</td>
<td></td>
<td>1.55</td>
<td>1.86</td>
<td>23.90</td>
<td>1.81</td>
<td>32.16</td>
<td>36.17</td>
<td>2.55</td>
</tr>
<tr>
<td>1,3-</td>
<td></td>
<td>7.36</td>
<td>1.53</td>
<td>38.36</td>
<td>11.68</td>
<td>15.62</td>
<td>17.34</td>
<td>8.12</td>
</tr>
<tr>
<td>16 Total</td>
<td></td>
<td>0.84</td>
<td>1.13</td>
<td>48.75</td>
<td>5.72</td>
<td>34.59</td>
<td>8.49</td>
<td>0.48</td>
</tr>
<tr>
<td>sn-2-</td>
<td></td>
<td>1.74</td>
<td>1.29</td>
<td>19.41</td>
<td>1.76</td>
<td>58.93</td>
<td>16.03</td>
<td>0.84</td>
</tr>
<tr>
<td>1,3-</td>
<td></td>
<td>0.39</td>
<td>1.05</td>
<td>63.42</td>
<td>7.70</td>
<td>22.42</td>
<td>4.72</td>
<td>0.30</td>
</tr>
<tr>
<td>20 Total</td>
<td></td>
<td>0.23</td>
<td>1.37</td>
<td>42.03</td>
<td>4.69</td>
<td>43.66</td>
<td>7.49</td>
<td>0.53</td>
</tr>
<tr>
<td>sn-2-</td>
<td></td>
<td>0.41</td>
<td>1.35</td>
<td>16.34</td>
<td>0.87</td>
<td>69.40</td>
<td>10.81</td>
<td>0.81</td>
</tr>
<tr>
<td>1,3-</td>
<td></td>
<td>0.14</td>
<td>1.38</td>
<td>54.88</td>
<td>6.60</td>
<td>30.79</td>
<td>5.83</td>
<td>0.39</td>
</tr>
<tr>
<td>24 Total</td>
<td></td>
<td>0.78</td>
<td>2.04</td>
<td>44.53</td>
<td>3.87</td>
<td>39.81</td>
<td>8.62</td>
<td>0.34</td>
</tr>
<tr>
<td>sn-2-</td>
<td></td>
<td>1.20</td>
<td>1.98</td>
<td>22.38</td>
<td>2.59</td>
<td>62.27</td>
<td>9.16</td>
<td>0.42</td>
</tr>
<tr>
<td>1,3-</td>
<td></td>
<td>0.57</td>
<td>2.07</td>
<td>55.61</td>
<td>4.51</td>
<td>28.58</td>
<td>8.35</td>
<td>0.30</td>
</tr>
</tbody>
</table>

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
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 triacylglycerols 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. Hitchcock 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 (Hitchcock 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 (i.e., 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 triacylglycerol 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 (Hitchcock 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 comparisons 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.

<table>
<thead>
<tr>
<th>Age of fruit in weeks after anthesis</th>
<th>Total triacylglycerol class</th>
<th>Proportion No. of fatty acid (mol %)</th>
<th>No. of double bonds per molecule</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>12:0</td>
<td>14:0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.26</td>
<td>1.92</td>
</tr>
<tr>
<td></td>
<td>saturated</td>
<td>3.39</td>
<td>3.86</td>
</tr>
<tr>
<td></td>
<td>monoene</td>
<td>6.82</td>
<td>2.03</td>
</tr>
<tr>
<td></td>
<td>diene</td>
<td>3.13</td>
<td>3.73</td>
</tr>
<tr>
<td></td>
<td>triene</td>
<td>29.59</td>
<td>1.37</td>
</tr>
<tr>
<td></td>
<td>polyene</td>
<td>57.07</td>
<td>0.47</td>
</tr>
<tr>
<td></td>
<td>8 0.37</td>
<td>4.64</td>
<td>5.32</td>
</tr>
<tr>
<td></td>
<td>saturated</td>
<td>14.40</td>
<td>1.82</td>
</tr>
<tr>
<td></td>
<td>monoene</td>
<td>26.37</td>
<td>1.35</td>
</tr>
<tr>
<td></td>
<td>diene</td>
<td>24.27</td>
<td>0.97</td>
</tr>
<tr>
<td></td>
<td>triene</td>
<td>15.44</td>
<td>4.11</td>
</tr>
<tr>
<td></td>
<td>polyene</td>
<td>19.51</td>
<td>0.51</td>
</tr>
<tr>
<td></td>
<td>12 0.54</td>
<td>5.42</td>
<td>1.64</td>
</tr>
<tr>
<td></td>
<td>saturated</td>
<td>11.02</td>
<td>2.57</td>
</tr>
<tr>
<td></td>
<td>monoene</td>
<td>11.12</td>
<td>3.04</td>
</tr>
<tr>
<td></td>
<td>diene</td>
<td>7.42</td>
<td>4.29</td>
</tr>
<tr>
<td></td>
<td>triene</td>
<td>21.08</td>
<td>1.93</td>
</tr>
<tr>
<td></td>
<td>polyene</td>
<td>49.36</td>
<td>0.86</td>
</tr>
<tr>
<td></td>
<td>16 96.63</td>
<td>0.84</td>
<td>1.13</td>
</tr>
<tr>
<td></td>
<td>saturated</td>
<td>11.00</td>
<td>1.14</td>
</tr>
<tr>
<td></td>
<td>monoene</td>
<td>37.98</td>
<td>0.61</td>
</tr>
<tr>
<td></td>
<td>diene</td>
<td>25.55</td>
<td>0.47</td>
</tr>
<tr>
<td></td>
<td>triene</td>
<td>11.28</td>
<td>0.45</td>
</tr>
<tr>
<td></td>
<td>polyene</td>
<td>11.80</td>
<td>0.59</td>
</tr>
<tr>
<td></td>
<td>20 406.72</td>
<td>0.23</td>
<td>1.37</td>
</tr>
<tr>
<td></td>
<td>saturated</td>
<td>12.94</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td>monoene</td>
<td>32.56</td>
<td>0.51</td>
</tr>
<tr>
<td></td>
<td>diene</td>
<td>29.75</td>
<td>0.35</td>
</tr>
<tr>
<td></td>
<td>triene</td>
<td>12.44</td>
<td>0.70</td>
</tr>
<tr>
<td></td>
<td>polyene</td>
<td>12.31</td>
<td>1.03</td>
</tr>
<tr>
<td></td>
<td>24 512.38</td>
<td>0.78</td>
<td>2.04</td>
</tr>
<tr>
<td></td>
<td>saturated</td>
<td>10.82</td>
<td>0.99</td>
</tr>
<tr>
<td></td>
<td>monoene</td>
<td>32.32</td>
<td>0.48</td>
</tr>
<tr>
<td></td>
<td>diene</td>
<td>30.84</td>
<td>0.96</td>
</tr>
<tr>
<td></td>
<td>triene</td>
<td>11.19</td>
<td>0.48</td>
</tr>
<tr>
<td></td>
<td>polyene</td>
<td>14.83</td>
<td>0.58</td>
</tr>
</tbody>
</table>
described elsewhere (section 3.1.2.2). The following five fractions or triacylglycerol classes were identified, 'saturated', 'monoene', 'dienes', '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 association 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 demonstrates 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

<table>
<thead>
<tr>
<th>Age of fruit in weeks after anthesis</th>
<th>Triacylglycerol class</th>
<th>Triacylglycerol (mol %)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Saturated</td>
<td>Monoene</td>
</tr>
<tr>
<td>4</td>
<td>64.9</td>
<td>59.66</td>
</tr>
<tr>
<td></td>
<td>35.1</td>
<td>40.34</td>
</tr>
<tr>
<td>8</td>
<td>74.53</td>
<td>14.74</td>
</tr>
<tr>
<td></td>
<td>25.47</td>
<td>85.26</td>
</tr>
<tr>
<td>12</td>
<td>82.09</td>
<td>94.73</td>
</tr>
<tr>
<td></td>
<td>17.91</td>
<td>5.27</td>
</tr>
<tr>
<td>16</td>
<td>95.53</td>
<td>5.05</td>
</tr>
<tr>
<td></td>
<td>4.47</td>
<td>94.95</td>
</tr>
<tr>
<td>20</td>
<td>76.87</td>
<td>97.87</td>
</tr>
<tr>
<td></td>
<td>23.13</td>
<td>2.13</td>
</tr>
<tr>
<td>24</td>
<td>89.53</td>
<td>97.28</td>
</tr>
<tr>
<td></td>
<td>10.47</td>
<td>2.72</td>
</tr>
</tbody>
</table>

190
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.
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 commercial 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

<table>
<thead>
<tr>
<th>Lipid class</th>
<th>Sterilizer condensate</th>
<th>Press fibre</th>
<th>Sludge</th>
<th>Crude palm oil</th>
<th>Solvent extracted oil from fresh mesocarp</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Neutral lipid (relative %)</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Triacylglycerol</td>
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<td>65.4</td>
<td>72.8</td>
<td>93.0</td>
<td>97.1</td>
</tr>
<tr>
<td>Diacylglycerol</td>
<td>11.5</td>
<td>16.6</td>
<td>10.1</td>
<td>4.5</td>
<td>2.0</td>
</tr>
<tr>
<td>Monoacylglycerol</td>
<td>9.6</td>
<td>5.6</td>
<td>6.2</td>
<td>0.9</td>
<td>0.2</td>
</tr>
<tr>
<td>Free fatty acid</td>
<td>24.0</td>
<td>12.5</td>
<td>10.9</td>
<td>1.5</td>
<td>0.7</td>
</tr>
<tr>
<td>Polar lipid (ppm total lipid)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phospholipid</td>
<td>6721</td>
<td>25975</td>
<td>6636</td>
<td>1443</td>
<td>5633</td>
</tr>
<tr>
<td>Glycolipid</td>
<td>13925</td>
<td>20311</td>
<td>1139</td>
<td>438</td>
<td>2492</td>
</tr>
</tbody>
</table>
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/cm² (equivalent to 130°C) 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°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°C to separate the crude palm oil from the watery
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>
<thead>
<tr>
<th>Fatty acid (wt. %)</th>
<th>Sterilizer condensate</th>
<th>Press fibre</th>
<th>Sludge</th>
<th>Crude palm oil</th>
<th>Solvent extracted oil from fresh mesocarp</th>
</tr>
</thead>
<tbody>
<tr>
<td>12:0</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>14:0</td>
<td>1.8</td>
<td>1.2</td>
<td>1.3</td>
<td>1.4</td>
<td>1.3</td>
</tr>
<tr>
<td>16:0</td>
<td>49.2</td>
<td>42.6</td>
<td>43.3</td>
<td>44.5</td>
<td>40.2</td>
</tr>
<tr>
<td>18:0</td>
<td>4.6</td>
<td>4.5</td>
<td>4.6</td>
<td>4.7</td>
<td>4.9</td>
</tr>
<tr>
<td>18:1</td>
<td>36.7</td>
<td>38.9</td>
<td>39.5</td>
<td>38.9</td>
<td>40.6</td>
</tr>
<tr>
<td>18:2</td>
<td>7.3</td>
<td>11.6</td>
<td>10.8</td>
<td>10.0</td>
<td>12.3</td>
</tr>
<tr>
<td>18:3</td>
<td>0.3</td>
<td>1.1</td>
<td>0.4</td>
<td>0.4</td>
<td>0.6</td>
</tr>
</tbody>
</table>
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>
<thead>
<tr>
<th>Process stream</th>
<th>Phospholipid</th>
<th>Glycolipid</th>
<th>Phospholipid</th>
<th>Glycolipid</th>
<th>Phospholipid</th>
<th>Glycolipid</th>
<th>Phospholipid</th>
<th>Glycolipid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sterilizer condensate</td>
<td>0.4</td>
<td>2.3</td>
<td>45.4</td>
<td>5.0</td>
<td>37.8</td>
<td>8.9</td>
<td>2.2</td>
<td></td>
</tr>
<tr>
<td>Press fibre</td>
<td>0.8</td>
<td>1.5</td>
<td>5.6</td>
<td>5.0</td>
<td>31.9</td>
<td>5.0</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>Sludge</td>
<td>0.2</td>
<td>0.4</td>
<td>31.1</td>
<td>1.5</td>
<td>40.6</td>
<td>24.2</td>
<td>2.1</td>
<td></td>
</tr>
<tr>
<td>Crude palm oil</td>
<td>0.6</td>
<td>1.4</td>
<td>35.9</td>
<td>3.6</td>
<td>30.8</td>
<td>17.7</td>
<td>24.3</td>
<td></td>
</tr>
<tr>
<td>Solvent extracted oil</td>
<td>0.2</td>
<td>1.1</td>
<td>36.2</td>
<td>4.6</td>
<td>42.6</td>
<td>11.2</td>
<td>6.2</td>
<td></td>
</tr>
</tbody>
</table>

Table 33: Fatty Acid Composition of Phospholipids and Glycolipids of Palm Oil Process Streams.
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>
<thead>
<tr>
<th>Lipid class</th>
<th>Process stream</th>
<th>Fatty acid (wt. %)</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>12:0</td>
<td>14:0</td>
<td>16:0</td>
<td>18:0</td>
<td>18:1</td>
<td>18:2</td>
</tr>
<tr>
<td>Triacylglycerol</td>
<td>Sterilizer condensate</td>
<td>0.1</td>
<td>1.4</td>
<td>44.9</td>
<td>5.2</td>
<td>37.9</td>
<td>9.6</td>
</tr>
<tr>
<td></td>
<td>Press fibre</td>
<td>0.7</td>
<td>2.2</td>
<td>47.6</td>
<td>3.6</td>
<td>36.5</td>
<td>9.2</td>
</tr>
<tr>
<td></td>
<td>Sludge</td>
<td>0.1</td>
<td>1.4</td>
<td>43.7</td>
<td>4.2</td>
<td>40.5</td>
<td>10.0</td>
</tr>
<tr>
<td></td>
<td>Crude palm oil</td>
<td>0.1</td>
<td>1.3</td>
<td>45.0</td>
<td>4.8</td>
<td>38.5</td>
<td>9.8</td>
</tr>
<tr>
<td></td>
<td>Solvent-extracted oil</td>
<td>-</td>
<td>1.4</td>
<td>41.2</td>
<td>4.9</td>
<td>41.0</td>
<td>11.2</td>
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<td>Diacylglycerol</td>
<td>Sterilizer condensate</td>
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<td>9.7</td>
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<tr>
<td></td>
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<td>0.5</td>
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<td></td>
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<td></td>
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<td>35.3</td>
<td>3.4</td>
<td>45.7</td>
<td>12.4</td>
</tr>
<tr>
<td></td>
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<td>1.4</td>
<td>30.7</td>
<td>3.6</td>
<td>45.4</td>
<td>16.9</td>
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<tr>
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<td>Sterilizer condensate</td>
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<td>36.4</td>
<td>5.7</td>
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<tr>
<td></td>
<td>Press fibre</td>
<td>4.7</td>
<td>3.2</td>
<td>39.5</td>
<td>5.9</td>
<td>36.5</td>
<td>9.1</td>
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<tr>
<td></td>
<td>Sludge</td>
<td>3.2</td>
<td>2.6</td>
<td>44.5</td>
<td>7.4</td>
<td>34.1</td>
<td>8.2</td>
</tr>
<tr>
<td></td>
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<td>3.8</td>
<td>38.9</td>
<td>5.8</td>
<td>35.7</td>
<td>9.2</td>
</tr>
<tr>
<td></td>
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<td>38.0</td>
<td>6.3</td>
<td>35.5</td>
<td>10.8</td>
</tr>
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<td>4.9</td>
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<td>5.6</td>
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<tr>
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<td>44.9</td>
<td>4.5</td>
<td>39.7</td>
<td>8.2</td>
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<td></td>
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<td>45.8</td>
<td>4.8</td>
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<td>37.5</td>
<td>6.2</td>
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<td>10.3</td>
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