PERGAMON

Progress in Lipid Research 39 (2000) 507–558

Progress in Lipid Research

www.elsevier.com/locate/plipres

Chemistry and biochemistry of palm oil

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Contents

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PII: S0163-7827(00)00015-1

Nomenclature

AAF	2-acetylaminofluorene
AHA	American Heart Association (the AHA diet has equal amounts of saturated, monounsaturated and polyunsaturated fatty acids
CHD	coronary heart disease
DAGAT	diacylglycerol 3-phosphate acyltransferase
HDL-C	high density lipoprotein cholesterol
HMGR	hydroxymethylglutaryl-CoA reductase
IV	iodine value
LDL-C	low density lipoprotein cholesterol
LM	laurate + myristate
LPAAT	lysophosphatidate accyltransferase
OT	obstruction times
PFAD	palm fatty acid destillate
RBD	refined bleached deordorised
SFC	solid fat content
TC	total cholesterol
TE	tocopherol equivalent
TRF	tocopherol-rich fraction

1. Introduction

The oil palm is a monocotyledon belonging to the species *Elaeis*. It is a perennial tree crop and the highest oil producing plant, yielding an average of 3.7 t of oil per hectare per year in Malaysia. The crop is unique in that it produces two types of oil. The fleshy mesocarp produces palm oil which is used mainly for edible purposes and the kernel produces palm kernel oil which has wide application in the oleochemical industry. The genus *Elaeis* comprises two species, namely *E. guineensis* and *Elaeis oleifera*. *E. guineensis* originates in West Africa and the commercial planting material is mainly of this species. *E. oleifera* is a stumpy plant of South American origin and its oil is characterised by a high oleic acid content.

Palm oil became the world's second most important vegetable oil after soybean oil in 1980 [1]. The oil may be designated as Malaysian, Indonesian, Nigerian, Ivory Coast or by its other sources. The origin of the oil often gives an indication of its identity and quality characteristics. Currently, most of the world's production of palm oil comes from south-east Asia, in particular Malaysia and Indonesia. Malaysian crude palm oil production increased from 8.3 million t in 1998 to 10.6 million t in 1999, maintaining the country's position as the world's largest supplier of the oil. Currently oil palm accounts for 13% of the total world production of oils and fats and is expected to overtake soybean oil as the most important vegetable oil.

2. Origin

E. guineensis originates from West Africa. It was first introduced to Brazil and other tropical countries in the fifteenth century by the Portuguese. However, its propagation did not take off until the nineteenth century when the Dutch brought seeds from West Africa to Indonesia resulting in the four seedlings planted in Bogor, Indonesia in 1848. The palms were *dura* and the progenies from these seedlings were planted as ornamentals in Deli and became known as Deli *Dura*. From there the oil palm was sent to the Botanical Gardens in Singapore in 1875, and subsequently brought to Malaya (as West Malaysia was then known) in 1878. The oil palm was initially planted in Malaya as an ornamental and the first commercial planting was only in 1917.

E. oleifera originates from South America. The mesocarp oil of *E. oleifera* has higher oleic and linoleic acid content and lower content of palmitic and other saturated acids. The iodine value (IV) ranges from 70–80. The current main interest in *E. oleifera* is in the potential of transmitting its useful characters to interspecific hybrids with *E. guineensis*. Both *E. guineensis* and *E. oleifera* have the same somatic chromosome number of 32 and hybridise easily [2]. The advantages of the F1 hybrids over *E. guineensis* are a more unsaturated oil and a lower height increment.

3. Fruit description

The oil palm fruit is a drupe, which forms in a tight bunch. The pericarp comprises three layers, the exocarp (skin), mesocarp (outer pulp containing palm oil) and endocarp (a hard shell enclosing the kernel (the endosperm) which contains oil and carbohydrate reserves for the embryo).

Shell thickness is controlled by a single gene [3]. The homozygote *dura* (sh^t sh^t) is thick-shelled and the homozygote *pisifera* (sh^-, sh^-) does not have a shell. A cross between *dura* and *pisifera* results in the heterozygote *tenera* which h thin shell surrounded by a fibre ring in the mesocarp. The thinner shell of the *tenera* results in more oil-bearing mesocarp. Since 1961, most of the planting materials have been *tenera* (*dura* × *pisifera*). The *pisifera* is not used as a commercial planting material as it is mostly infertile.

4. Biochemistry of palm oil

4.1. Fruit development and oil deposition

Fruit development and oil deposition in the oil palm have been described by Hartley [4] and Thomas et al. [5]. Fruit development starts at approximately 2 weeks after anthesis (WAA). At 8 WAA, the endosperm of the seed is still liquid and turns semi-gelatinous at 10 WAA [4,6]. Oil deposition in the endosperm starts at approximately 12 WAA and is almost complete by 16 WAA [6,7]. During this period the endosperm and endocarp slowly harden and by 16 WAA the endocarp is a hard shell enclosing a hard whitish endosperm — the kernel. Oil deposition in the mesocarp starts at approximately 15 WAA and continues until fruit maturity at about 20 WAA

[6,8]. In Nigerian oil palm, maximum lipid accumulation occurs at 18–22 WAA [9]. The fruits on a bunch do not ripen simultaneously owing to slight variation in the time of pollination. The period of receptivity of the florets in an anthesising female inflorescence is 2–5 days. Fruits at the end of each spikelet ripen first and those at the base last. Fruits on the outside of the bunch are large and deep orange when ripe while the inner fruits are smaller and paler. Evidence for further accumulation of lipid in the bunch following the onset of the first fruit abscission has been contradictory. Some workers reported that oil continued to be deposited in the bunch after the first abscission [10] while others have reported that one loose fruit is the signal for full ripeness of the bunch with no further oil synthesis [11]. More recently, Sambanthamurthi et al. [8] also reported that oil accumulation in the bunch is complete at the first sign of fruit abscission. Any ''increase'' in oil content after this is apparent as a consequence of desiccation as the fruit ripens.

4.2. Changes in lipid class and fatty acid composition during development of the oil palm mesocarp

Oo et al. [6] and Bafor and Osagie [9] studied the changes in lipid class and composition in the developing mesocarp of *E. guineensis* var. *tenera* and var. *dura*, respectively. The results of Oo et al. [10] are represented in Tables 1 and 2 and are essentially similar to those obtained by Bafor and Osagie [9]. Phospholipids are the major lipid class before oil deposition, accounting for about 60% of the total lipids at 8–12 WAA. The phospholipids form the larger portion of the polar component of the immature oil palm mesocarp. At maturity, however, glycolipids form the major component of the polar lipids [9].

Bafor and Osagie [12,13] reported that the major phospholipid of immature (8 WAA) mesocarp is phosphatidylcholine (PC), followed by phosphatidylinositol (PI) and lysophosphatidyl-choline (LPC) with PC accounting for about 51% of the total phospholipids. Phosphatidylethanolamine (PE), which was not detected at eight WAA, accounted for 20% of the total phospholipids at 18 WAA. PC was the major fraction (28%) in mature mesocarp, followed by PI, PE, diphosphatidylglycerol (DPG), phosphatidic acid (PA) and phosphatidylglycerol (PG). LPC remained a trace.

Table 1
Changes in lipid content of developing oil palm mesocarp [10][a]

WAA	Moisture content (g/100 g fr. Weight)	Lipid content (g/100 g fr. weight)						
		TL	TAG	FA	1,3-DAG	1,2-DAG	MAG	PL
8	87.85	0.09	0.01 (7.10)	0.01 (9.30)	0.01 (8.10)	0.01 (13.60)	Trace (1.60)	0.05 (60.30)
12	88.42	0.14	0.02 (13.90)	0.01 (4.80)	0.01 (8.40)	0.02 (13.20)	Trace (1.80)	0.08 (57.80)
16	81.46	6.4	5.08 (79.30)	0.51 (8.00)	0.11 (1.70)	0.28 (4.40)	0.08 (1.20)	0.34 (5.30)
20	37.45	47.77	36.75 (76.90)	6.17 (12.90)	0.39 (0.80)	2.30 (4.80)	1.76 (3.70)	0.41 (0.90)
Overripe	22.82	66.69	19.32 (29.00)	39.06 (58.80)	2.29 (3.40)	1.66 (2.50)	0.21 (0.30)	4.15 (6.20)

[a] Figures in parenthesis give the % of each lipid class in the sample. WAA, weeks after anthesis; TL, total lipids; FA, fatty acids; TAG, DAG, MAG, mono-, di-, tri-acylglycerols; PL, polar lipids.

Although the percentage composition of phospholipids decreased as the fruit developed, their absolute content remained virtually constant throughout fruit development, indicating that these membrane lipids were synthesised very early during fruit development.

Table 2
Changes in fatty acid composition of lipid classes at different stages of development of oil palm mesocarp [10][a]

Weeks after anthesis	Lipid class	Percentage of total fatty acids									
		10:0	12:0	14:0	16:0	18:0	18:1	18:2	18:3	20:3	22:0
8	Total lipids	4.20	3.10	1.00	27.50	4.40	22.20	24.00	13.60		
12		1.40	1.50	1.00	27.00	4.50	22.70	23.90	18.00		
16		0.10	1.70	0.40	35.20	5.40	42.60	13.90	0.80		
20		0.40	5.50	1.10	40.80	5.00	35.90	11.30	0.00		
Overripe		0.00	0.80	1.50	44.20	5.40	38.70	9.40	0.00		
8	Triacylglycerols	0.00	0.70	1.40	43.20	3.20	18.90	20.60	10.80	1.20	
12		0.10	0.70	1.30	25.80	1.40	24.50	25.70	20.60	0.00	
16		0.40	0.80	0.60	38.90	4.80	42.60	11.90	0.00	0.00	
20		0.00	0.10	1.40	45.40	5.20	36.70	11.10	0.00	0.00	
Overripe		0.00	0.30	1.40	56.30	3.20	33.30	5.60	0.00	0.00	
8	Fatty acids	0.00	1.40	2.30	49.80	2.60	20.30	7.00	8.70	7.80	
12		0.00	3.50	3.00	25.40	0.40	45.00	9.50	1.40	11.90	
16		0.00	0.60	0.00	28.30	0.80	54.90	15.40	0.00	0.00	
20		0.20	0.20	2.50	58.80	6.00	24.70	7.60	0.00	0.00	
Overripe		0.00	0.20	1.50	35.40	0.50	45.20	17.20	0.00	0.00	
8	1,3-Diacylglycerols	0.00	33.30	7.30	52.40	3.50	3.40	0.00			
12		1.00	18.00	8.20	42.40	5.20	25.50	0.00			
16		0.10	2.10	0.60	68.10	5.80	21.90	1.80			
20		1.10	0.90	2.80	51.30	5.90	29.70	8.30			
Overripe		0.00	0.20	1.90	55.80	4.60	31.20	6.20			
8	1,2-Diacylglycerols	0.20	1.40	2.20	74.20	4.10	7.50	0.00	0.00	10.40	
12		0.10	1.30	1.70	62.50	3.60	12.80	13.60	3.00	1.30	
16		0.00	0.20	0.10	35.70	1.90	45.40	16.70	0.00	0.00	
20		0.30	0.00	1.30	41.80	4.60	37.50	14.50	0.00	0.00	
Overripe		0.00	0.40	1.60	55.50	3.40	31.40	7.80	0.00	0.00	
8	Monoacylglycerols	0.20	0.40	3.90	60.00	3.80	14.00	7.60	2.30	3.40	4.30
12		0.10	1.70	1.80	44.30	2.90	14.70	22.90	11.70	0.00	0.00
16		0.00	0.60	0.80	66.30	8.50	18.50	5.30	0.00	0.00	0.00
20		0.70	1.80	1.40	67.00	9.70	14.80	4.70	0.00	0.00	0.00
Overripe		0.10	2.20	2.00	59.10	5.60	28.80	7.20	0.00	0.00	0.00
8	Polar lipids	0.00	0.30	0.50	40.90	2.00	14.00	28.60	13.70	0.00	
12		0.00	0.30	0.60	32.50	2.10	17.20	24.80	22.30	0.00	
16		0.20	1.00	0.30	33.40	1.90	24.50	28.00	10.80	0.00	
20		1.30	0.30	1.20	45.60	4.30	18.90	14.80	13.60	0.00	
Overripe		0.50	2.00	1.40	40.80	0.70	33.40	14.60	5.40	1.10	

[a] Fatty acids are denoted by the number of carbon atoms: the number of double bonds.

Monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG) were the main glycolipids of immature mesocarp, accounting for 62 and 38%, respectively of the total glycolipids. MGDG was the main glycolipid in mature mesocarp. Other glycolipids identified were esterified glycoside, steryl glycoside, cerebrosides and DGDG.

In the commercial Malaysian *tenera*, the neutral lipids, especially triacylglycerols (TAG), increase rapidly from 16 WAA and parallel the accumulation of total lipids, reaching their maximum at 20 WAA. The polar lipids simultaneously decrease to less than 1% of the total lipids at 20 WAA. The Nigerian *dura* follows a similar pattern except that rapid TAG accumulation occurs from 18–22 WAA.

Palmitoleic and linolenic acids are present in significant amounts in the early stages of lipid synthesis. These are typical chloroplast and membrane fatty acids, reflecting a high ratio of chloroplast and cellular synthesis to storage lipid synthesis. These fatty acids, however, are undetectable after 16 WAA, probably greatly diluted by the accumulation of storage lipids.

The immature mesocarp contains large amounts of chlorophyll which decline by about 17 WAA, accompanied by a massive accumulation of carotenes as the fruit ripens [13,14]. Also characteristic of the immature green mesocarp are large amounts of sterols [13]. As the fruit matures, the sterols decrease as a consequence of dilution by the tremendous amount of TAG synthesised.

Monoacylglycerols (MAG) are not involved in TAG biosynthesis and their presence in tissue extracts is probably a consequence of lipolytic activity. As seen in Table 1, the level of MAG is low throughout fruit development in the oil palm mesocarp. The percentages of both 1, 2- and 1, 3-diacylglycerol (1,2- and 1,3-DAG) decrease with progressive accumulation of TAG. 1,2-DAG is the immediate precursor for TAG and its concentration in the mesocarp represents its steady state concentration in the biosynthetic pathway.

The major fatty acids of palm oil TAG are 16:0, 18:1 and 18:2. In the younger fruit (8–12 WAA), 18:3 is an additional major fatty acid. In the ripe fruit (20 WAA), palmitic acid is the major fatty acid accounting for 44% of the total fatty acid composition followed by oleic and linoleic acids which account for 39 and 10%, respectively. The level of oleic acid is significantly higher and palmitic acid lower at 16–18 WAA compared to the ripe fruit [8]. This is probably because of higher palmitoyl-ACP thioesterase activity in the ripe fruit. The stereospecific distribution of the fatty acyl residues of palm oil is indicated in Table 3. This distribution is largely determined by the specificities of the acyltransferase enzymes of the Kennedy Pathway. The first enzyme in the Kennedy pathway, glycerol-3-P acyltransferase (GPAT) catalyses the esterification of acyl-CoAs to the sn-1 position of the glycerol backbone resulting in the formation of 1-acyl sn-glycerol-3-phosphate or lysophosphatidate [15]. The action of 1-acyl-sn-glycerol-3-phosphate acyltransferase (LPAAT) at the sn-2 position produces phosphatidate [16]. Phosphatidate phosphohydrolase hydrolyses phosphate from phosphatidate to yield 1,2-diacylglycerol. The final step in the Kennedy pathway is catalysed by 1,2-diacylglycerol acyltransferase (DAGAT) which adds an acyl group to the sn-3 position to produce triacylglycerol (TAG).

In plants, GPAT appears to be the rate-controlling enzyme that limits carbon entry into the Kennedy Pathway [17]. Although GPAT prefers saturated moieties, its specificity is much less than that of LPAAT [18,19]. In crude palm oil, the C50 and C52 TAG species account for 43 and 41%, respectively of the total TAG composition [20]. The major components of C50 are palmitoyl, palmitoyl, oleoyl (PPO) and POP. The major constituents of C52 are POO and OOP. Based

Table 3
Positional distribution of fatty acids (%) in palm oil [235]

Fatty acid	Position 2	Position 1, 3
C14:0	0.96	1.42
C16:0	16.03	62.77
C18:0	1.37	5.73
C18:1	62.68	24.52
C18:2	18.95	5.52

on this distribution, it appears that all three positions of the glycerol molecule can accommodate either palmitic acid or oleic acid. However, there is some preference for palmitic acid over oleic acid at sn-1. Manaf and Harwood [21,22] solubilised and purified GPAT from microsomal fractions of oil palm mesocarp and calli and found that although oil palm GPAT can use both palmitoyl-CoA and oleoyl-CoA, palmitoyl-CoA is the preferred substrate.

LPAAT exhibits strong substrate specificity in most oilseed species. In safflower, this enzyme prefers linoleate to oleate and discriminates almost totally against saturated fatty acids [16]. In rapeseed, erucate is an extremely poor substrate compared to oleate [23,24]. The high composition of PPO in palm oil suggests that the sn-2 position, which is usually specific for unsaturated fatty acids, is less specific in this plant. Thus, although oil palm LPAAT appears to have a preference for oleic acid, it can accept palmitoyl-CoA as substrate. Similarly, in palm kernel oil, the major TAG species are those of carbon numbers 36, 38 and 40. C36 comprises mainly lauric acid, while C38 and C40 are mostly combinations of lauric and myristic acids. Here again, it is obvious that the sn-2 position, and hence oil palm kernel LPAAT, does not exhibit high specificity for unsaturated fatty acids. While GPAT and LPAAT are also involved in phospholipid biosynthesis, the third enzyme, DAGAT, is unique to TAG biosynthesis. This enzyme has some selectivity but, in general, a broad specificity [25]. Oo and Chew [26] detected DAGAT activity in both the microsomes and oil bodies of oil palm mesocarp with activities two to three times higher in the oil bodies. Both microsomal and oil body preparations required Mg^{2+} for maximum activity. In both preparations, the enzyme was active with myristoyl-CoA, palmitoyl-CoA, stearoyl-CoA and oleoyl-CoA. When presented with a mixture of two acyl-CoAs, the microsomal enzyme showed no selectivity but the oil body enzyme had a preference for oleoyl-CoA over palmitoyl-CoA. Oo et al. [27] and Oo and Chew [26] also reported GPAT and LPAAT activity in both microsomes and oil body preparations. Umi Salamah et al. [28] reported that DAGAT of oil palm callus cultures preferred palmitoyl-CoA as substrate.

4.3. Key enzymes in regulation of fatty acid composition in the oil palm mesocarp

Palmitic acid accounts for 44% of the total fatty acid composition of palm (mesocarp) oil. In no other commerical vegetable oil does palmitic acid accumulate to this extent. Any attempt to alter the fatty acid composition by genetic manipulation requires an understanding of the regulation of fatty acid synthesis. In the oil palm, the pertinent question to be answered is why palmitic acid accumulates in the mesocarp.

4.4. Beta-ketoacyl ACP synthase II (KAS II)

KAS II is a condensing enzyme exclusively responsible for the conversion of palmitic acid to stearic acid [29]. There is, therefore, considerable interest in this enzyme with respect to its role in determining the ratio of C16 to C18 fatty acids [30].

Investigation of KAS II activity in the developing mesocarp from 12–21 WAA showed that activity increased from 15 WAA reaching a maximum at 20 WAA [31–33]. This is similar to the pattern of TAG biosynthesis in oil palm mesocarp which starts at 16 WAA and reaches a maximum at around 20 WAA [6].

The high palmitate content relative to stearate in palm oil suggested that KAS II may be a rate-controlling enzyme. The Palm Oil Research Institute of Malaysia (PORIM) is fortunate to have the world's largest oil palm germplasm collection. Fruits from various palms from this collection have been screened for KAS II activity against fatty acid composition and iodine value (IV). IV is a measure of the level of unsaturation of an oil. A strong positive correlation was observed between KAS II activity and IV.KAS II activity was also positively correlated with the levels of C18:1 and C18:2 individually. However, the correlation was much stronger with the combined level of these two fatty acids. Interestingly, the level of C16:0 was negatively correlated to KAS II activity as well as to the level of C18:1. These findings provide strong evidence that limiting KAS II activity contributes towards palmitic acid accumulation in the oil palm mesocarp [33–35]. The results also indicate that Δ 9 desaturase is not limiting in the oil palm mesocarp and efficiently converts Δ 9 stearoyl ACP to oleoyl ACP. Oleate desaturase is also quite active in converting oleic acid to linoleic acid as seen from the strong correlation between KAS II activity and C18:2 individually or in combination with C18:1. Increasing KAS II activity is thus likely to result in an increase in oleic acid as well as linoleic acid content . Anti-sensing the oleate desaturase gene may thus be necessary to obtain high oleic acid without a concomitant increase in linoleic acid.

KAS II was purified about 10,000-fold from oil palm mesocarp [31,33]. The pure enzyme had optimum activity between pH 4.5 and 5.0. Maximum activity was observed at 30°C with a significant reduction at higher temperatures. Divalent ions had a significant effect on KAS II activity. Mg^{2+} at 10 mM stimulated KAS II activity 6–10-fold while Cu^{2+} and Ca^{2+} reduced the activity. Field experiments are in progress to investigate the effect of these cations on fatty acid composition and oil content. Questions to be answered include what role fertilizers such as Mg^{2+} play in affecting fatty acid composition. Would soils rich in magnesium promote higher unsaturation?

4.5. Acyl ACP thioesterases

Acyl ACP thioesterases play a very important role in the termination of chain elongation. These enzymes cause the release of fatty acids from ACP so that they can be exported out of the plastid into the cytoplasm where they are incorporated into triacylglycerols. The high palmitic and oleic acid content of palm oil suggested the presence of acyl ACP thioesterases with high specificity for palmitoyl ACP and oleoyl ACP in the oil palm mesocarp. Investigation of thioesterase activity in crude extracts of oil palm mesocarp showed a preference for palmitoyl ACP followed by oleoyl ACP as substrates (Fig. 1) [36–38]. The results confirmed that palmitic acid is

cleaved from palmitoyl ACP in the growing fatty acid chain, resulting in palmitic acid moving out of the plastid and being esterified to TAGs by the action of acyltransferases in the cytoplasm (endoplasmic reticulum). This mechanism would thus result in accumulation of TAGs containing high levels of palmitic acid. Similarly, the high activity of thioesterase towards oleoyl ACP explains the high levels of oleic acid in oil palm mesocarp. There is some thioesterase activity towards stearoyl ACP and very low activity towards lauroyl ACP and myristoyl ACP in crude mesocarp extracts. Stearic, lauric and myristic acids are present only in small amounts in palm oil (Table 4). With the advent of genetic manipulation, it is now possible to tailor the fatty acid compositions of oils. Increasing oleic acid at the expense of palmitic acid is one such target. It was thus of interest to investigate whether palmitoyl ACP thioesterase and oleoyl ACP thioesterase activities reside on a single protein or on separate proteins. If on a single protein, manipulation for lower palmitic acid would result in a corresponding decrease in oleic acid. Similarly, manipulation for increased oleic acid would also elevate the level of palmitic acid. However, if they were different proteins, then they could be manipulated independently. Protein purification was thus carried out and two major peaks were resolved by chromatography. Thioesterase activity towards palmitoyl ACP and oleoyl ACP was thus confirmed to be on two separate peaks [39,40], i.e. they

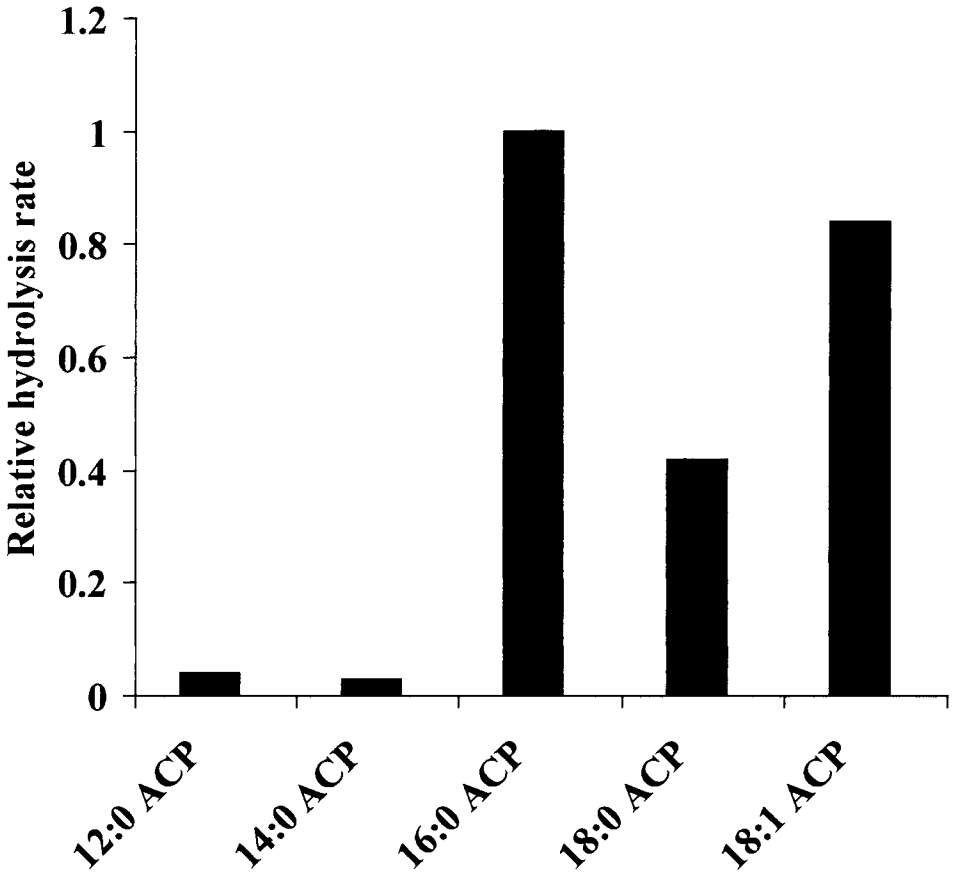

Fig. 1. Thioesterase activity in oil palm mesocarp.

are two separate proteins and would therefore be amenable to genetic manipulation independently.

4.6. Stearoyl ACP desaturase

Oleic acid is formed by the aerobic desaturation of stearic acid by the action of Δ 9 stearoyl ACP desaturase. This enzyme is highly specific for stearoyl ACP (as substrate) but palmitoyl ACP can act as a poor substrate to produce palmitoleic acid. This explains why palmitate and oleate are the main products of de novo fatty acid biosynthesis. Palm oil contains 39% oleic acid and $< 5\%$ stearic acid indicating that the Δ 9 stearoyl ACP desaturase of oil palm mesocarp is very active and effectively converts almost all the stearoyl ACP to oleoyl ACP. Despite the fact that palm oil contains 44% palmitic acid, palmitoleic acid only accounts for about 0.1–0.3% of the total fatty acids, indicating that palmitoyl ACP is a poor substrate for Δ 9 stearoyl ACP desaturase. However, under certain conditions, the overall desaturase changes its characteristics dramatically. It was shown that protoplasts isolated from both mesocarp and embryogenic cultures of oil palm synthesised palmitoleic acid in excess of 30% of the total fatty acid composition [41,42]. Stearic acid content also increased, showing that the total desaturase activity had changed its preference for stearoyl ACP and was also instead acting on palmitoyl ACP. The C18:1 content was about 30%. However, almost all of this was *cis*-vaccenic acid which must have formed by elongation of palmitoleic acid. There is pharmaceutical demand for palmitoleic acid as it has antithrombotic properties. *Cis*-vaccenic acid also has industrial applications. It may thus be useful to produce these fatty acids on a large scale in the oil palm.

4.7. Lipase activity in the oil palm mesocarp

The level of free fatty acids (FFA) is a major determinant of oil quality. Oxidation of free fatty acids results in rancidity and impairment of oil quality. Lipase (triacylglycerol acylhydrolase) is the first enzyme involved in the degradation of triacylglycerols. The increase in FFA levels in palm oil in the fruit is attributed to the action of a lipase. However, evidence as to the nature of the lipase has been contradictory. Oo [43] and Tombs and Stubbs [44] reported the absence of endogenous lipase in oil palm mesocarp. The latter workers attributed FFA increase in oil palm

Table 4
Typical fatty acid composition (%) of Malaysian palm oil [51]

Fatty acid chain length	Mean	Range observed	Standard deviation
12:0	0.3	0–1	0.12
14:0	1.1	0.9–1.5	0.08
16:0	43.5	39.2–45.8	0.95
16:1	0.2	0–0.4	0.05
18:0	4.3	3.7–5.1	0.18
18:1	39.8	37.4–44.1	0.94
18:2	10.2	8.7–12.5	0.56
18:3	0.3	0–0.6	0.07
20:0	0.2	0–0.4	0.16

mesocarp to microbial activity. Hartley [45] however, suggested the presence of a very active endogenous lipase in the oil palm. Abigor et al. [46] described the partial characterisation of a lipase from the oil palm mesocarp, while Mohankumar et al. [47] reported the histochemical localisation of oil palm fruit lipase. The presence of an active endogenous lipase in the oil palm was firmly established by two reports [48,49]. Sambanthamurthi et al. [49] also demonstrated that the lipase is activated by cold treatment. This finding was in contradiction to Henderson and Osborne's [48] report that the lipase in the ripe oil palm fruit is sensitive to chilling inactivation at 8°C. However, it was later established by Sambanthamurthi et al. [50] that the inhibition in enzyme activity observed by Henderson and Osborne [48] was a consequence of the large increase in FFA at low temperature. Lipase activity in the oil palm mesocarp is synchronised with TAG biosynthesis with activity first being observed at 16 WAA and reaching a maximum at 21 WAA. The lipase is probably therefore an inducible and ripening associated gene. The endogenous lipase activity declines after 21 WAA. However, there continues to be a significant increase in FFA level in the overripe fruit (beyond 21 WAA) attributable to microbial infestation. The oil palm lipase is located in the oil body fraction and has optimal activity at pH 7.5. The enzyme is only stable in a hydrophobic environment and loses activity in the absence of a non-polar environment. The soluble fraction does not have detectable lipase activity. Maximum in vitro activity (using tri-[^{14}C]oleate as substrate) was observed at 18°C. However, with an in vitro assay (measurement of total FFA in the fruit), maximum activity was observed at 5°C, implying possible structural change at 5°C leading to enhanced interaction of the enzyme with the substrate. Low temperature activation varies with the genotype and is very much less pronounced in a water-saturated environment. Water stress may thus have an effect on the oil palm lipase. Since the oil palm is a tropical crop, the physiological significance of this chilling-induced enzyme is not clear, nor, for that matter, the physiological role of any lipase in the oil palm fruit. The mesocarp is already decomposed in the natural state before the seed germinates [43]. In the development of the fruit and its subsequent germination, there is no occasion when an endogenous lipolysis of the accumulated mesocarp oil takes place physiologically. When the fully ripe fruit drops from the bunch, the mesocarp is either eaten by small animals or rots away in the tropical undergrowth. Germination only takes place many months later, and depends on the oil stored in the seed kernel which remains intact during this period. It does not depend on the oil stored in the mesocarp. It may thus be important to bear in mind that lipases are powerful tools for catalysing not only hydrolysis but also esterification and transesterification reactions. The possible role of the mesocarp lipase in transesterification during lipid synthesis would be an interesting subject for further study. The synchronisation of lipase activity with TAG biosynthesis supports this postulation.

5. Chemistry of palm oil

Like all oils, TAGs are the major constituents of palm oil. Over 95% of palm oil consists of mixtures of TAGs, i.e. glycerol molecules, each esterified with three fatty acids. During oil extraction from the mesocarp, the hydrophobic TAGs attract other fat- or oil-soluble cellular components. These are the minor components of palm oil such as phosphatides, sterols, pigments, tocopherols and trace metals. Other components in palm oil are the metabolites from the biosynthesis of TAGs and products from lipolytic activity. These include the MAGs, DAGs and FFAs.

5.1. Fatty acids of palm oil

The fatty acids are any of a class of aliphatic acids, such as palmitic (16:0), stearic (18:0) and oleic (18:1) in animal and vegetable fats and oils. The major fatty acids in palm oil are myristic (14:0), palmitic, stearic, oleic and linoleic (18:2). The typical fatty acid composition of palm oil from Malaysia [51] is presented in Table 4. The ratio of palmitic/stearic acid in palm oil can vary according to a number of factors. Clegg [52] reported that the palmitic and stearic acid content in *E. guineensis* oil varied because of geographical influences (Table 5). Palm oil has saturated and unsaturated fatty acids in approximately equal amounts.

5.2. Triacylglycerols

Most of the fatty acids of palm oil are present as TAGs. The different placement of fatty acids and fatty acid types on the glycerol molecule produces a number of different TAGs. There are 7–10% of saturated TAGs, predominantly tripalmitin [53]. The fully unsaturated TGs constitute 6 to 12% [53,54]. As stated earlier, the sn-2 position has a specificity for unsaturated fatty acids . Therefore, more than 85% of the unsaturated fatty acids are located in the sn-2 position of the glycerol molecule. Table 6 shows the percentage distribution of individual TAGs of palm oil obtained by subjecting lipolysis data to computer assisted statistical analysis. Analyses of TAGs using capillary columns chemically bonded with methyl silicone phase [55] and reversed phase high-performance liquid chromatography [56] have also been used to resolve the different combinations of saturated and unsaturated triacylglycerols. The distribution of fatty acids in positions 1,2 and 3 of the glycerol molecule was shown in Table 3.

High temperature gas-liquid chromatography is used to differentiate triacylglycerols according to the total carbon number of their three fatty acid moieties. A typical Malaysian refined palm oil has a triacylglycerol carbon number profile as shown in Table 7 [57].

The triacylglycerols in palm oil partially define most of the physical characteristics of the palm oil such as melting point and crystallisation behaviour.

6. Minor constituents of palm oil

The minor constituents can be divided into two groups. The first group consists of fatty acid derivatives, such as partial glycerides (mono- and diacylglycerols), phosphatides, esters and sterols. The second group includes classes of compounds not related chemically to fatty acids.

Table 5
Variation in palmitic and stearic acid contents (%) of palm oil from different countries [52]

Acid	Zaire	Indonesia	Malaysia
Palmitic	41–43	46–50	46–51
Stearic	4.4–6.3	2.0–4.0	1.5–3.5

These are the hydrocarbons, aliphatic alcohols, free sterols, tocopherols, pigments and trace metals.

Most of the minor components found in the unsaponifiable fraction of palm oil are sterols, higher aliphatic alcohols, pigments and hydrocarbons. The other minor components, such as partial glycerides and phosphatides, are saponifiable by alkaline hydroxide.

6.1. Partial glycerides

The partial glycerides do not occur naturally in significant amounts except in palm oil from damaged fruits. Such oils would have undergone partial hydrolysis resulting in the production of free fatty acids, water and the partial glycerides.

Table 6
Triacylglycerol composition (%) of Malaysian *tenera* palm oil[a]

No double bond			One double bond			Two double bond			Three double bonds			Four double bonds		
	A	B		A	B		A	B		A	B		A	B
MPP	0.29	0.5	MOP	0.83	1.4	MLP	0.26	–	MLO	0.14	0.2	PLL	1.08	0.8
PMP	0.22	0.2	MPO	0.15	0.2	MOO	0.43	0.7	PLO	6.59	6.0	OLO	1.71	1.4
PPP	6.91	7.2	POP	20.02	23.7	PLP	6.36	6.3	POL	3.39	3.1	OOL	1.76	1.5
PPS	1.21	1.0	POS	3.50	3.1	PLS	1.11	0.8	SLO	0.60	0.4	OLL	0.56	–
PSS	0.12	0.1	PMO	0.22	–	PPL	1.17	1.0	SOL	0.30	0.2	LOL	0.14	0.1
PSP	–	0.7	PPO	7.16	6.9	OSL	0.11	–	OOO	5.38	5.1			
			PSO	0.68	0.6	SPL	0.10	0.1	OPL	0.61	0.5			
			SOS	0.15	–	POO	20.54	21.5	MOL	–	0.1			
			SPO	0.63	0.5	SOO	1.81	1.4						
						OPO	1.86	1.6						
						OSO	0.18	0.2						
						PSL	–	0.1						
Others	0.16			0.34	0.3		0.19	0.6		0.15	–		0.22	
Total	9.57	9.7		33.68	35.8		34.12	34.6		17.16	15.6		5.47	3.8

[a] A, Based on Kan-Ichi Hayakawa's computation [54]; B, based on Van der Waal's method [234]; H, myristate; P, palmitate; S, stearate; O, oleate; L, linoleate.

Table 7
Triacylglycerol composition (%) of Malaysian palm oil by carbon number [57]

Carbon No.	Mean	Range observed	Standard deviation	Coefficient of variation (%)
C44	0.07	0.00–0.2	0.06	84.8
C46	1.18	0.70–2.00	0.19	16.7
C48	8.08	4.70–9.7	0.72	8.9
C50	39.88	38.9–41.6	0.54	1.3
C52	38.77	33.10–41.0	0.62	1.6
C54	11.35	10.3–12.10	0.37	3.3
C56	0.59	0.50–0.80	0.10	17.1

Different isomers of MAGs and DAGs are found in palm oil. 1-MAGs are more stable than their β-isomers. As in most vegetable oils, the α,α'-DAGs (or 1,3 DAGs) are the predominant DAGs in palm oil. Several workers [58–60] have quantified the DG content of palm oil. Jacobsberg and Oh [59] found 5.6 and 7.6% DAGs in the oil of unbruised and bruised fruits, respectively. Bhakare and co-workers [61] analysed neutral lipids of palm oil from *dura* and *pisifera* palms grown in India. They reported 4.5 and 3.8% DAGs, and 1.2 and 1.0% MAGs in the *dura* and *pisifera* oils, respectively. Goh and Timms [58] showed that there was no correlation between the FFA and DAG contents and found the DAG content ranging from 5.5 to 7.1% with a mean of 6.5% in crude palm oil. The average levels of DAGs reported for refined palm oil, olein and stearin were 6, 6.1 and 4.4%, respectively [60].

Palm oil contains mainly three types of DAGs — C32 (dipalmitoylglycerol or PP), C34 (palmitoyloleoylglycerol or PO) and C36 (dioleoylglycerol or OO). The major DAG is C34 at 54.4%, followed by 33.0% of C36 and 12.6% C32 [60]. In fresh oils, PO varies from 1.3 to 3.3% depending on the ripeness of the fruits. In commercial oils, PO ranges from 3.0 to 3.3%. OO is at 0.5–0.8% in fresh oils and 2.1% in over-ripe fruits. In commercial oils, it ranges from 1.4 to 2.4%. PP is at 0.1–1.2% in commercial oils while in freshly extracted oil, the level can be $<0.2\%$.

Siew and Ng [62] reported that the composition of DAGs in palm oil was dependent on the degree of fruit ripeness and the extent of hydrolytic degradation. More DAGs were found in ripe and over-ripe fruits, and in fruits which were not processed immediately (Table 8). In oils from immature fruits, 17–18 WAA, the level of 1,3 DAGs was very low ($<0.5\%$). The higher level of 1,2 DAG was derived from the TAG biosynthesis pathway [63]. As the fruit matured, there was a gradual increase of 1,3 DAGs. Jacobsberg and Oh [59] noted that the ratio of the 1,3 to 1,2 isomer in unbruised fruits was low (0.38) compared to that found in bruised fruits (1.53) and commercial oils (1.47–1.63). As the ratio rose more than could be expected from the change in total TAG content, the authors concluded that there was isomerisation of 1,2 DAGS to 1,3 DAGs in bruised fruits and commercial oils. Siew [60] was in agreement with the results of Jacobsberg and Oh [59] when she reported commercial palm oil contained 5.8% DAGs with a high 1,3–1,2 ratio of 2.3. Siew and Ng [62] suggested that the ratio of 1,3–1,2-DAG was indicative of the storage conditions and quality of the oil. They recorded the lowest ratio (0.2) in oil from fresh fruits while the oil from fruits which were stored for 10 days had a higher ratio (1.0). Commercial oils showed values ranging from 1.8 to 3.3. This ratio and the DAG content could therefore be used as quality indices for the fractionation of palm oil as oil with a low ratio and total DAG content is easier to fractionate. A quantitative analysis of diacylglycerols in palm oil extracted from fruits at different stages of maturity is shown in Table 8.

Although the DAGs preferentially partition into the olein phase when palm oil is fractionated, there are still differences in the distribution of the component DAGs. The saturated PP or C32 concentrated in stearin and the unsaturated OO or C36 in the olein [62]. The slow crystallisation of palm oil during fractionation has long been a cause of much concern in the palm oil industry. DAGs are rather difficult to remove by refining because of their low volatility. The DAGs in palm oil affect its physical properties such as crystallisation. They interact with the TAGs to form eutectic mixtures, reducing the yield of high melting TAGs (the stearin fraction) in the fractionation process. DAGs also slow down transformation of the crystals from the α-form to the β'-form and, subsequently, to the β-form. This results in mixed and poor separation of the various fractions of palm oil [64]. Siew [60] reported the dual role of DAGs in palm oil. DAGs inhibit the

Table 8
Composition of isomeric diacylglycerols (Weight percentage in total oil) in crude palm oil from different aged-fruits, fruits[a] stored for 10 days and different sources [62]

Diacyl-glycerol	Under-ripe[b]		Ripe[c]		Over-ripe[d]		Fresh oil	Production[e] tank oil	Commercial oil[f]
	0 day	10 days	0 day	10 days	0 day	10 days			
1,2 isomer	2.8	4.3	2.9	3.4	4.2	3.1	2.2	1.7–2.4	1.1–2.2
1,3 isomer	0.6	1.5	0.6	3.5	1.6	3.1	0.4	2.0–3.6	2.9–5.0
Total diacylglycerol	3.4	5.8	3.5	6.9	5.8	6.2	2.6	4.4–5.6	4.0–7.5

[a] Fruits stored in the field.
[b] 19-week old fresh fruit bunches.
[c] 21-week old fresh fruit bunches.
[d] 23-week old fresh fruit bunches.
[e] Five samples from mills.
[f] 24 Samples from mills and refineries.

isothermal crystallisation of palm oil during fractionation. Conversely, DAGs can also enhance the crystallisation or clouding of palm olein when the oil is subjected to temperature fluctuations. In particular, the PP tends to crystallise out during temperature fluctuations thereby inducing subsequent crystallisation of the TAGs. Variation in the composition of DAGs has been implicated in the inconsistent properties of palm-based margarines and confectionery products made with palm oil.

The content of MAGs in palm oil is low, usually below 1%. The presence of MAGs, together with free fatty acids, was reported to promote water solubility in crude palm oil [64]. Ooi and Leong [65] identified the major fatty acids of MAGs in palm oil as palmitic and oleic acids.

6.2. Cyclic esters

Lactones are cyclic esters produced from unsaturated acids, hydroxy acids or other fatty acid derivatives. Although lactones are commonly associated with milk and milk products, they are also found in vegetable fats and oils. The characteristic and desirable flavour of butterfat is partly due to lactones such as δ-decalactone and δ-dodelactone. Lactones are the major cyclic esters in palm oil. A study by Van Der Ven and de Jong [66] on the isolation of optically active lactones from animal and vegetable fats showed the presence of δ-C_{11}, δ-C_{15}, γ-C_{10} and γ-C_{12} lactones in palm oil. The δ-lactones were the predominant components. They reported as much as 1 mg/kg lactones in palm oil but were not able to measure the optical rotation of the lactones.

7. Nonglyceride constituents

Several minor nonglyceride compounds are found in palm oil. Table 9 gives the levels of these minor components in the oil. The nonglyceride fraction of palm oil consists of sterols, triterpene alcohols, tocopherols, phospholipids, chlorophylls, carotenoids and volatile flavour components, such as aldehydes and ketones.

7.1. Triterpene alcohols

The unsaponifiable matter in palm oil contains a small proportion (about 0.02%) of triterpene alcohols [67]. These are a complex group of plant constituents which consist mainly of five condensed cyclohexane rings with 30 carbon atoms. They can be separated from the sterols by chromatography and the few identified in crude palm oil include cycloartenol, 24-methylenecycloartanol, cycloartanol and β-amyrin [68].

7.2. 4-Methyl sterols

Cycloartenol and 24-methylene cycloartanol in plants represent the biosynthetic origin of 4-methyl sterols. These sterols are present in small quantities in the triterpenic fractions of the oil. Itoh et al. [69] reported the presence of obtusifoliol, cycloeucalenol, gramisterol and citostadienol in palm oil. These sterols do not appear to play any specific biological role and are probably biosynthetic intermediates between evolvable triterpenic alcohols and sterols.

7.3. Sterols

Sterols are tetracyclic compounds with generally 27, 28 or 29 carbon atoms. They make up a sizeable portion of the unsaponifiable matter in oil. The total content of sterols in palm oil is

Table 9
Ranges in content for various components in the unsaponifiable fraction from a Zaire plantation palm oil [236]

Component	%	mg/kg (in palm oil)
Carotenoids		
α-carotene	36.2	
β- carotene	54.4	
γ-carotene	3.3	500–700
Lycopene	3.8	
Xanthophylls	2.2	
Tocopherols		
α-tocopherols	35	
γ-tocopherols	35	
δ- tocopherols	10	500–800
ε + η-tocopherols	10	
Sterols		
Cholesterol	4	
Campesterol	21	~300
Stigmasterols	21	
β-sitosterol	63	
Phosphatides		500–1000
Total alcohols		
Triterpenic alcohol	80	~800
Aliphatic alcohol	20	

about 0.03%. Bhakare et al. [61] reported that Indian palm oil from *dura* and *pisifera* palms contains the levels of β-sitosterol, brassicasterol, campesterol and stigmasterol shown in Table 10. They could not detect any cholesterol in the samples although very small amounts have been reported [68,70] in palm oil. Cholesterol (2.2–6.7%), Δ5-avenasterol (0–2.8%) Δ7–stigmasterol (0–2.8%) and Δ7-avenasterol (0–4%) were also found in the sterol fraction (326–627 mg/kg) of palm oil [70].

Most of the sterols are relatively inert and do not appear to contribute to any important property and behaviour of palm oil. However, Δ5-avenasterol has been reported to show antioxidant activity in edible oils [71].

7.4. Vitamin E

Vitamin E is a fat-soluble vitamin, which comprises two major homologous series of compounds (tocochromanols), known as tocopherols and tocotrienols. The tocopherols are structurally characterised by a saturated side chain in the chroman ring, whereas the tocotrienols possess an unsaturated phytyl side chain. Four homologs of each type are known to exist in nature and they have different degrees of antioxidant and vitamin E activities.

Vegetable oils, especially the seed oils, are rich sources of tocopherols. Vitamin E has traditionally been extracted from the residues of the soybean refining industry. Tocotrienols, on the other hand, are predominantly found in palm oil and in cereal oils such as barley and rice bran oils. With the emergence of palm oil as the second largest edible oil in the world markets, technological advances have been made enabling the extraction of tocotrienols from palm oil which is currently available commercially.

The vitamin E content in crude palm oil ranges between 600–1000 parts per million (ppm) [20] and is a mixture of tocopherols (18–22%) and tocotrienols (78–82%). The major tocotrienols occurring in palm oil are alpha-tocotrienol (22%), gamma-tocotrienol (46%) and delta-tocotrienol (12%) [72].

The vitamin E content of palm oil is partially lost as a result of processing. For example, it has been reported that refined bleached deodorized (RBD) palm oil, palm olein and palm stearin retain approximately 69, 72 and 76% of the original level of vitamin E in the crude oils, respectively. However, there is a large variation in these estimates within the refining industry because differences in the plant conditions as well as the plant design influence the amount of vitamin E lost during refining. It has been observed that vitamin E tends to partition preferentially into the olein fraction during fractionation of palm oil. For example, the concentration of vitamin E in RBD palm olein and RBD palm stearin were 104–135% and 57–75%, respectively of the original level of vitamin E in the source RBD palm oil.

Table 10
Composition (weight%) of the sterol fraction from *dura* and *pisifera* palms [61]

Variety	Brassicasterol	Campesterol	Stigmasterol	β-Sitosterol
Dura	3.2	18.4	8.3	70.1
Pisifera	4.3	19.8	6.4	69.5

Losses mainly occur in the deacidification stage of palm oil refining and may be minimised by the incorporation of a sieve tray or a packed column pre-stripper into the conventional refining process. The vitamin E thus lost during processing is concentrated in the palm fatty acid distillate (PFAD), a by-product of the physical refining of palm oil. PFAD has been identified as a good source of raw material for the recovery of palm vitamin E. In addition, PFAD is relatively cheap and readily available throughout the refining industry. Laboratory analysis of PFAD from crude palm oil, palm olein and palm stearin refining has shown that on average 5252, 6895 and 4235 ppm of vitamin E, respectively were present.

7.5. Pigments

The pigmentation of palm fruits is related to their stage of maturity. Two classes of natural pigments occur in crude palm oil — carotenoids and chlorophylls.

Palm oil from young fruits contains more chlorophyll and less carotenoids than oil from mature or ripe fruits [73]. The pigments in palm oil are involved in the mechanisms of autoxidation, photooxidation and antioxidation.

7.6. Carotenoids

Carotenoids are highly unsaturated tetraterpenes biosynthesised from eight isoprene units. Their more favoured state is the all-*trans*. Carotenoids are divided into two main classes: carotenes which are strictly polyene hydrocarbons, and xanthophylls, which contain oxygen. The oxygen in xanthophylls may be in the form of hydroxy (e.g. zeaxanthin and lutein), keto, epoxy or carboxyl groups. The simplest carotene is lycopene.

Crude palm oil has a rich orange-red colour due to its high content of carotene (700–800 ppm). The major carotenoids in palm oil are β- and α-carotene which account for 90% of the total carotenoids [74]. There are about 11 hydrocarbon carotenoids in processed palm oil fractions [75]. The various types and composition of carotenoids (Table 11) extracted from oils of different palm species were studied by Yap et al. [76]. They found 13 types of carotenoids with the major ones, α-carotene and β-carotene, accounting for 54–60% and 24–60% of the total carotenoids, respectively. No significant difference in the types of carotenoids was found in the oils of *E. oleifera* and *E. guineensis*, and their hybrids and backcrosses to *E. guineensis*. The study also showed that *E. guineensis* contained a higher level of lycopene compared to *E. oleifera* and its hybrids with *E. guineensis*.

Carotenoids are the precursors of vitamin A, with β-carotene having the highest provitamin A activity. Palm oil has 15 times more retinol equivalent than carrot and 300 times more than tomato [77]. Carotenes are sensitive to oxygen and light. The oxidation of carotenes is accelerated by hydroperoxides generated from lipid oxidation, leading to discoloration and bleaching. Among the products formed from the oxidative deterioration of carotenoids are α- and β-ionones, β-13 and β-14-apocarotenals and β-13-apocrotenone.

In refining crude palm oil, the carotenoids are first partially removed by adsorption on an activated earth, following which high temperature steam deodorisation destroys the chromogenic properties of the remaining carotenoids to produce a light yellow refined palm oil. With carotene as a rich source of Vitamin A, a process was developed [78] to produce a deacidified and deo-

Table 11
Carotene profiles of palm oil extracted from *Elaeis guineensis*, *Elaeis oleifera* and their hybrids [76][a]

	Carotene composition (%)						
	M	P	D	MP	MD	MD×P	T
Phytoene	1.12	1.68	2.49	1.83	2.45	1.30	1.27
Cis-β-carotene	0.48	0.10	0.15	0.38	0.55	0.42	0.68
Phytoene	Trace	0.90	1.24	Trace	0.15	Trace	0.06
β-carotene	54.08	54.39	56.02	60.53	56.42	51.64	56.02
α-carotene	40.38	33.11	54.35	32.70	36.40	36.50	35.16
Cis-α-carotene	2.30	1.64	0.86	1.37	1.38	2.29	2.49
ζ-carotene	0.36	1.12	2.31	1.13	0.70	0.36	0.69
γ-carotene	0.09	0.48	1.10	0.23	0.26	0.19	0.33
δ-carotene	0.09	0.27	2.00	0.24	0.22	0.14	0.83
Neurosporene	0.04	0.63	0.77	0.23	0.08	0.08	0.29
β-zeacarotene	0.57	0.97	0.56	1.03	0.96	1.53	0.74
α-zeacarotene	0.43	0.21	0.30	0.35	0.40	0.52	0.23
Lycopene	0.07	4.50	7.81	0.05	0.04	0.02	1.30
Total carotene (parts per million)	4592	428	997	1430	2324	896	673

[a] M, *E. oleifera* (Melanococca); P, *E. guineensis (pisifera)*; D, *E. guineensis (dura)*; T, *E. guineensis (tenera)*, D×P.

dorised red palm oil which retains as much as 80% of the original carotenoids. A red palm oil produced from this process, bearing the trade name 'CAROTINO', is available in the market.

7.7. Chlorophylls

Besides the carotenoids, the other important groups of pigments in palm oil are the chlorophylls. These are the green chlorophyll *a* and chlorophyll *b* and the brown pheophytin *a* and pheophytin*b*. Structurally, the chlorophyll molecule contains a porphyrin (tetrapyrrole) nucleus with a chelated magnesium atom in the centre. Chlorophylls are fat-soluble as a result of a phytol chain attached to one of the porphyrin rings.

In crude palm oil, the higher level of carotenoids visually masks the presence of chlorophylls.

An investigation by Ikemefuna and Adamson [14] on chlorophyll and carotenoid changes in the fruit of *E. guineensis* showed that chlorophyll did not disappear completely in ripe fruits. The green and ripe fruits of *tenera* and *dura* palms all contained chlorophyll *a* and chlorophyll *b* in varying amounts (Table 12) but chlorophyll *a* in the ripe fruit was reduced by 80–90% from the green fruit. The loss of chlorophyll *b* was less — only 50–75%. Strecker et al. [79], in their paper on physically refined oils, reported a value of about 800 μg/kg chlorophyll for crude palm oil but found no residual chlorophyll in refined palm products. A subsequent study by Usuki et al. [80] showed that refined palm olein contained as much as 583 μg/kg total chlorophyll consisting of chlorophyll *a* (30 μg/kg), chlorophyll *b* (114 μg/kg), pheophytin *a* (341 μg/kg) and pheophytin *b* (98 μg/kg). Tan et al. [73] found that the chlorophyll content in crude palm oil, expressed as pheophtyin *a* [81], ranged from 250 to 1800 μg/kg. The liquid (olein) fraction of crude palm oil contained more chlorophyll because of the preferential partitioning of chlorophyll into it [82]. In a 1-year survey on crude palm oil collected from mills and refineries in Malaysia, Tan et al. [51] analysed

Table 12
Chlorophyll content (mg/kg) of two fruit forms of *E. guineensis* at three different stages of development [14]

Pigment		*Tenera*			*Dura*		
		Green 1–2 months	Mature 3–4 months	Ripe 5–6 months	Green 1–2 months	Mature 3–4 months	Ripe 5–6 months
Chlorophyll *a*	Mean	28.9	20.7	4.3	26.5	22.7	2.4
	Range	12–48	3–34	0.3–7.3	13–54	16–34	0.7–3.7
Chlorophyll *b*	Mean	18.6	15.3	7.3	19	11.8	4.6
	Range	8–33	3–20	0.3–13	14–35	5–17	1.3–7.2

the total chlorophyll content in 1300 samples using a laser-induced technique. They observed a range of 897–4000 μg/kg. A high chlorophyll content would indicate oil from unripe fruits.

Chlorophylls and their derivatives are photosensitisers. These chemical species absorb light and activate, or ''sensitise'', either the unsaturated oils or molecular oxygen to induce photosensitised oxidation. The term ''photosensitised oxidation'' is synonymous with oxidation with singlet oxygen which initiates oxidation by the production of free radicals. Chlorophylls in oils are undesirable because of their adverse effects on oxidative deterioration, hydrogenation and bleachability. As far back as 1938, Coe [83] suggested that rancidity in vegetable oils might be correlated with chlorophylls which act as photosensitisers to liberate free hydrogen in a photochemical reaction. Abraham and Deman [84] reported that chlorophyll slowed down the hydrogenation rate while Koritala [85] found that oils containing pheophytin turned green after hydrogenation. Once chlorophylls from oil-bearing seeds or fruits are co-extracted into the oil, they are difficult to remove by conventional alkali treatment and bleaching processes [86].

Like the carotenoids, chlorophyll pigments are partially removed by bleaching earths. The efficiency of bleaching earths for the refining of crude palm oil can be better measured by the adsorption of chlorophyll from the oil. A significant negative correlation was found between chlorophyll adsorption and the colour of the refined oil [87]. All these reports implied that a more involved and expensive process would have to be used to bleach, heat treat or hydrogenate vegetable oils containing high levels of chlorophyll before acceptable colours are attained.

7.8. Polar lipids

Besides TAGs (neutral lipids), palm oil also contains polar lipids such as glycolipids and phospholipids. Glycolipids are the major polar lipids (1000–3000 ppm) [88]. This class of lipids consist of various types of long chain sugar derivatives which may contain a DAG, a ceramide backbone or a phosphorylated polysaccharide-lipid complex. Glycolipids can therefore be classified as those based on glycerol, ceramides or lipopolysaccharides. The major glycolipid (26.8% of total glycolipids) is the glycerol-based MGDG which has one sugar linked glycosidically to DAG. The major fatty acid in MGDG is linolenic acid. DGDG is the second major glycolipid in palm oil (23.1%). Besides MGDG and DGDG, steryl glycoside and acylated steryl glycoside were also detected in Indian palm oil [89].

Phospholipids are present in relatively small quantities (5–130 ppm) in palm oil as compared with other vegetable oils. According to Kulkarni et al. [90], the main phospholipids of Indian palm oil are phosphatidylcholine (34%–35%), phosphatidylethanolamine (22–26%), phosphatidylinositol (21–25%), cardiolipin (7–8%) and phosphatidylglycerol (5–7%). They reported that the predominant fatty acids in all these phosphatides were palmitic, stearic, oleic and linoleic acids. In addition to the phospholipids found by Kulkarni et al. [89], Goh and coworkers [90] found phosphatidylglycerol in the mesocarp oil of *E. gunineensis*. They reported that solvent extracted mesocarp oil contained 1000–2000 ppm phospholipids. However, phospholipids were only present at levels of 20–80 ppm in commercial crude palm oil. Goh et al. [90] also found other minor phospholipid components such as phosphatidic acid, diphosphatidylglycerol, lysophosphatidylethanolamine and traces of lysophosphatidylcholine and phosphatidylserine.

A detailed study on the phosphorus compounds in palm oil by Siew [91] showed that phospholipids form a relatively minor proportion of the total phosphorus content in crude palm oil. According to Siew [91], most of the phosphorus appears as inorganic orthophosphates and that there is a strong correlation between the phosphorus and iron contents in the crude oil. She reported that the major phosphorus compounds in refined palm oil are the phosphoric acids, phosphorylated DAGs such as phosphatidic acids, and possibly polyphosphates formed from the heating of residual phosphoric acid. Organic phosphates are formed as artifacts from the residual phosphoric acid and mixtures of diacylglycerols during deodorisation. The organic phosphates formed are mainly of two types: phosphatidic acids and polyphosphatidic acids. She concluded that although the former may be residual phosphatidic acids in crude oils, they were more likely formed from the phosphorylation of DAGs. The latter might be formed from phosphorylation of MAGs at the 1,2- or 1,3- position of MAGs.

The phosphorus from inorganic phosphates and that from phospholipids appear to play different roles in palm oil. Phospholipids have been reported to show antioxidant effects [92–94]. Their antioxidant-synergistic effects [95] can be attributed to the sequestering of soluble prooxidant metal ions to form inactive species [96]. Hudson and Mahgoub [95] also showed a synergism between phospholipids and naturally occurring antioxidants such as α-tocopherol and quercetin. Hydratable insoluble metal ions could also be dispersed by phospholipids through miscellar action. Since phospholipids and glycolipids cause reverse micelle, vesicle or emulsion droplet formation, phospholipids can remove prooxidant metal ions and their hydrophilic salts from the lipid phase to reduce oxidation.

Inorganic phosphates, e.g. phosphoric acid, increase the rate of hydrolysis of refined palm oil. Siew [91] observed a gradual increase in inorganic orthophosphates on storage of palm oil at elevated temperatures, and hypothesised that the increase was from the release of phosphoric acid from hydrolysis of polyphosphoric acids or organic phosphates. Hydrolysis of the oil is then catalysed by the released phosphoric acid.

Although phospholipids have been implicated in oxidative instability [95], refining problems and losses [97] and colour problems [97,98], these deleterious effects of phospholipids were not observed in palm oil. Gee [88] showed that bleachability of crude palm oil is largely unrelated to the phospholipid content or the total phosphorus content. This observation was later corroborated by Siew [91].

The role and effects of phospholipids are conflicting, acting as an antioxidant synergist by chelating traces of prooxidant metal, and as a prooxidant by dispersing metal impurities respon-

sible for reduced oil stability. In general, the phosphorus content of crude and refined palm oil is considered an essential quality specification because of the indirect effect on oil quality through the association of phosphorus with metals and free fatty acids.

7.9. Volatile components from palm oil oxidation

Kuntom [99] carried out a detailed study of the volatiles produced from palm oil oxidation. She used the headspace technique of adsorption on Tenax to monitor the development of volatile compounds in palm oil samples. The volatiles were subsequently separated by capillary gas chromatography and identified by mass spectrometry. She found that the most significant compounds in the volatiles of oxidised palm oil were the alkanals $C_{4\text{-}9}$, *trans*-2-alkenals $C_{5\text{-}8}$, 2-alkyls furans $C_{1,2,4,5}$, and also aliphatic and aromatic hydrocarbons. The dominant aldehyde was n-hexanal which was proposed as a good parameter for monitoring palm oil oxidation.

7.10. Flavour compounds

Fresh crude palm oil has a typical nutty odour. Kuntom [99] characterised the natural flavour and rancid flavour by extracting the flavour compounds using steam distillation followed by gas chromatographic analyses. She found small amounts of aldehydes, with hydrocarbon terpenes and monoxygenated terpenes predominating in the natural (or fresh) palm oil flavour compounds. The terpenes included linalol, trans-allo-ocimene and β-cyclocitral. Besides terpenes, naphthalene and its derivatives, 1,2,3,4-tetrahydronaphthalene were also detected.

The flavour compounds of rancid palm oil were found to be mainly aldehydes and ketones. These included aliphatic aldehydes, C_4–C_{10}, and the unsaturated *trans*-2-alkenals of C_6–C_9. Other aldehyes detected were the *trans*-2-nonenal, *trans,cis*-2-4-decadienals, *trans,trans*-2,4-hexadienal and *trans,cis*-2,4- hexadienal. Among the ketones present were 2-alkanone of $C_{5,9,10}$, 2,2,6-trimethylcyclohexanone, oct-3-en-2-one and γ-heptalactone.

7.11. Hydrocarbons

Squalene is a hexaunsaturated hydrocarbon with 30 carbon atoms and is found mostly in vegetable and animal fats. It is a precursor in sterol biosynthesis and exhibits an antioxidant activity [100] Crude palm oil contains about 200–350 ppm squalene. Other hydrocarbons present in small quantities are sesquiterpene and diterpene hydrocarbons. These hydrocarbons, together with the volatiles, are removed during refining of the crude oil.

7.12. Metals

Trace metals may be present as complexes surrounded by proteins, phospholipids and lipids or non-lipid carriers. In crude palm oil, trace metals can originate from contamination by soil and fertilisers. Trace metals can also be picked up from the palm oil mill, storage tanks, road tankers, pipelines and ships' tanks [101]. The use of stainless steel for certain mill machineries which are subjected to constant wear and tear should help to reduce metal contamination.

Trace metals can also be present as suspended solid impurities in the oil [102]. Iron can be present in colloidal admixtures with protein and cellulosic matter, or with other micro-particulate materials such as those containing compounds of calcium, magnesium or phosphate.

Iron and copper are pro-oxidants and their high levels in palm oil should be avoided. These metals catalyse the decomposition of hydroperoxides to free radicals. Of the two, copper is the more potent being ten times more active than iron. Copper accelerates the hydroperoxide destruction rate thereby increasing the production of secondary oxidation products, while iron increases the rate of peroxide formation. The average level of iron reported in crude palm oil was 4.4 ppm while that for copper was 0.06 ppm [103]. These levels are reduced by refining — to 0.5–1.0 ppm and <0.1 ppm, respectively [104].

Other metals reported in palm oil are manganese, cadmium and lead [105]. The pro-oxidant activity of manganese lies between that of copper and iron and it is present at about 1 ppm in crude palm oil [106]. Cadmium and lead are found in very low concentrations [104] and their effects on oxidation appeared to be negligible.

8. Chemical reactions of palm oil

8.1. Hydrolysis

Triacylglycerols constitute 95% of palm oil. Therefore, the chemistry of palm oil is dominated by the reactions of the ester group. The ester linkages in triacylglycerols can be hydrolysed to yield partial glycerides, free fatty acids and glycerol depending on how far the reaction is allowed to proceed. Since the crude palm oil trade is based on specifications which include the FFA content, factors which affect the rate of hydrolysis of the oil in the palm fruit and the extracted oil are of great concern to the palm oil industry.

Hydrolysis can be from microbial lipolysis, autocatalysis, or enzymatic lipolysis. Microbial lipolysis is caused by the microorganisms which enter the fruit and liberate the enzyme lipase. Improper storage of fruits and delayed processing favour the multiplication of the microorganisms and hydrolysis of the oil.

Water must be present for autocatalytic hydrolysis to occur. The rate of the hydrolytic reaction depends on the temperature, moisture content and initial free fatty acid concentration. Enzymatic hydrolysis is caused by endogenous lipase in the fruits. Bruised fruits display more lipolytic activity than undamaged fruits [107]. Over-ripe fruits, processing delay and rough handling of palm fruit bunches all contribute to oil acidification. Thus, good harvesting and handling must always be practiced to minimise hydrolysis. A number of publications on the subject of FFA content and hydrolysis in palm oil products are available [108–110].

8.2. Saponification

Alkaline hydrolysis of an organic ester, or saponification produces an alkaline salt and alcohol. When a triacylglycerol or fatty acid is treated with alkali, it yields the salt of the alkali metal (soap) and glycerol. This is the basic reaction in the making of soap and glycerine from palm oil. Saponification is also the basis for two important analytical determinations. It is used to deter-

mine the acidity and saponification number of fats and oils. The saponification number indicates the average molecular weight or equivalent weight of fatty materials in the oil. It is, therefore, an identity characteristic of an oil or fat and palm oil has a saponification number, or value, between 192 and 205 [51].

8.3. Interesterification

The group of reactions in which an ester of a fatty acid is made to react with fatty acids, alcohols or other fatty acid esters to produce esters of differing composition from the original ester, is broadly termed "interesterification". This reversible reaction allows for modification of the physical properties of fats, such as the melting behaviour, crystalline characteristics, solid fat content and plasticity, while retaining the chemical and nutritional properties. Interesterification is the route for production of speciality fats such as shortenings and confectionery fats. The following sections outline some of the interesterification reactions commonly carried out on palm oil.

8.4. Alcoholysis

This is a reaction in which another group replaces the alkoxyl group of the glyceride molecule. Alcoholysis proceeds in steps: first using DAGs, followed by MAGs as intermediates. TAG alcoholysis by methanol in the presence of sodium produces methyl esters and glycerol. Methanolysis is of major importance in the transformation of fats to methyl esters as the latter are increasingly replacing fatty acids in industrial oleochemical reactions. Alcoholysis is the basic reaction used in the preparation of methyl esters for determining the fatty acid composition of palm oil. The reaction is also one of the two steps of interesterification used for commercial production of methyl esters from crude palm oil. The esters have very similar fuel properties to diesel fuel [111] and have been extensively evaluated in diesel engines.

Esterification can also be catalysed by enzymes. Using *Candida cylindracea,* methanolysis was successfully carried out on palm oil and its products to produce alkyl esters for the oleochemical industry [112].

8.5. Transesterification

Palm oil can be transesterified with another oil to give a blend with very different properties from the original oil. The process essentially redistributes the triacylglycerol fatty acids. The use of chemical and enzymic transesterification with alkyl esters to increase the unsaturation in palm oil has been explored by a number of workers [113,114]. The production of methyl esters from crude palm oil by Choo and co-workers [115] is a two step reaction involving firstly, esterification of the free fatty acids present in the oil into methyl esters (alcoholysis) followed by transesterification of the neutral glyceride mixture directly into methyl esters.

8.6. Oxidation

The action of oxygen on unsaturated fatty acids causes their deterioration to allylic derivatives. This chemical reaction, which usually occurs at ambient temperature, is referred to as autoxida-

tion. The autoxidation of oils is autocatalytic and takes place largely via a self-propagating free radical mechanism. Production of the first few radicals to start the propagation reaction can be by the decomposition of preformed hydroperoxides from metal catalysis, heat or light exposure or mechanisms which involve singlet oxygen.

The role of singlet oxygen at the initiation step of oxidation is through its direct reaction with the double bonds of the fatty acids. This photo-oxidation reaction is influenced by the light source and the characteristic transmission, absorption and reflectance of light absorbing components in the oil. In vegetable oils, photosensitisers generate singlet oxygen from triplet oxygen in the presence of light. Chlorophylls in vegetable oils are known to be efficient photosensitisers. Unlike in autoxidation, the hydroperoxides formed during photo-oxidation or singlet oxygen oxidation may be unconjugated.

Antioxidants function by rendering ineffective the factors which promote oxidation or by interfering with one or more steps involved in oxidation. Thus, the actions of tocopherols, tocotrienols and carotenoids are through their free radical scavenging as well as their singlet oxygen quenching activities. Carotenoids quench singlet oxygen through energy transfer from the latter to the former while the quenching mechanism of tocopherols is by a charge transfer [116].

The mechanisms, reaction products and conditions of oxidation during palm oil processing are outlined by Kuntom [117]. Unlike polyunsaturated oils, palm oil is more resistant to oxidation because of its higher level of saturated fatty acids. It is the unsaturated fatty acids in the oil which are susceptible to oxidation. In addition, it contains natural antioxidants such as tocopherols and carotenoids.

The ease of bleaching an oil is also dependent upon the degree and type of oxidation which has occurred.

8.7. Halogenation

The addition of iodine (a halogen) to double bonds of unsaturated fatty acids is the basis of the determination IV as a measure of unsaturation. The IV, however, only gives a true representation of the unsaturation when the double bonds are unconjugated and the molecular structure is not severely hindered for halogenation to take place.

8.8. Hydrogenation

Hydrogenation of oils, sometimes known as ''hardening'', is an addition reaction involving the ethylene bonds of the TAG molecule. In simple terms, it can be described as the saturation of double bonds in the unsaturated fatty acids with hydrogen. Hydrogenation of double bonds can be total or partial and the use of a catalyst is necessary to speed up the reaction. The specific surface area of a catalyst determines the speed of reaction, while the surface structure defines the selectivity. Full or partial hydrogenation can be carried out depending upon the solid fat content (SFC) required of the hydrogenated product. The SFC is the amount of solid fat present in the oil at any given temperature.

Total or full hydrogenation results in saturation of the double bonds in the oil. Partial hydrogenation can cause a change from the '*cis*' form to the '*trans*' form of unsaturated bonds (geometric isomerisation), a change in the position of the unsaturated bonds (positional isomerisation) and formation of conjugated systems of unsaturated bonds in polyunsaturated fatty

acid chains (conjugation). Formation of *trans* double bonds depends on the catalytic metal used and the hydrogenation conditions (temperature, pressure, stirring rate). Nickel-sulphur catalysts lead to the formation of more *trans* isomers than conventional nickel catalysts [118], while low temperature hydrogenation with more active precious metals can limit the formation of *trans* isomers.

The fatty acid composition of palm oil (≈1:1 saturated to unsaturated fatty acids) is such that the oil is semi-solid at normal room temperature. This property and the oil melting range permit its use as a major component in margarine and shortening without hydrogenation. Thus, for most practical purposes, palm oil does not need hydrogenation. Nonetheless, the use of this process has been explored to maximise the utilisation of palm oil and its fractions in edible food products. Palm oil can be hydrogenated with used nickel catalyst or a sulphur-poisoned catalyst to increase its SFC at 10°C without a significant increase in its melting point. The SFC of the hydrogenated product at 10°C and above is significantly increased with an IV drop of only 2–3 units [118]. Palm stearin is an excellent and economic starting material for certain food and non-food applications where fully hydrogenated fats are required. Cake shortenings made from palm oil products such as hydrogenated or interesterified palm oil, in combination with butterfat, produced cakes with better baking properties than cakes made with 100% butterfat [119]. While the butterfat gave the cakes the desired buttery flavour, the palm products enhanced the baking performance. Some hydrogenated products of palm oil and its products are also suitable for application in a number of high premium speciality products, such as toffee and confectionery fats.

9. Nutritional properties of palm oil and its components

Almost 90% of the world palm oil production are is as food. This has made demands that the nutritional properties of palm oil and its fractions be adequately demonstrated. The fatty acid composition of palm oil has thus been the focus of attention in determining its nutritional adequacy in relation to coronary heart disease (CHD) risk factors. As mentioned earlier, palmitic acid (44%) is the major saturated fatty acid in palm oil and this is balanced by almost 39% monounsaturated oleic acid and 11% polyunsaturated linoleic acid. The remainder is largely stearic (5%) and myristic (1%) acids. This composition is significantly different from palm kernel oil (obtained as a co-product during the processing of oil palm fruits) which is almost 85% saturated. The results of a large number of nutrition trials in animals and humans have been published. These studies have yielded results that not only demonstrate the nutritional adequacy of palm oil and its products but have also caused transitions in the understanding of the nutritional and physiological effects of palm oil, its fatty acids and minor components.

Dietary fats (and fatty acids) are known to modulate plasma lipids and lipoproteins. This concept has been extensively researched upon since the early 1950s and evidence has steadily accumulated hypothesising a positive correlation between saturated fat intake and increased levels of plasma total cholesterol (TC) in humans. The classical Keys et al. [120,121] and Hegsted et al. [122] equations indicated that the three saturated fatty acids — lauric, myristic and palmitic were equally cholesterol raising. Hegsted [123] originally showed that myristic acid was more cholesterolaemic than palmitic acid in humans. Nevertheless, this conclusion was subsequently revised after a series of experiments with modified triglycerides. The regression equations that

predicted plasma cholesterol response on the basis of energy contributed by the sum of saturated and polyunsaturated fatty acids in one's diet assumed that the monounsaturates were neutral but that dietary cholesterol affected plasma cholesterol besides the fatty acids. (For equations see Table 13.)

As a result of these and other findings, there has been a drive to educate consumers to choose fats containing fatty acids that can help maintain normal blood cholesterol levels. Such recommendations are embodied in almost every major national health report focused at reducing the incidence and mortality from CHD. Consumer awareness of these dietary recommendations has been demonstrated in the marketplace by a switch from animal saturated fats to polyunsaturated and monounsaturated oils. Such changes are however, often determined and conditioned by the end uses and functionality of the oils and fats concerned. The replacement of butter with margarine and the trend towards increased consumption of polyunsaturated margarine and other low saturates containing fat-rich products was seen as a positive stride in reducing CHD incidence. Newer data has now shown that hydrogenation of liquid polyunsaturated and monounsaturated oils used in such product formulations results in the formation of *trans* fatty acids that increase the lipid associated risk factors for CHD. Since palm oil contains 44% of its composition as saturated palmitic acid, it is generally assumed that TC elevation following its long-term consumption would be imminent. Indeed, early human studies [124–128] have reported that palmitic-acid enriched diets, derived mostly from palm oil, resulted in higher TC and low density lipoprotein cholesterol (LDL-C) than did diets enriched either in monounsaturated oleic or polyunsaturated linoleic acids. However, on closer examination it was also apparent that the TC and LDL-C values of these volunteers after the palm oil diets were actually lower than their baseline starting values. This suggests that the palm oil enriched diets were able to beneficially modulate the plasma lipoprotein profiles in comparison to the basal diets of the volunteers. Newer studies (in animal and human models) to be examined below have since produced results that are in variance to the above. At least one epidemiological study has reported that normal TC values are possible in a dietary environment in which palm oil was the predominant fat source [129]. The issue is further confounded by several studies that reported effects of triglyceride species [130] and minor components (Qureshi et al. [131]) in palm oil on cholesterol modulation.

The minor components of interest in palm oil are the vitamin E and carotenoids. The technology to isolate and concentrate these components has led to their use in several studies aimed at

Table 13
Regression equations predicting the effect of fatty acid classes on serum cholesterol concentrations in man (adapted from [173])

Equation 1	$\Delta SC = 2.74\Delta S - 1.31\Delta$	[120]
	$(\Delta SC = 2.40S\Delta S^{\dagger} - 1.20\Delta P + 1.5\Delta C^{1/2})$	[121]
Equation 2	$\Delta SC = 2.16S\Delta S - 1.65\Delta P + 0.065\Delta C$	[122]
	$(\Delta SC = 2.74S\Delta S^{\dagger} - 1.83\Delta P + 0.071\Delta C)$[a]	

[a] ΔSC, the change in serum cholesterol in mg/dl, ΔS, changes in % energy for all the SFA, $\Delta S^{\dagger}$, changes in % for saturated fatty acids with 12, 14 and 16 carbons only, ΔP, the change in % for polyunsaturated fatty acid (principally linoleic acid) and ΔC denotes the change in dietary cholesterol in mg/1000 kcal.

understanding their physiological effects. Accordingly, the emphasis has been on the cholesterol lowering effects of palm oil tocotrienols, the pro-vitamin A activity of red palm oil and palm carotene concentrates and the antioxidant and anti-cancer properties of both the palm vitamin E and carotenes. These findings are currently supported by a large volume of scientific publications, which are discussed below.

10. Effects of palm oil and its fractions on blood lipids and lipoproteins

10.1. Animal studies

10.1.1. Rat studies

The effects of palm oil on blood lipids and lipoproteins have been examined by comparison with the more unsaturated oils. Kris-Etherton et al. [132] compared the cholesterolaemic effects of diets containing 10% w/w palm, olive, safflower and corn oils. Rats fed olive oil had significantly higher levels of plasma TC than rats fed the corn oil diet, whereas there were no significant differences in the TC levels of rats fed corn, safflower and palm oils. In the presence of dietary cholesterol however, Sugano and Immaizumi [133] demonstrated a significant increase in TC in rats fed a palm oil or palm olein diet compared to a safflower oil diet. Ikeda et al. [134] examined the effect of palm and safflower oils in association with different types of dietary fibre on cholesterol absorption in rats. Cholesterol absorption was elevated by palm oil in association with insoluble fibre but this effect was not evident when the insoluble fibre was substituted with a soluble fibre.

The effect of palm oil on pre- and post-prandial lipid levels have also been examined by Groot et al. [135] who observed higher pre- and post-prandial plasma triglyceride levels in rats fed a palm oil diet compared with a sunflower oil diet. It was suggested that this effect might be related to the more saturated triglyceride species present in palm oil. Haave et al. [136] examined the effect of dietary fat contributed by palm oil, olive oil or safflower oil in pregnant rats. The foetal free plasma cholesterol levels were not significantly different between the diets; however, the foetal liver 3-hydroxy-3-methylglutaryl-Co-enzyme A (HMG-CoA) reductase activity of dams fed safflower oil and olive oil were higher than that of the palm oil fed rats.

Sundram et al. [137] examined palm oil, palm olein, palm stearin, corn oil and soyabean oil for their effects on lipoprotein cholesterol distribution in rats fed diets containing 40% energy as fat. Plasma cholesterol levels of rats fed soyabean oil were significantly lower than those of rats fed corn oil, palm oil, palm olein and palm stearin. Significant differences between the plasma cholesterol content of the rats fed corn oil and rats fed the three palm oil diets were not evident. HDL-cholesterol was raised in rats fed the three palm oil diets compared to the rats fed either corn oil or soyabean oil. The resulting TC/HDL-C ratio was more favourably decreased by the three palm oil diets.

These early studies, all conducted in the rat model, were aimed at understanding the basic effects of palm oil and its fractions on cholesterol regulation and metabolism. The results from these rat studies were often not conclusive enough, since the rat is predominately a HDL animal model (i.e. the bulk of circulating TC in the rat is carried in its high-density lipoprotein fraction) and, hence, is difficult to induce hypercholesterolaemia or atherosclerosis. In recognising these

limitations, subsequent studies were attempted in other animal models such as the hamster, gerbil and non-human primates.

10.1.2. Hamster and gerbil studies

In recent years the hamster, especially the golden Syrian hamster and the gerbil, have been used extensively as an animal model to elucidate sterol synthesis and LDL metabolism [138]. Using these models, the effects of dietary fatty acids and dietary cholesterol on plasma cholesterol synthesis and LDL metabolism in vivo have been attempted. In the hamster model, dietary cholesterol feeding induces significant changes in plasma total and LDL-C, whereas these parameters are relatively unaffected in the rat model. Several hamster studies in which palm oil was included as the dietary fat have been carried out and these are examined below.

Lindsey et al. [139] examined the qualitative effect of different fats including palm oil and specific fatty acids on plasma lipids and lipoproteins in young male hamsters. Diets contained 13% energy as fat with variations in the polyunsaturated/saturated fatty acid ratios. Replacing 12:0 + 14:0 from coconut oil with 16:0 from palm oil induced a significant increase in HDL-C and a decrease in LDL-C. Similar beneficial shifts were evident in the LDL/HDL cholesterol ratio and the plasma apolipoprotein B/A1 ratio. This study also postulated that the major saturated fatty acid in palm oil, namely palmitic acid, might enhance the production of beneficial HDL-C. This was corroborated by the observation that the highest apolipoprotein A1 and LDL-receptor mRNA abundance was associated with the palm oil fed hamsters.

Pronczuk et al. [140] compared the relative effects of 16:0 (derived from palm oil) and 12:0 fatty acids on plasma lipids in the gerbil. An 8% energy exchange between 16:0 and 12:0 was achieved in a 40% fat energy diet in which all other fatty acids were held constant. Both diets resulted in similar plasma cholesterol, triglycerides and HDL-C. When the same diet was fed to *Cebus* monkeys similar effects on the lipids and lipoproteins were again evident. This study suggested that the hypercholesterolaemic response to the combined 12:0 + 14:0 fatty acids which co-occur in nature (e.g. in coconut oil, palm kernel oil and milk fat) was probably confined to myristic acid (14:0) in the mixture. This hypothesis has gained further credence from the results of several well-controlled human studies (see below).

Hayes et al. [141] examined the cholesterolaemic potential of different fats in the fat-sensitive gerbil. They reported that a *trans* containing hydrogenated soyabean oil diet induced hypercholesterolaemia that was intermediate in response to palm stearin and coconut oil diets. In keeping with previous observations, the coconut oil diet induced significantly higher cholesterol levels than 16:0-rich palm stearin.

10.1.3. Non-human primate studies

Hayes et al. [142] fed three species of monkeys cholesterol-free diets in which the fatty acid classes were strictly controlled. Although the diets provided equal amounts of saturates, monounsaturates and polyunsaturates, the quality of the saturates was different: 12:0 + 14:0 from palm kernel and coconut oils was systematically replaced by 16:0 from palm oil. A highly saturated fat diet (> 80% saturates) and an American Heart Association (AHA)-type diet (equal saturates, monounsaturates and polyunsaturates) were included as the positive and negative controls, respectively. As 12:0 + 14:0 was replaced by 16:0 from palm oil, TC and LDL-C was decreased. Additionally, replacing 50% of the polyunsaturate from the AHA diet with 16:0 from palm oil

failed to elicit the expected increases in TC and LDL-C whereas a similar replacement with 12:0 + 14:0 in the AHA diet significantly increased TC, LDL-C and the LDL/HDL cholesterol ratio. Furthermore, the observed TC showed an essentially perfect fit with the predicted TC based on the Keys–Hegsted equations if 16:0 from palm oil was considered neutral. This study formed the basis for the Hayes and Khosla [143] hypothesis of 1992, depicting thresholds for polyunsaturate effects on cholesterol and neutrality of 16:0.

In another study using *Cebus* monkeys, Khosla and Hayes [144] evaluated the cholesterolaemic response of palmitic acid in the presence and absence of dietary cholesterol. Palm oil was used as the dietary source of palmitic acid. They exchanged 10% of the fat energy between 16:0 and 18:1 while maintaining 14:0 and 18:2 levels constant in the diet. Plasma lipid concentrations and lipoprotein metabolism (LDL and HDL kinetics) were unaffected by 16:0 when cholesterol free diets were used. Similar results were also apparent in another study using *Cebus* and rhesus monkeys fed cholesterol-free diets. Plasma lipid concentrations were identical in animals fed the 16:0-rich (palm oil) and 18:1-rich (canola oil) diets. The palm oil diet also resulted in the lowest LDL/HDL cholesterol ratio, which was significantly better than the 18:2-rich control diet [144].

Khosla et al. [145] assessed the impact of replacing 12:0 + 14:0 from coconut oil with palmitic acid (16:0) from palm oil in an AHA step-1 diet using rhesus monkeys. These test diets had a polyunsaturated/saturated ratio of 0.99 whereas the control diet representing an average American diet had a polyunsaturated/saturated ratio of 0.57. Feeding the 16:0 diet elicited significant reductions in TC, LDL-C, LDL apoB concentrations and a reduced LDL apoB pool size compared to the control diet. In contrast the 12:0 + 14:0 diet had no significant effect on these atherogenic parameters compared to the control diet. In a similarly designed *Cebus* monkey study, Khosla et al. [146] evaluated the effects of palmitic acid from palm oil with *trans* fatty acids from hydrogenated soyabean oil for their cholesterolaemic effects. Relative to the control average American diet, the palmitate diet significantly reduced the atherogenic TC/HDL-C ratio whereas the *trans* diet had no effect. However, the *trans* diet was characterised by a significant decrease in the beneficial HDL-cholesterol relative to the palmitate diet.

African green monkeys fed cholesterol-enriched diets and varying levels of fatty acids were evaluated by Rudel et al. [146] for their atherosclerotic tendencies. A high level of dietary cholesterol was fed in order to elevate TC and induce atherosclerosis. Feeding very high levels of saturated fat (palmitic-rich palm oil) in the presence of a high level of dietary cholesterol induced the highest TC levels. However, when cholesterol was omitted from the saturated fat diet, TC plummeted within 2 weeks from ~390 to ~200 mg/dl. Sundram et al. (1997) demonstrated a significant increase in TC and LDL-C in rabbits fed cholesterol-free semipurified diets containing 12:0 + 14:0 or *trans* fatty acids but not in rabbits fed 16:0-rich palm olein diets. When dietary cholesterol was added to the diets in a follow-up study, atherosclerotic lesions were apparent in 5/8 and 6/8 of the rabbits fed the 12:0 + 14:0-rich diet and the *trans*-rich diet, respectively. In contrast, atherosclerotic lesions were significantly lower and apparent in only 2/8 of the palm olein fed rabbits and 1/8 of the AHA-blend fed rabbits [147,148].

10.2. Human studies

One of the earliest clinical trials evaluating palm oil was pioneered by Ahrens et al. [149] who fed two of their subjects a liquid formula diet containing 40% energy as palm oil. The TC levels

of both these subjects fed palm oil were significantly higher than during a corn oil period. Nevertheless, the TC values after the palm oil period was lower than the baseline values. Grande et al. [150] showed that a palm oil enriched diets resulted in higher TC than a diet predominated by stearic acid derived from cocoa butter. This study was also noteworthy in that it confirmed Key's earlier observation that stearic acid lacked a cholesterol raising effect.

Anderson et al. [124] fed 12 volunteers diets containing 35% saturated fat contributed by two parts of palm oil and one part coconut oil and compared its cholesterolaemic effects with a polyunsaturated safflower oil diet. The safflower oil diet resulted in lower serum TC than the saturated fat diet. However, the saturated fat diets actually resulted in approximately 10% lower serum TC levels than the subjects' habitual diets. In 1984, Baudet et al. [125] undertook a dietary trial with Benedictine nuns to evaluate the effect of 30% fat calories contributed predominantly (two thirds) by palm oil, sunflowerseed oil, peanut oil or milk fat on serum lipid and lipoprotein levels. The sunflowerseed oil diet reduced serum TC and LDL-C significantly compared with all other diets. Serum TC and LDL-C were essentially similar after the palm oil and peanut oil diets whereas milk fat resulted in significantly higher TC and LDL-C levels than all the other test diets.

Mattson and Grundy [128] fed 20 male volunteers a liquid formula diet containing 40% calories contributed either by palm oil, high oleic safflower oil or high linoleic safflower oil. After 4 weeks, the high oleic and high linoleic safflower oil diets produced significantly lower TC and LDL-C than the palm oil diet. HDL-C on the palm oil and high oleic safflower oil diets were similar but HDL-C on the high linoleic safflower oil diet was significantly lower.

In a follow-up study, Grundy and Vega [127] fed 11 patients liquid formula diets containing 40% fat calories (high fat) and compared their effects with a 20% fat calories (low fat) diet. The high fat diets were formulated with coconut oil, palm oil or high oleate safflower oil. The 11 patients were then subdivided into two groups in which seven were fed the coconut oil diet while the remaining four the palm oil diet. The patients were also rotated through the high-oleate safflower oil and low-fat diets. TC and LDL-C were significantly lower on the high oleate safflower oil diet compared with all other test diets. The four patients on the palm oil diet had TC, LDL-C and HDL-C values that were lower than the coconut oil diets and the habitual diets of these patients.

Bonanome and Grundy [126] evaluated the impact of palm oil, high oleic safflower oil and an interesterified fat blend (43% 18:0 and 40% 18:1) using liquid formula diets in 11 elderly patients. The diets contributed 40% fat calories and were consumed by the subjects for 3 weeks in a random order. Mean TC and LDL-C after the palm oil diet were significantly higher than the values of either the high oleic safflower oil or the high stearate interesterified fat. Cholesterol levels after the palm oil period were 11% lower than the entry (habitual) levels but this was discounted by the authors who suggested that lowering of the cholesterol levels of subjects on admission to a metabolic ward was a commonly observed phenomenon. The study was also important in that it concluded that stearic acid had a neutral impact on cholesterol and lipoprotein levels in humans.

Laine et al. [151] compared the effects of palm oil, corn oil, soybean oil and lightly hydrogenated soybean oil added to cholesterol rich diets containing 35% fat energy in 24 normocholesterolemic students. Cholesterol levels after the corn oil, soybean oil and lightly hydrogenated soybean oil were lower by 14, 13 and 9%, respectively compared with the palm oil diet. The analysis of data was, however, complicated by the higher levels of dietary cholesterol consumed during the palm oil period.

These studies (summarised in Table 14) are often cited as examples of the cholesterol raising property of palm oil, which contains almost 50% of its fatty acids as saturates. On closer examination of these studies, several fallacies have been pointed out. For example, these studies were characterised by:

1. the use of liquid formula diets in which fats contributed almost 40% energy, which is considered excessive for normal human metabolic needs (30% energy as fat is considered optimum),
2. the use of relatively older subjects with moderate to severe hypercholesterolemia; and
3. feeding of atypical diets in which the target fatty acid often represented an excessive intake of the total fatty acids.

The above may have led to plasma lipid changes that seemingly established the cholesterol-raising effects of palm oil. However, most latter date studies in which solid-food diets were used with more realistic fatty acid exchanges and mildly hypercholesterolaemic to normocholesterolaemic younger subjects, the cholesterol raising attribute of palm oil was either muted or disappeared. In contrast to the older studies, recent trials have used palm olein, the liquid fraction of palm oil rather than palm oil itself. Whether the switch to palm olein having a higher unsaturated fatty acid composition (reduced palmitic, increased oleic and linoleic acids) resulted in the muted cholesterol response in the subjects is not clearly defined. Some of these recent studies are discussed below.

Table 14
Human studies comparing dietary palmitic and oleic acids[a]

Ref: Study characteristics	Dietary fatty acids % energy				C	TC	LDL-C mg/dL	HDL-C	LDL/HDL-C ratio
	16:0	18:0	18:1	18:2					
[128]	17.4	2.0	15.8	3.9	0	224	143	39	3.7
20M, Age 59; SC = 263;	2.0	1.0	29.3	7.0	0	197	119	38	3.1
40% energy; LF									
[126]	18.0	1.9	15.5	3.8	100	202	140	42	3.6
11M, Age 64; SC = 227;	2.2	0.9	31.9	4.8	100	181	119	44	3.0
40% energy; LF									
[169]	17.4	1.7	16.0	4.0	0	200	152	35	4.3
14M, Age 63; SC = NR;	1.9	0.9	30.3	6.2	0	172	128	32	4.0
40% energy; LF									
[171]	5.2	2.3	17.8	5.3	64	215	151	38	4.0
27M ; SC = 221; 37%	9.8	2.3	12.9	5.7	73	226	161	42	3.8
energy; SF									
[171]	4.6	1.8	20.3	2.7	64	216	150	43	3.5
27M, Age 49; SC = 225;	8.0	2.0	17.6	2.7	66	224	157	44	3.6
37% energy; SF									

[a] C, dietary cholesterol intake; TC, total plasma cholesterol; LDL-C, low density lipoprotein; HDL-C, high density lipoprotein; M, males; S,C starting plasma cholesterol; NR, not recorded; LF, liquid formula diet; SF, solid food diet.

Although palm oil is only a minor component in the European and American diets, it has been singled out as a major contributor to CHD risk in these populations. It was therefore hypothesised that maximum substitution of palm oil in a European population will have no detrimental effects on major lipid and lipoprotein risk factors for CHD. Maximum replacement of dietary palm oil was evaluated for its effects on some aspects of the cardiovascular risk profile of healthy male volunteers, using a double blind crossover design [152]. Maximum replacement (about 70%) with palm oil had no effects on serum total cholesterol or most lipoprotein fractions, including low-density lipoprotein cholesterol. HDL-2 cholesterol was, however, significantly elevated after the palm oil diet relative to the control diet. The palm oil diet caused a significant reduction in the ratio of LDL/HDL cholesterol. It also caused a significant increase in serum apolipoprotein A1 and a significant decrease in apolipoprotein B compared to the control diet. Consequently, the ratio of B/A1 apolipoprotein was decreased significantly. This study clearly indicated that palm oil when used to replace the normal fats in a Western (Dutch) diet had no adverse effects on serum and lipoprotein lipids whereas a possible beneficial effect on apolipoprotein distribution was indicated.

10.2.1. Effects of palm olein as part of a low-fat healthy diet

Palm olein when consumed as part of a low-fat ($<30\%$ energy) has been evaluated for its cholesterolemic effects in a number of human studies. Marzuki et al. [153] evaluated the effect of consuming foods containing either palm olein or soybean oil in young healthy volunteers. In normal healthy volunteers, the level of serum TC and LDL-C was not significantly altered by the palm olein or soybean oil diets. In a similar study [154], when volunteers were switched from a coconut oil diet to a palm olein or a corn oil diet, serum TC decreased by 36 mg/dl and 51 mg/dl, respectively. Hence, a reduction in serum TC was observed on the consumption of a palm olein or corn oil diet relative to a coconut oil diet. Ghafoorunissa et al. [155] substituted palm olein for groundnut oil in the typical Indian diet contributing 27% energy as fat. This effectively doubled the availability of saturated fatty acids and decreased by half the linoleic acid content of the diet. In spite of these major shifts in the fatty acid composition from the use of palm olein, plasma levels of cholesterol and lipoproteins were not altered in this population.

10.2.2. Effects of palm olein in comparison to oleic rich oils

Monounsaturated oils rich in oleic acid are currently touted to be the healthiest of the edible fats in the human diet. While olive, rapeseed and Canola contain in excess of 60% of their composition as *cis*-oleic acid, palm olein has about 48% of this monounsaturated fatty acid. The question of whether this level of oleic acid in palm olein is adequate to result in a lipoprotein-cholesterol profile that protects against CHD was examined in a series of human trials. Ng et al. [156] evaluated the effects of palm olein and olive oil on serum lipids and lipoproteins in comparison to a coconut oil diet. Each test oil was served as the sole cooking oil and contributed two thirds of the total fat intake. The coconut oil diet significantly raised all the serum lipid and lipoprotein parameters, i.e. TC, LDL-C and HDL-C. However, the one-to-one exchange between palm olein (rich in 16:0) and olive oil (rich in 18:1) resulted in similar TC, LDL-C and HDL-C values. This showed that in healthy normocholesterolaemic humans, palm olein could be exchanged for olive oil (high oleic) without adversely affecting serum lipids and lipoprotein levels. Choudhury et al. [157] managed a 5% energy exchange between palm oil (16:0-rich) and olive oil

(18:1-rich) in 21 healthy normocholesterolaemic Australian men and women consuming a low fat (30% energy) and low dietary cholesterol (< 200 mg/day) diet. Under these conditions, TC and LDL-C were not significantly different between the two oils, so that when 16:0 in palm oil was replaced with 18:1 in olive oil, the expected increase in TC and LDL-C were not evident. In a previous human study, Truswell et al. [158] also reported a similar effect between palm olein and Canola oil.

Sundram et al. [159] fed 23 healthy normocholesterolaemic male volunteers carefully designed whole food diets containing Canola oil (18:1-rich), palm olein (16:0-rich) or an AHA Step 1 diet AHA, all contributing approximately 31% energy as fat and <200 mg dietary cholesterol/day. The AHA oil blend was obtained by blending soyabean oil (50%), palm oil (40%) and Canola oil (10%) which resulted in a 1:1:1 ratio of the Saturates, Monounsaturates and polyunsaturates. Serum TC, VLDL-C and LDL-C were not significantly affected by these three diets despite manipulations of the key fatty acids. The high 18:1 Canola and high 16:0 palm olein resulted in almost identical plasma and lipoprotein cholesterol. Only HDL-C after the AHA diet was significantly raised compared with the other two diets. The findings of the above study have now become the subject of a patent (Sundram et al. [147]) advocating a balanced fatty acid ratio for maintaining a proper LDL/HDL-cholesterol ratio that could be cardio-protective.

In contrast to the above studies, Zock et al. [160] reported that replacing 10% energy from 16:0 with 18:1 in normocholesterolaemic subjects significantly lowered TC and LDL-C. This Dutch study did not use natural fat sources. The 18:1-rich diet was prepared by blending high 18:1 sunflower oil, fully hydrogenated sunflower oil and high 18:2 sunflower oil and interesterified palm oil mixed with other edible oils. The 16:0-rich diet was formulated by blending fractionated palm oil, cottonseed oil, and fully hydrogenated sunflower oil. The feeding of fat blends containing atypical triglyceride moieties may have been partially responsible for the observed increases in TC and LDL-C. By contrast, when Sundram et al. [161] maximally replaced the habitual Dutch diet with palm oil, TC and LDL-C were unaffected. The palm oil diet however resulted in significant improvements in the HDL2-C and the apolipoprotein A1/B ratio signalling some cardiovascular benefits rather than the converse to be true for palm oil.

The above mentioned studies focused on the oleic acid content in the different oils tested (palm olein, canola, rapeseed and olive) for their cholesterol modulating properties. Without doubt, oleic acid has been proven to have cholesterol-lowering properties that are said to equal or better than that of the polyunsaturates. However, the optimum amount of oleic acid that is required to ascertain beneficial lipoprotein profiles has not yet been defined. In this context, palm olein containing 44–48% oleic acid was equal in its plasma cholesterol and modulating lipoprotein effect to those of higher oleic acid containing oils including olive (70%), canola (65%) and rapeseed (60%). This augers well for palm olein and its apparent lack of cholesterolemic effects.

10.2.3. Effects of palm olein in comparison to saturated fats

The human diet contains a mixture of fats, and, therefore, mixtures of fatty acids. The net effect of such a mixture on TC and/or the individual lipoproteins will be the sum effects of all the fatty acids, some acting in opposite directions to each other. It is therefore important to decipher the key cholesterol modulating fatty acids to determine the cholesterolaemic index of the fat or oil consumed. Fortunately, several recent human studies have focused on these issues and have

provided additional observations that tend to support the Hegsted [123] observation that saturated fatty acids differ in their cholesterol regulating ability. Some of these studies that used palm oil as a source of 16:0 in their test diets are described below.

Sundram et al. [162] fed 17 normocholesterolaemic subjects whole food diets that exchanged 5% energy between 16:0 and 12:0 + 14:0 (lauric + myristic, LM). Compared with the LM diet, the 16:0 rich diet produced a 9% lower TC concentration reflected primarily by a lower (11%) LDL-C concentration. Heber et al. [163] evaluated diets enriched with palm oil, coconut oil or hydrogenated soybean oil for 3-week test periods in healthy American males. Significant increases in TC, LDL-C and apolipoprotein B were apparent following consumption of the coconut oil diet but not the palm oil and hydrogenated soybean oil diets. In the Ng et al. [154,156] studies, coconut oil enriched diets were compared to palm olein. In both populations, coconut oil feeding resulted in significant increases in TC and LDL-C compared with the palm olein feeding.

These studies compared the effects of 12:0 + 14:0 (LM) occurring naturally in coconut oil and palm kernel oil. They suggest that the cholesterolaemic effect due to 16:0 (palmitic acid) is significantly lower than that of a LM combination. Coconut oil is almost 85% saturated and it has been suggested that the higher cholesterol values after a coconut oil diet may be simply due to the lower availability of linoleic acid. This suggestion has been discounted in the recent study of Sundram et al. [164]. Despite the incorporation of a high level of 18:2 (5.6% en) in the LM diet, it induced significantly higher concentrations of TC and LDL-C in healthy volunteers compared to a 16:0-rich palm olein diet (3.3% en as 18:2) [165]. (Figs. 2 and 3)

The higher TC and LDL-C levels induced by the LM diets are inconsistent with the values expected based on the Keys–Hegsted equations [121–123], which predict that identical TC concentrations would result from both fatty acids. However, it is arguable that the simplified combination of the different dietary saturates effects in the Keys–Hegsted regressions tend to overestimate the importance of 16:0 and underestimate the impact of 12:0 + 14:0. The question that remains is which of the two fatty acids namely 12:0 and 14:0 is more cholesterolaemic? The separation of 12:0 and 14:0 from natural fat sources is difficult since they tend to co-occur. Hayes and Khosla [143] optimised the content of 12:0 or 14:0 in their animal studies so that actual cholesterolemic effects of individual saturated fatty acids could be better understood. They found that 14:0 was almost four times more cholesterol raising than 12:0. This allowed them to postulate that myristic acid (14:0) was the most potent cholesterol raising fatty acid in the human diet and palmitic acid (16:0) was relatively neutral.

Wood et al. [166] reported the effects of butter, hard margarine, sunflower oil and palm oil (crude and refined) on lipoprotein profiles of American subjects consuming 38% energy as fat. The test fats provided almost 50% of the total fat intake, which was equivalent to 16% energy. Diets containing crude or refined palm oil did not elevate the TC relative to the habitual American diet while LDL-C was unaffected relative to all other test diets. However, refined palm oil caused a significant increase in the beneficial HDL-C and its associated apolipoprotein A1 level. The resulting LDL/HDL-cholesterol ratio was superior on this refined palm oil diet. In comparison, sunflower oil resulted in significant reductions in TC, LDL-C and the desirable HDL-C and apolipoprotein A1.

Using 15 normocholesterolaemic women fed solid-food diets, Schwab et al. [167] failed to find any difference in plasma lipid levels following a 4% energy exchange between 12:0 and 16:0. Temme et al. [168] reported the effects of feeding diets enriched in lauric and palmitic acids on

plasma lipids. The subjects consumed solid food diets that exchanged 8% energy between lauric and palmitic acids. The lauric acid diet resulted in higher TC and LDL-C than the palmitic acid diet but this could not be explained by the somewhat higher myristic acid content in the diet. Accordingly, the plasma lipid changes appear to suggest that lauric acid per se was more cholesterol raising than palmitic acid. In the Denke and Grundy study [169], the 12:0-rich diet (contributing 17.6% energy) raised TC by 9 mg/dL compared with a diet containing 17.4% en as 16:0. The increase in TC due to the 12:0 diet occurred exclusively in LDL-C. These data, therefore, suggest that the cholesterolaemic effects of 16:0 derived from palm oil/palm olein are lower than those of 12:0 and 14:0 derived from natural fats including coconut oil, palm kernel oil and butter fat.

From the above studies the effect of palmitic acid (the major saturated fatty acid in palm oil products), on plasma lipoprotein cholesterol is becoming better understood. Indeed, if palmitic acid is hypercholesterolemic, then an increased endogenous synthesis or a decreased clearance rate of cholesterol should be evident. The human study of Cook et al. [170] investigated the relationship between endogenous synthesis of cholesterol and the content of palmitic acid in the diet contributed by palm oil. High levels of palmitic acid in the diet did not significantly affect serum total and LDL-cholesterol levels. Fractional synthetic rate of cholesterol was not different between dietary treatments (high versus low palmitic acid content). This suggested that there was no relation between endogenous synthesis of cholesterol and palmitic acid content in the diet.

The saturated fatty acids in palm oil consist of palmitic and stearic acids (44 and 5%, respectively). Grundy and Vega [127] have previously advocated the neutrality of stearic acid. The above mentioned studies with palm oil, in essence suggest that the saturated fatty acids in palm oil do not contribute to increase plasma cholesterol loads. By simple deduction, this suggests that palmitic acid behaves as a neutral fatty acid and therefore none of the fatty acids in palm oil are cholesterol raising.

10.3. Effects of palm olein in comparison to trans *fatty acids*

Controversy continues over the significance of *trans* fatty acids in human nutrition, particularly concerning their negative impact on the plasma lipoprotein profile and its untoward implications for atherogenesis. *Trans* fatty acids can deleteriously affect lipoproteins by increasing TC, LDL-C, lipoprotein Lp(a) and decreasing HDL-C relative to their *cis* isomers. This has raised the need to replace hydrogenated fats with natural solid fats in a large number of food formulations. The nutritional efficacy of the solid fats replacing hydrogenated fats should be such that they do not adversely affect plasma lipids and other CHD risk factors. In this context, palm oil can be considered a suitable alternative.

Nestel et al. [171] compared a *trans* elaidic rich fat with a 16:0-rich blend (16:0 contributed mainly by palm oil). Both test blends resulted in higher TC and LDL-C than an oleic-rich control diet. There was essentially no difference in TC and LDL-C between the elaidic-rich and palm oil-rich diets. HDL-C was however significantly raised on the 16:0-rich diet and the resulting LDL/HDL-C ratio were more favourable on the palm oil diet than the *trans* diet. This led the authors to conclude that there is little benefit from avoiding the use of palm oil by substituting *trans*-fatty acids in food formulations. Sundram et al. [164] undertook a direct comparison of *trans* elaidic fat designed to replace the saturates (16:0, 12:0 + 14:0) in foods and food processing. Feeding of elaidic acid at 5.5% energy significantly elevated TC and LDL-C relative to the 16:0-rich (palm

olein) and 18:1-rich fats and uniquely depressed HDL-C and increased lipoprotein Lp (a) relative to all the fats tested (including 12:0 + 14:0). The 16:0 and *cis* 18:1-rich diets elicited identical effects on lipoproteins. The impact of *trans* elaidic acid on the lipoprotein profile of humans appeared to be worse than that of saturates occurring in natural oils and fats. In a follow-up study the content of *trans* elaidic acid was reduced by increasing the content of 18:1 and 18:2 *trans* isomers which were again compared with a palm olein enriched-diet. Despite these changes, the cholesterol elevating properties of the *trans* fatty acids persisted over that of palm olein containing significantly higher levels of palmitic acid [172].

Evidence implicating the adverse nutritional effects of *trans* fatty acids is increasing steadily. In the long run, the industry will be forced to seek alternatives for hydrogenated fats. Such alternatives must first be proven nutritionally safe and yet meet the physico-chemical requirements of the food products. The solid fat profile of palm oil makes it a natural contender to replace hydrogenated fats in solid-fat food formulations. The use of palm oil in such products could virtually eliminate their *trans* fatty acid content. The desired fatty acid composition in the product can also be easily achieved by blending palm and other oils. For example, this has been demonstrated previously in the AHA-blend [159] in which palm olein contributed 40% of the blend's composition and this resulted in an optimum LDL/HDL-cholesterol ratio.

The above mentioned studies suggest that the cholesterolaemic properties of palm oil and palm olein are dependent on several set points. Palm oil and palm olein have been shown to be hypocholesterolaemic compared to diets contributing variable amounts of lauric and myristic fatty acids. This augurs well for the hypothesis that the cholesterolaemic effects of the saturated fatty acids are not equal [173]. Indeed, the neutrality of stearic acid has long been advocated. In comparison to diets enriched by canola, rapeseed and olive oils, palm olein appears to be comparable in its ability to modulate the lipids and lipoproteins. The studies that lend credence to this observation were conducted with normal healthy volunteers consuming moderate fat energy loads (30% energy) and moderate dietary cholesterol (<300 mg/day). When hypercholesterolaemic subjects and high fat ($>40\%$ energy as fat) liquid formula diets were used palm oil appeared to raise TC and LDL-C.

Palm oil and palm olein will also continue as important ingredients in food applications requiring solid fats without hydrogenation. It certainly seems nutritionally superior to hydrogenated fats by not increasing TC and LDL-C while even aiding in the increase of the beneficial HDL-cholesterol. Apart from its fatty acids, the minor components present in palm oil, especially the tocotrienols, have been reported to reduce TC and LDL-C [174] through their ability to suppress HMG-CoA reductase activity. These findings merit a re-evaluation of the nutritional effect of palm oil and palm olein on blood lipids and lipoproteins especially since they are poised to continue their importance as major edible oils for human consumption worldwide.

11. Effect of palm oil on arterial thrombosis, cardiac arrhythmia and prostanoid synthesis

11.1. Arterial thrombosis

Hornstra et al. [175] undertook several studies to evaluate palm oil effects on arterial thrombosis using the aorta loop technique in rats. These data have also been reviewed [176]. Obstruc-

tion times (OT) was measured as the time lapsed between the insertion of a canula loop in the abdominal aorta of the animal and its occlusion by a thrombus formation. In general, rats fed diets rich in polyunsaturated fatty acids had longer OT than those fed saturated fatty acid diets. Rats fed palm oil diets had similar OTs to that of rats fed the more polyunsaturated oil diets including rapeseed, linseed and sunflowerseed oils. Hence, in the aorta loop model palm oil behaved like a polyunsaturated oil. Hornstra [176] further suggested that the antithrombotic effects of palm oil seemed at least in part, to be related to the amount and composition of unsaponifiables in the oil.

Rand et al. [177] measured collagen activated platelet aggregation in rats fed palm oil or sunflowerseed oil (50% fat energy). They reported greater platelet aggregation in the sunflowerseed oil fed rats compared to the palm oil fed rats. Platelet aggregation induced by adenosine diphosphate, collagen or PAF-acether was also reported to be comparable in rabbits fed palm oil, sunflowerseed oil, olive oil, linseed oil and fish oil [175]. Vles [178] demonstrated that palm oil feeding resulted in a higher degree of atherosclerosis lesions than the administration of rapeseed or sunflowerseed oils. However, when the protein was altered from casein to soya protein, this difference was no longer apparent even when the rabbits were fed for 1.5 years [175].

11.2. Cardiac arrhythmia

Using a rat model of ischemic cardiac arrhythmia following surgical occlusion of a major coronary artery, it has been demonstrated that saturated fats promote, whereas polyunsaturated oils, especially fish oils rich in n-3 fatty acids protect against cardiac arrhythmia. Charnock et al. [179] found that diets supplemented with either chemically refined or physically refined palm oil gave intermediate values between saturated sheep kidney fat and polyunsaturated sunflowerseed oil during ischemic stress. However, during reperfusion of a previously ischemic heart both palm oils were as effective as sunflowerseed oil in reducing premature ventricular beats. The number of animals displaying severe ventricular fibrillation was also reduced after palm oil feeding compared to feeding saturated sheep kidney fat.

11.3. Prostanoid synthesis

Prostanoids, often called local hormones, are a series of structurally related compounds derived from certain polyunsaturated fatty acids. The prostanoids formed by blood platelets and blood vessels play an important role in thrombosis and atherosclerosis. The quantity and type of dietary fat, its polyunsaturated/saturated ratio and ratio of n-3/n-6 fatty acids modulate the synthesis of prostanoids. Activated blood platelets produce thromboxane A_2, that is a platelet aggregator, and hence have a prothrombotic effect. Prostacyclin (PGI_2) produced by the vessel walls, on the other hand, deactivates blood platelets and breaks up platelet aggregates. As a result of these opposing effects, the balance between the pro- (TxA_2) and anti- (PGI_2) prostaglandins are known to influence atherosclerotic thrombotic tendencies.

Hornstra et al. [175] reported that in rats, a palm oil diet caused a significant reduction of TxA_2 in collagen activated platelets compared to a sunflowerseed oil diet in rats. The production of PGI_2 was not significantly different between treatments and palm oil feeding significantly reduced the TxA_2/PGI_2 ratio. Similarly, Rand et al. [177] reported a significantly lower thromboxane/

prostacyclin ratio in platelets of rats fed palm oil. Sugano and Immaizumi [133] showed that when diets contained 20% palm oil, safflower oil or olive oil, the PGI_2/TxA_2 ratio was highest for safflower oil, intermediate for palm oil and lowest for olive oil fed rats. It was also shown that evening primrose oil containing 74% linoleic acid and 9% linolenic acid increased thromboxane production and the thromboxane/prostacyclin ratio in rats compared to a more saturated palm oil. Abeywardena et al. [180] hypothesised that the beneficial modulation of the thromboxane/prostacyclin ratio by palm oil may be related to the unsaponifiable fractions in palm oil and especially so the tocotrienols. This has to some extent been confirmed by Sugano and Immaizumi [133] who showed that the ratio of aortic prostacyclin to plasma thromboxane following a palm oil diet in rats was not simply predictable on the basis of the fatty acid composition of the oil alone.

12. Influence of palm oil on carcinogenesis

There appears to be a positive correlation between the quantity and quality of fat consumed and incidence of cancers of the breast, colon and prostrate. Many of these epidemiological observations relating fat consumption to different types of cancers have been reproduced in laboratory animals. For example, polyunsaturated fatty acids, especially those rich in the n-6 series derived from vegetable seed oils, have tumour promoting effects in mammary cancer models. This was, therefore, the basis for evaluating the effects of palm oil enriched diets on the initiation, promotion and progression stages of mammary carcinogenesis.

Using a rat model made carcinogenic by the administration of 7,12 dimethylbenz(a) anthracene (DMBA), Ip et al. [181] demonstrated that tumour incidence and final tumour numbers increased proportionally as saturated fatty acids (coconut oil/palm oil) in the diet were decreased and polyunsaturated fatty acid content increased. This observation, along with previous findings [182], showed that mammary tumour progression and incidence was sensitive to the level of linoleic acid (18:2 n-6) in the diet. In moderately exercised rats, a diet containing 2.8% corn oil and 21.8% palm oil resulted in lower carcinogenesis indices (increased latency period, decreased tumour incidence and tumour numbers) than did a low-fat, low-linoleic acid diet containing 5% corn oil. Buckman et al. [183] evaluated the effects of a 5 or 20% palm oil diet compared with either a 5 or 20% safflower oil diet in mice made spontaneous cancerous by the injection of mammary tumour cells. After 21 days, the mean volume of tumours in mice fed the 20% safflower oil diet was almost double that of the 20% palm oil fed rats and six-fold that of mice fed the 5% palm oil diet. Of interest too was the observation that the 5% palm oil fed rats had significantly lower mean tumour volumes than the 5% safflower oil fed rats. These studies while emphasising the promotional role of linoleic acid in tumourgenesis, also suggest an inhibitory effect of palm oil.

Dietary fats may also play an influential role during the initiation phase of carcinogenesis. This was demonstrated by Sylvester et al. [184] who fed diets containing palm oil, corn oil, beef tallow or lard (45% calories) ad libitum to rats for 4 weeks prior to and 1 week following DMBA administration. The rats were then fed the control diet (corn oil at 11% calories) for the remaining 19 weeks and then sacrificed. Palm oil feeding resulted in the lowest tumour numbers. Although both lard and beef tallow have saturation levels somewhat similar to that of palm oil, the resulting tumour numbers were significantly higher than both palm and corn oils. This

suggests that dietary fats may modulate tumourgenesis through mechanisms independent of their fatty acid compositions.

Sundram et al. [185] fed female Sprague-Dawley rats treated with DMBA, semisynthetic diets containing 20% w/w of palm (either crude, refined or metabisulphite treated), corn and soyabean oils for five months. Rats fed 20% corn or soyabean oil diets had significantly higher tumour incidence and tumour yield than rats fed palm oil. This suggested that palm oil did not promote chemically induced tumourgenesis in the rats compared to the corn and soyabean oil diets. A possible reason was the content of n-6 linoleic acid in these diets which was present at significantly higher levels in the corn and soyabean oils compared to the palm oil.

The non-promoting effects of palm oil on certain types of experimental carcinogenesis may in part be related to the minor constituents present in it. Of these, the carotenoids and tocotrienols are of interest. β-carotene has long been postulated to be beneficial as an anticancer agent. Crude palm oil is one of the richest natural sources of carotenoids and through improved processing techniques much of the original carotenoid content can be retained in the processed oil. The design of this study [185] had therefore speculated that the crude palm oil containing carotenoids and palm vitamin E (tocopherols and tocotrienols) would have a greater non-promoting effect on rat mammary cancer compared to the refined or metabisulphite treated palm oil. However, this was not obvious in the above study, since tumour numbers and tumour yield were similar among the three different palm oils used.

13. Minor components in palm oil and their health effects

Some of the minor components in palm oil include the carotenoids, tocopherols, tocotrienols, sterols, phosphatides, triterpenic and aliphatic alcohols. Although these minor components account for less than 1% of the oil's constituents, they nevertheless play significant roles in maintaining its stability and quality. In addition, some of these minor components especially the carotenoids and vitamin E (tocopherols and tocotrienols), are important nutritionally.

Crude palm oil contains between 500 and 700ppm carotenoids. The major components are alpha- and beta-carotene. These carotenoids have pro-vitamin A activity. Unfortunately, in an effort to meet the consumer's perception of refined oil (golden yellow colour) the carotenoids are often thermally degraded and removed during the deodorisation stage of the refining process. In crude palm oil, carotenoids appear to offer some protection against oxidation by themselves being oxidised first prior to the oxidative attack on the triacylglycerols.

Crude palm oil is also a rich source of vitamin E (600–1000ppm). Unlike the seed oils the vitamin E in palm oil occurs largely as tocotrienols. The vitamin E content of palm oil is partially lost as a result of processing. RBD palm oil, palm olein and palm stearin retain approximately 69, 72 and 76% of the original level of vitamin E in the crude oils, respectively. However, there is a large variation in these estimates within the refining industry because differences in the plant conditions as well as the plant design influence the amount of vitamin E lost during refining. It has been observed that vitamin E tends to partition preferentially into the olein during fractionation of RBD palm oil. For example, the concentrations of vitamin E in RBD palm olein and RBD palm stearin are 104–135% and 58–75%, respectively of the original level in the source RBD palm oil.

Sitosterol, campesterol, stigmasterol and cholesterol largely constitute the phytosterols in palm oil. As in the case of all other edible oils of vegetable origin, cholesterol content in palm oil is negligible and the phytosterol levels are further reduced on refining.

14. Palm vitamin E

Refined palm oil contains about 350–450 ppm vitamin E, is present as the RRR-α-tocopherol (30%) and tocotrienol (70%) isomers. In contrast, other oils such as corn, soya and sunflower are good sources of the tocopherols but contain no tocotrienols. Historically, vitamin E activity (one international unit, IU) has been defined as 1 mg of all rac-a-tocopheryl acetate while 1 mg of RRR- α-tocopherol equalled 1.49 IU. In addition, vitamin E activity in foods is expressed as the α-tocopherol equivalent (α-TE) which is the activity of 1 mg of RRR- α-tocopherol [186]. On this basis, conversion factors for each mg of the different tocopherols and toctrienols present in palm oil to α-TE are as follows: α-tocopherol, 1.0; gamma-tocopherol; 0.5; delta-tocopherol, 0.1; alpha-tocotrienol, 0.3 and beta-tocotrienol, 0.05. The factors for gamma and delta tocotrienols are presently unknown. These conversion factors are based on the ability of each isomer to overcome specific vitamin E deficiency symptoms such as foetal resorption, muscular dystrophy and encephalomalacia. Since these factors are based on rat foetal resorption assays [187], their relevance to humans is often questionable. In addition, their biological activity may be based on their antioxidant activities, but this too appears misleading. For example, α-tocotrienol has only one third the biological activity of α-tocopherol, yet it has a higher [188] or equivalent [189] antioxidant activity. However, alpha-tocotrienol has now been reported to exhibit greater protection of red blood cells against oxidative hemolysis than α-tocopherol. It also showed a higher inhibitory effect on rat liver microsomal lipid peroxidation induced by adriamycin than α-tocopherol [190].

The tocopherols in palm oil and other dietary sources are absorbed, transported and metabolised as previously documented [191]. The tocotrienols, on the other hand, are handled differently. Ikeda et al. [192] reported that α-tocotrienol in rats is enhanced compared to α-tocopherol. In hamsters, Hayes et al. [141] suggested that feeding a mixture of tocotrienols and tocopherols enriched from palm oil increased the preferential secretion of α-tocopherol in lymph. Plasma concentrations of tocotrienols, especially in the fasted state, are difficult to detect as they are metabolised very rapidly. Hayes et al. [141] reported the presence of tocotrienols in all tissues except the brain of hamsters fed palm tocotrienol fractions tocopherol rich fraction (TRF) with adipose tissue was especially rich in tocotrienols. Mouse skin also appears unique in its ability to accumulate appreciable amounts of tocotrienols. The presence of α- and gamma-tocotrienols has been reported by Podda et al. [193] even when the diet was not especially enriched with tocotrienols. Skin has been suggested to be an important storage and excretory site for vitamin E and the accumulation of tocotrienols could be especially beneficial in protecting the lipid barrier of the stratum corneum [65].

There is now a growing interest in the nutritional and physiological properties of vitamin E in palm oil, especially those of the tocotrienols. This has recently been reviewed by Theriault et al. [194]. Qureshi et al. [131] first isolated tocotrienols from barley and suggested that alpha-tocotrienol in barley exerted a dose dependent inhibition of HMG-CoA reductase (HMGR) activity,

which regulates cholesterol synthesis in the liver. Parker et al. [195] suggested that alpha-, gamma- and delta-tocotrienols act post transcriptionally to lower the mass of HMGR in HepG2 cells.

Subsequently, Qureshi et al. [174] used tocotrienol-rich fractions (palm vitee) from palm oil to evaluate a possible hypocholesterolaemic effect in humans. In a double blind crossover study using 20 hypercholesterolaemic subjects (total cholesterol, TC >6.2 mmol/L) palmvitee supplementation caused significant reductions in TC and LDL-C. Apolipoprotein B decreased by 9–11%, serum thromboxane by 25% and platelet factor PF4 by 16% in comparison to the placebo corn oil supplementation. Tan et al. [196] demonstrated that administration of just one palm vitee capsule, containing 18 mg tocopherols and 42 mg tocotrienols, significantly lowered TC and LDL-C in hypercholesterolaemic subjects. Hypercholesterolaemic pigs with inherited hyperlipemias fed palm tocotrienol rich fractions also showed significant decreases in TC, LDL-C, apolipoprotein B, TxB2 and PF4 [197]. It has been proposed that a combination of gamma-tocotrienol and alpha-tocopherol in a ratio similar to that present in palm oil deserves further evaluation as a potential hypolipaemic agent for people at atherogenic risk.

Serbinova et al. [188] demonstrated that palm oil vitamin E afforded greater protection against ischemia/reperfusion injury of isolated Langendorff hearts than tocopherols. This was manifested through a complete suppression of LDH enzyme leakage from the ischemic hearts, decrease in adenosine triphosphate and creatine phosphate levels and inhibition of formation of endogeneous lipid peroxides by palm vitamin E. Palm tocotrienols also demonstrated a higher recycling efficiency and greater uniformity of distribution in membrane layers. These properties offered a much higher anti-oxidant potency for tocotrienols (especially d-γ-tocotrienol) than tocopherol isomers.

Palm tocotrienols may have possible anti-cancer properties. Sundram et al. [185] suggested that crude palm oil was more effective than refined palm oil in increasing the tumour latency period in DMBA treated rats. This was attributed to the presence of tocotrienols and carotenoids in the crude oil. When the vitamin E content in palm oil was removed, significantly more tumours were apparent [198]. Addition of palm vitamin E to corn oil (500 or 1000ppm) resulted in a lower tumour incidence and yield compared to rats fed corn oil alone.

A series of studies also investigated the in vitro effects of tocotrienols on human breast cancer cells. Compared to α-tocopherol (500 ug/ml concentration), which had no growth inhibition of human breast cancer cells, palm TRF inhibited the incorporation of (3H) thymidine into human breast cancer cells by 50% (at a concentration of 180 ug/ml) [199]. Osterogen-receptor negative and positive human breast cancer cells were used to test the efficacy of individual palm tocotrienols at varying concentrations. These individual tocotrienols showed even greater inhibitory effects on these cells and at much lower concentrations than TRF. Insulin growth factor (IGF) binding proteins have been reported to play a role in mediating IGF induced growth of breast cancer cells. Gamma and delta tocotrienols were shown to decrease the expression of the IGF binding proteins in addition to their anti-proliferating effect. Guthrie et al. [200] demonstrated that tocotrienols inhibited the proliferation of oestrogen receptor positive MCF-7 human breast cancer cells and that the combination of tocotrienols with tamoxifen was more effective than either tocotrienols or tamoxifen alone. There also appears to be a synergy in the inhibition of human cancer cells between palm tocotrienols and flavonoids. Combinations of tocotrienols, flavonoids and tamoxifen proved to be even more effective than the individual components [201].

Wan Zurinah et al. [202] suggested that palm TRF reduced the severity of hepatocarcinogenesis in rats treated with 2-acetylaminofluorene. Hepatic cell damage was reduced by TRF along

with decreased activities of plasma glutamyltranspeptidase. The degree of severity of hepatocarcinoma was reduced in rats supplemented with TRF for 9 months compared to 1 or 2 months. Palm tocotrienols also inhibited proliferation of Caski epithelial cancer cells whereas α-tocopherol had no effect. The anti-proliferative effect of palm tocotrienols was attributed to an increase in apoptosis as measured by increased DNA fragmentation.

Palm tocotrienols have also been reported to be effective against transplantable mice tumours [203]. Mice injected with mixtures of tocopherols and tocotrienols extracted from palm oil had significantly improved survival rates following intra-peritonial transfer of IMC carcinoma cells. On the other hand, alpha-tocopherol produced only a modest increase in the survival time. Survival of mice receiving the tocotrienols was increased in a dose dependent manner. Komiyama et al. [204] hypothesised that the antitumour effects of palm tocotrienols may be mediated through a direct cytotoxic activity or through an ability to stimulate the host immune system. Komiyama and Yamaoka [204] demonstrated growth inhibition of human and mouse tumour cells exposed to tocotrienols while the onset of subcutaneous lymphoma in HRS/J hairless mice was delayed [205] by 2–4 weeks. Gamma and delta-tocotrienols derived from palm oil also inhibited the growth of Epstein–Barr virus [206].

15. Palm carotenoids

The mesocarp of the oil palm fruit yields a deep red coloured palm oil, which contains 700–800ppm carotenoids [45]. The characteristics of these palm carotenoids and technological advances aimed at producing red palm oil and palm carotene concentrate have been discussed earlier. The pro-vitamin A activity of carotenoids has been known for a long time. Only a few carotenoids are provitamins, and those that are vary in their bioavailability. The relative biopotencies of only a few of these provitamins have been estimated by rat bioassays and the most important is β-carotene- both in terms of its bioactivity and widespread occurrence. β-carotene is the most important vitamin A precursor in human nutrition and provides the major source of vitamin A in many developing countries. On a worldwide basis, about 60% of dietary vitamin A is estimated to come from pro-vitamin A.

15.1. Pro-vitamin A activity of palm carotenoids

Drummond and colleagues [207] demonstrated that crude palm oil was an excellent source of vitamin A as only small quantities were able to promote growth and cure xerophthalmia in young rats. Further, they found that the decolourised fraction of palm oil was biologically inactive and the carotene content was destroyed during hydrogenation. The vitamin A activity in the palm oil was later attributed to the carotenoids [208].

Rosedale and Oliveiro [209] investigated the fat-soluble vitamins of red palm oil. Their interest stemmed from the high incidence of xerophthalmia in Tamil children in Malaya and the proneness of adults to respiratory infections due to vitamin A deficiency. Palm oil was shown to be effective in replacing vitamin A in the diet and its activity was found to be equal that of cod liver oil rich in vitamin A. They also drew the conclusion that it was unlikely that an adequate supply of vitamin A could be secured by a mixed vegetable diet, unless some oil such as red palm oil was also consumed.

There was also renewed interest in palm oil and red palm oil elsewhere. β-carotene rich red palm oil was used in dietary intervention studies to evaluate its possible role in the prevention of vitamin A deficiency among populations at risk in India in the 1930s [210]. Children 5–10 years old with keratomalacia were treated twice a day with an emulsion prepared with red palm oil. Each dose contained 0.6ml of red palm oil for 15 days. The red palm oil treatment compared well with the results obtained by treating another group of children (with keratomalacia) with cod liver oil containing a similar dose of vitamin A.

It was also observed that red palm oil could be blended with different edible oils at 5–12% without modifying the original taste of the oil, but with some slight colour change. The carotene content ranges from 30–70ug/g, the vitamin potency being two to three times greater than that of a good quality butter [211]. Arising from these encouraging findings, the League of Nations Intergovernmental Conference on Rural Hygiene in the late 1930s recommended the possibility of making use of red palm oil as a source of pro-vitamin A in certain countries.

Red palm oil is the richest natural source of β-carotene, a precursor of vitamin A, in addition to providing energy density to the diet. Rukmini [212] summarised some aspects of the health and nutritional effects of red palm oil and the results of a comprehensive safety evaluation was carried out by the Indian Council for Medical Research at the National Institute of Nutrition. The purpose of this work was to recommend use of the oil in supplementary feeding programmes. Based on the results obtained, it was recommended that developing countries should have no hesitation in creating strategies to increase the use of red palm oil in combating vitamin A deficiency. The importance of red palm oil in the treatment of vitamin A deficiency has been reiterated by many others [213–216]. These are easy to produce, available all year round, inexpensive and accessible sources of vitamin A for most of the developing world.

15.2. Absorption of carotenoids

Dietary fat is a critical factor influencing absorption of carotenoids, since carotenoids are only absorbed in the presence of bile salts and proper micelle suspension [217]. Fat in the diet also provides the vehicle for transporting carotenoids. Protein status also influences absorption [218]. Since the carotenoids in red palm oil are present in a lipid solubilised free form, a higher rate of absorption is possible [219]. The *trans* isomers constitute the major fraction and are better converted to vitamin A compared to the *cis* isomers, thereby enhancing their utilisation [220]. Palm oil is rich in palmitate, which is required for the esterification and transport of retinol to the liver. Vitamin E is essential for normal in vivo utilisation of vitamin A besides increasing its storage by three to six times [219].

Roels et al. [218] reported palm oil administration to have cured night blindness in Indonesian children with mild protein malnutrition. Their serum retinol levels, after treatment with palm oil were the same as in children receiving adequate amounts of vitamin A acetate. Children 2 to 5 years old apparently utilise carotene from red palm oil better than carotene from other sources [221]. In more recent times, the efficacy of utilising red palm oil as a source of pro-vitamin A in combating vitamin A deficiency in school children in India and lactating mothers in Honduras has been established [222,223]. Crude palm oil is acceptable to pre-school children in India as an edible grade oil (31). Children showed considerable improvement in overcoming vitamin A deficiency. The fifteenth meeting of the International Vitamin A Consultative Group in Guatemala

[224], recommended among other measures, the use of red palm oil because its carotenoid content seems to be particularly bioavailable. Similar recommendations have also been made in a United Nations report [225]. Dietary intervention with red palm oil thus offers a good degree of protection from severe vitamin A deficiency.

15.3. Palm carotenoids and cancer prophylaxis

Doll and Peto [226] identified diet as one of the major factors in the aetiology of cancer. Cancer epidemiological studies have provided evidence that cancer chemopreventive agents exist naturally in our diets. High intakes of vegetables and fruits are known to be associated with lower risk of cancer of the lung and gastrointestinal tract. The protective effect may relate to different components present in fruits and vegetables [227,228]. Although, over 1000 compounds have been tested, the retinoids and carotenoids have received the most attention. A number of epidemiological studies have demonstrated an inverse correlation between dietary intake or blood level of vitamin A/carotenoids and cancer risk, as well as an anti-carcinogenic effect for these compounds [229]. The data further indicate that a wide range of cancer sites may be influenced by these carotenoids.

The inhibition of chemical carcinogenesis by palm oil carotenoids with reference to benzo(a) pyrene metabolites in vivo and in vitro in rat hepatic cells has been reported by Tan and Chu [230]. It has also been reported that palm carotenoids exhibit an inhibitory effect on the proliferation of a number of human cancer cells. These include the neuroblastoma, GOTO, pancreatic cancer PANC-1, glioblastoma A172 and gastric cancer HGC-27 [231,232]. Of significant interest from these studies was the observation that palm alpha-carotene and a palm carotene concentrate were protective whereas synthetic beta-carotene was tumour promoting. Murakoshi et al. [233] isolated palm alpha-carotene and a palm carotene concentrate and showed its ability to inhibit liver, lung and skin tumours in mice. The same effect could not, however, be attributed to synthetic beta-carotene. Similar superior inhibitory effects for alpha-carotene were apparent in a chemically induced skin tumour progression model. Overall, these results lead to the conclusion that the natural bouquet of carotenoids, in palm oil has promising chemopreventive activities against cancer.

Acknowledgements

We thank the Director-General of Malaysian Palm Oil Board (MPOB) for his permission to publish this paper. We are indebted to our colleague Mr. Andrew Chang for his valuable comments and suggestions during the preparation of this review.

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