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BIOCHEMICAL FACTORS THAT CONTROL OIL COMPOSITION IN THE OIL PALM

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Fatty acid biosynthesis is essentially the same in all plant species. Yet, different plants have unique fatty acid profiles which differentiate one vegetable oil from another. The oil palm produces different oils in the mesocarp and kernel. These two oils have distinct fatty acid profiles. Palm oil is rich in palmitic and oleic acids while palm kernel oil is a rich source of lauric and myristic, i.e. medium chain fatty acids. The role of different biochemical factors in controlling fatty acid composition in the oil palm was investigated. The studies confirmed that fatty acid composition was not controlled by a single enzyme. Instead, a number of enzymes work in concert to produce the distinct fatty acid composition of palm and palm ***kernel oil. Beta ketoacyl ACP synthase II, acyl ACP thioesterases and acyltransferases have an important role in determining the fatty acid profiles of palm and palm kernel oil.***

INTRODUCTION

The oil palm is a unique crop in that it produces two distinct types of oil. The oil palm fruit produces palm oil in the mesocarp and palm kernel oil in the seed. Both these storage oils have unique fatty acid profiles. The mesocarp accumulates predominantly palmitic (C16:0) and oleic (C18:1) acids while the kernel accumulates medium chain fatty acids, i.e. lauric (C12:1) and myristic (C14:1) acids.

The pathway for fatty acid biosynthesis is essentially the same in all plants ***(Figure 1)***. Palmitic and the shorter chain fatty acids are produced from acetyl-CoA and malony-CoA by a series of two carbon addition reactions which are catalyzed by a complex of enzymes known as fatty acid synthase (FAS). Palmitic acid is

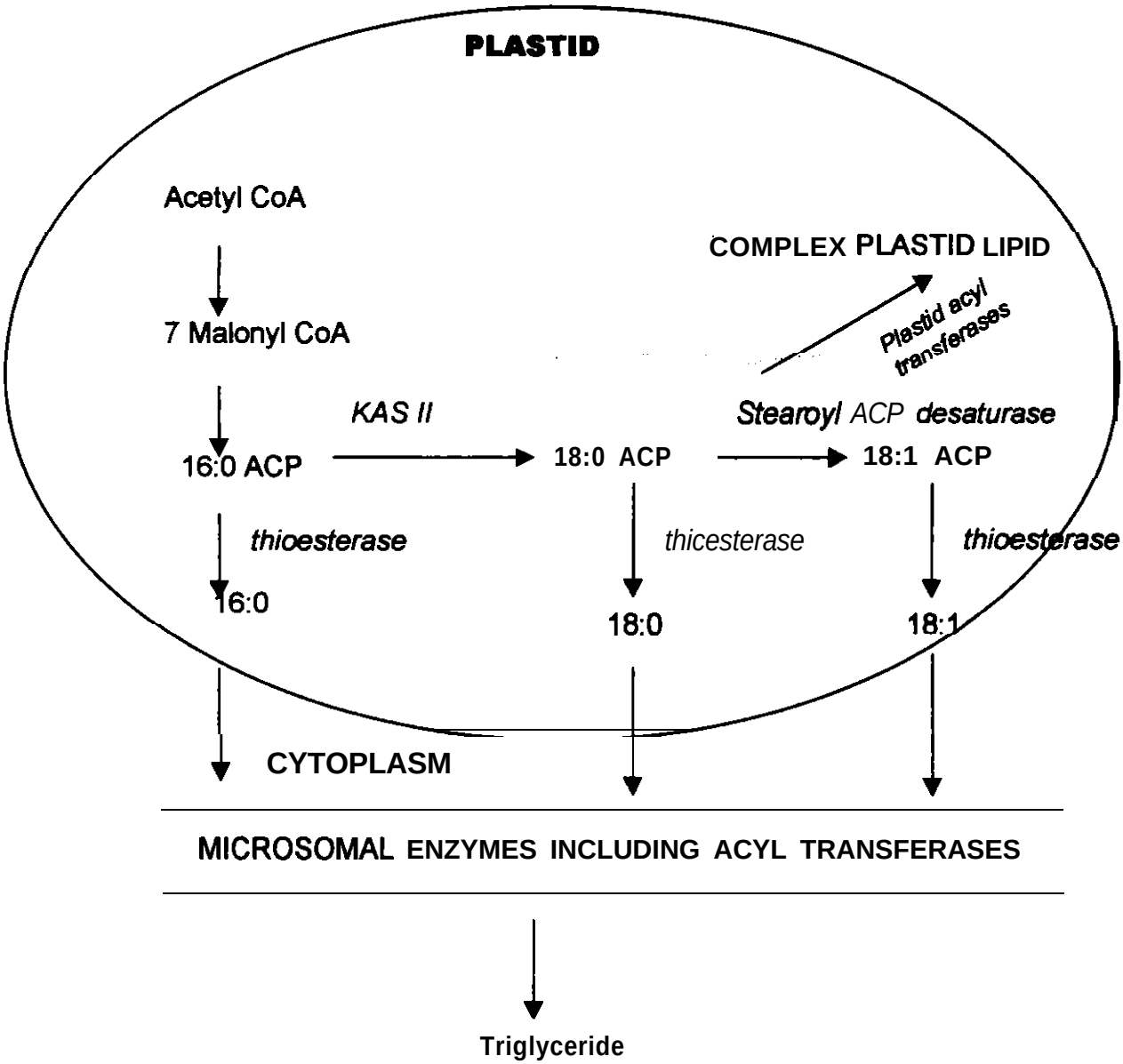

Figure 1. Fatty acid biosynthesis.

then elongated to stearic acid (C18:0) by a condensing enzyme known as β-ketoacyl ACP synthase II (KAS II). Desaturation of stearic acid by A9 stearoyl ACP desaturase results in the formation of oleic acid (C18:1). All these reactions require acyl carrier protein (ACP) as a cofactor and take place in the plastid. Enzymes known as acyl ACP thioesterases cleave the fatty acid from ACP. The released fatty acids can then move out of the plastid into the cytoplasm where other modification reactions occur.

While this mechanism of synthesis is similar for all plant lipids, the fatty acid profiles of different commercial oils are obviously different. What determines the chain length of a fatty acid and what are the mechanisms that determine fatty acid composition of a particular oil?

In the oil palm, the question to be raised is why palmitic acid accumulates in the mesocarp accounting for about 44% of the total fatty acid composition whereas it generally does not accumulate in the storage oils of other plant species. Both the mesocarp and kernel are part of the fruit, yet the kernel produces

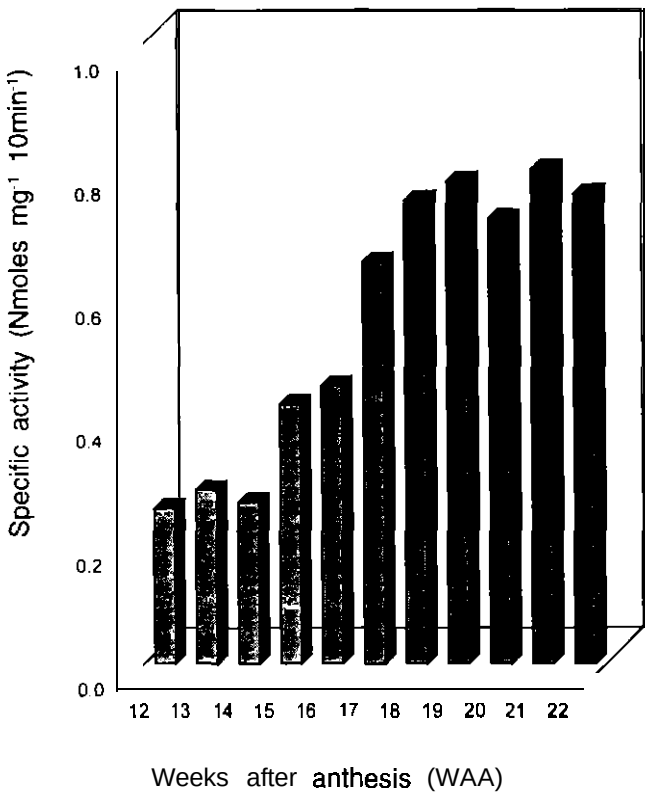

Figure 2. Relationship between KAS II activity and fruit ripening.

mainly lauric and myristic acids. What is the mechanism(s) that differentiates the oil *in* these two tissues?

These questions prompted us to investigate the role of different fatty acid biosynthetic enzymes in determining the fatty acid composition of palm and palm kernel oil. This paper briefly reviews the biochemical factors that control oil composition in the oil palm.

BETA-KETOACYL ACP SYNTHASE II (KAS II)

This is a condensing enzyme that adds two carbon atoms to palmitic acid resulting in the formation of stearic acid. Another condensing enzyme, KAS I catalyzes most of the condensation reactions during succcessive rounds of the chain-lengthening process. KAS II however, is exclusively responsible for the conversion of palmitic acid to stearic acid (Harwood, 1988). Therefore, there is considerable interest in this enzyme with respect to its role in determining the ratio of Cl6 to C18 fatty acids (Kridl et al., 1992).

KAS II Activity during Fruit Development

Oil palm fruits *from 12* weeks after anthesis (WAA) to 22 WAA were screened for KAS II activity. Activity was first observed around 15 WAA and showed a progressive increase until it reached a maximum at 20-21 WAA (Figure *2*) (Umi Salamah, 1995; Umi Salamah and Sambanthamurthi, 1996a). This was similar to the pattern of triglyceride synthesis in oil palm mesocarp which starts at 16 WAA and reaches a maximum around 20 WAA (00 et *al.*, 1985).

Relationship between KAS II Activity and Unsaturation Level in the Oil Palm Mesocarp

Various oil palm fruits from PORIM's germplasm collection were screened for KAS II activity, fatty acid composition and iodine value (I.V.). Iodine value is a measure of the level of unsaturation of the oil. *Table 1* shows the relationship between KAS II activity, fatty acid composition and I.V. *Table 2* shows the multiple regression analysis. Positive correlation *was* observed between KAS II activity and I.V. Positive correlation was also observed between KAS II activity and the level of C18:1 and C18:2 individually. However, the correlation was much stronger when these two fatty acids were considered in combination. The total level of unsaturated Cl8 fatty acids (C18:1 + C18:2 + C18:3) also correlated positively with KAS II activity. However, KAS II activity had little effect on C18:3 individually thus, indicating that the strong positive correlation observed for KAS II vs (C18:1 + C18:2 + C18:3) was actually contributed by C18:1 and C18:2. Interestingly, the level of C16:0 was negatively correlated to KAS II activity and the level of C18:1. These findings provide strong evidence that limiting KAS II activity contributes towards palmitic

TABLE 1. RELATIONSHIP BETWEEN KAS II ACTIVITY IODINE VALUE AND FATTY ACID COMPOSITION

Sample	KAS II activity	Iodine value	Fatty acid composition (%)						
			C16:0	C18:0	C18:1	C18:2	C18:3	C18:1 +	C18:2 + C18:3
E. guineensis	2.15	60.80	30.80	6.60	49.00	11.70	0.50	61.20	
E. guineensis	1.85	57.30	36.50	4.50	50.30	8.00	0.21	58.50	
E. guineensis	1.19	57.80	36.10	7.40	43.90	11.70	0.30	55.90	
E. guineensis	1.01	54.60	38.40	5.60	46.30	7.80	0.96	55.06	
E. guineensis	0.45	50.10	39.80	4.00	47.70	7.60	0.21	55.50	
Surinam hybrids	3.93	67.80	24.10	n.d	n.d	n.d	n.d	n.d	
Surinam hybrids	3.90	64.10	24.00	2.60	65.70	5.90	0.82	71.62	
Surinam hybrids	3.39	63.80	22.20	2.60	69.20	5.40	0.40	75.00	
Surinam hybrids	2.25	62.10	26.90	2.30	63.90	5.40	0.61	69.90	
Surinam hybrids	1.52	58.90	40.50	4.40	38.30	14.60	▪	52.90	
E. oleifera	7.51	88.50	17.70	0.82	56.80	22.70	1.10	80.60	
E. oleifera	***6.90***	85.80	13.40	0.86	68.70	15.20	1.10	85.00	
E. oleifera	5.60	84.30	16.40	0.90	61.80	18.70	0.97	81.50	
E. oleifera	5.30	83.40	15.00	0.87	59.90	21.50	1.30	82.70	
E. oleifera	4.13	84.00	18.90	1.30	60.20	17.30	0.98	78.50	

Key: n.d: not determined

TABLE 2. REGRESSION ANALYSIS OF RELATIONSHIP BETWEEN KAS II ACTIVITY, FATTY ACID COMPOSITION AND IODINE VALUE (IV)

Variable	Correlation coefficient	Regression coefficient	t value	P
KAS II *vs* I.V.	0.94151	0.156525	10.074	<0.001
KAS II us C16:0	0.92004	-0.208826	-8.466	<0.001
KAS II *vs* C18:1	0.66193	-0.151869	3.059	<0.01
KAS 11 us C18:2	0.69005	-0.259508	3.303	<0.01
KAS II *vs* C18:3	0.76302	4.220126	4.089	<0.01
KAS II *vs* (C18:1 + C18:2 + C18:3)	0.92004	-0.174740	8.186	<0.001
KAS II us C18:1 and C18:2	0.95030	0.149917 (for 18:1)	6.960 (for 18:1)	<0.001
		0.256442 (for 18:2)	7.264 (for 18:2)	
KAS II *vs* C18:1, C18:2 and C18:3	0.95039	0.147070 (for 18:1)	4.775 (for 18:1)	<0.001
		0.251861 (for 18:2)	5.031 (for 18:2)	
		0.121757 (for 18:3)	0.136 (for 18:3)	

acid accumulation in the oil palm mesocarp. The results also indicate that A9 desaturase is not limiting in the oil palm mesocarp and efficiently converts A9 stearoyl ACP to oleoyl ACP. Oleate desaturase is also quite active in converting oleic acid to linoleic acid in the oil palm mesocarp 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 increase in oleic acid as well as linoleic acid content. Antisensing of the oleate desaturase gene may thus be necessary to obtain high oleic acid without a concomitant increase in linoleic acid.

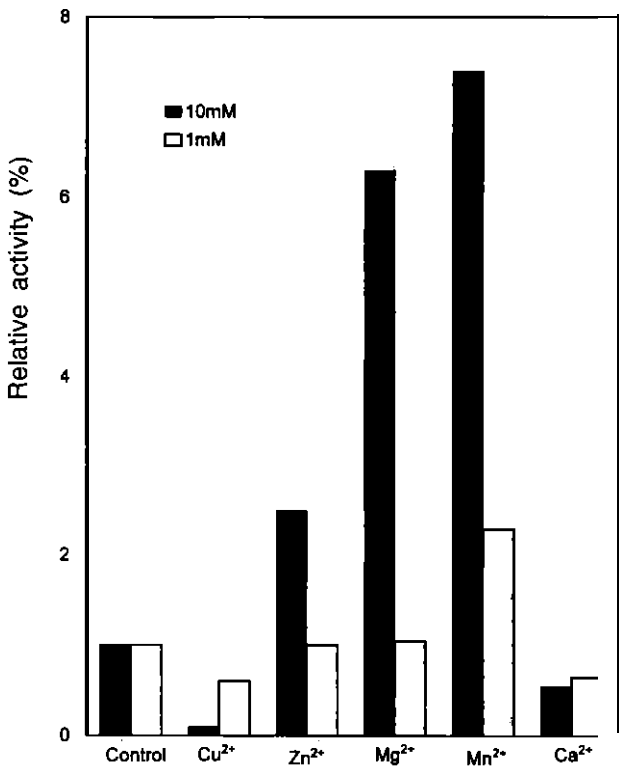

Figure 3. Effect of divalent cations on KAS II activity.

Fig. 4: Effect of EDTA on KAS II Activity

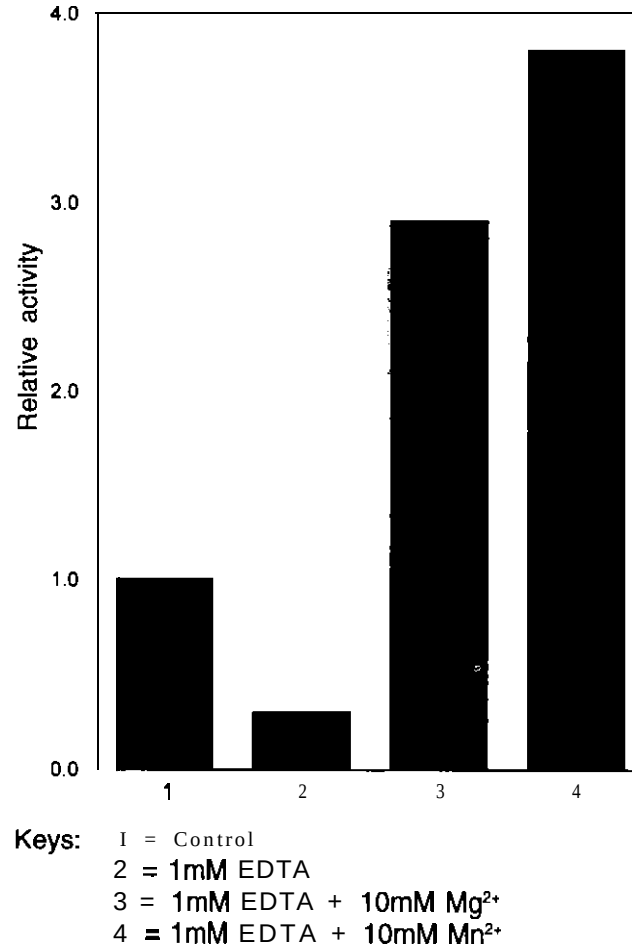

Figure 4. Effect of EDTA on KAS II activity.

Purification and Characterization of KAS II

KAS II was purified about 10 000- fold from oil palm mesocarp (Umi Salamah, 1995). The pure enzyme had optimum activity between pH 4.5 and 5.0. Maximum activity was observed at 30°C with significant reduction in activity at higher temperatures. The influence of divalent cations on KAS II activity was determined in the presence of various cations at 1mM and 10mM concentration respectively. Activity increased 3- to lo-fold in the presence of Zn^{2+}, Mg^{2+} and Mn^{2+} at both concentrations (Figure 3). The highest stimulation was observed with Mn^{2+}. Exogenously supplied EDTA (chelating agent) decreased KAS II activity by approximately 50% (Figure 4). However, prior incuba-

tion of KAS II with divalent cations (Mn^{2+} or Mg^{2+}) prevented/reduced the inhibitory action of this chelating agent. Cu^{2+} and Ca^{2+} at both concentrations reduced KAS II activity significantly.

While the above results were obtained under in vitro conditions, it would be interesting to know how these results translate to field conditions. Would there be a decrease in oleic acid content at temperatures above 30°C since KAS II activity decreases and there is strong positive correlation between KAS II activity and oleic acid content? Similarly, what role do fertilizers such as Mg^{2+} play in affecting fatty acid composition? Would soils rich in magnesium promote higher unsaturation?

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 can be incorporated into triglycerides (storage oil).

Acyl ACP Thioesterases in the Oil Palm

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 marked preference for palmitoyl ACP followed by oleoyl ACP as substrates (Figure 5) (Sambanthamurthi and Oo, 1990; Abrizah et *al.*,1992; Abrizah and Sambanthamurthi, 1995). The results confirmed that palmitic acid was cleaved from palmitoyl ACP in the growing fatty acid chain, thus resulting in palmitic acid moving out of the plastid and being esterified to triglycerides by the action of acyltransferases in the cytoplasm (endoplasmic reticulum).

This mechanism would thus result in accumulation of triglycerides 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 was some thioesterase activity towards stearoyl ACP and very low activity towards

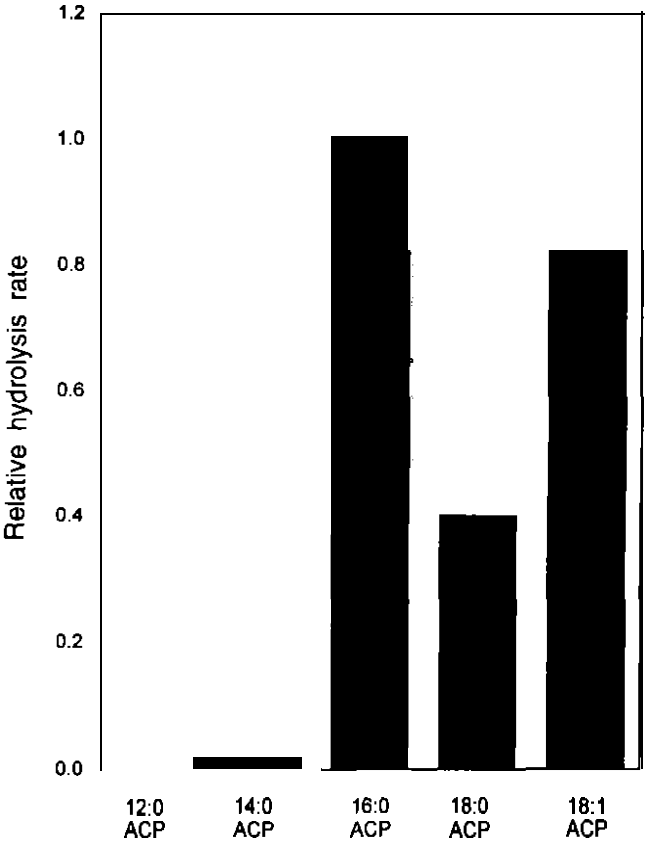

Figure 5. Thioesterase activity in oil palm mesocarp.

lauroyl ACP and myristoyl ACP in crude mesocarp extracts. Stearic, lauric and myristic acids are present in only small amounts in palm oil (*Table* 3). With the advent of genetic manipulation, it is now possible to tailor fatty acid compositions of different oils. Increasing the level of oleic acid at the expense of palmitic acid is one such target. It was thus of interest to us to investigate whether palmitoyl ACP thioesterase and oleoyl ACP thioesterase activities resided on a single protein or separate proteins. If it was a single protein, then manipulation for lower palmitic acid would result in a corresponding decrease in oleic acid.

Similarly, manipulation for increased oleic acid would result in elevated levels of palmitic acid. However, if they were different proteins, then they could be manipulated independently. Protein purification was thus carried out and

TABLE 3: PERCENTAGE FATTY ACID COMPOSITION OF PALM OIL'

Fatty acid	Percentage composition (mean)
12.0	0.2
14.0	1.1
16.0	44.0
16.1	0.1
18.0	4.5
18.1	39.2
18.2	10.1
18.3	0.4
20.0	0.4
Iodine value	53.3

'Extraction from Tan and Oh (1981).

TABLE 4: FATTY ACID COMPOSITION OF PALM KERNEL OIL'

Fatty acid	Percentage composition (mean)
C6	0.3
C8	4.4
C10	3.7
C12	48.3
C14	15.6
C16	7.8
C18	2.0
C18:1	15.1
C18:2	2.1
Other	0.2
Iodine value	17.8

'Extraction from Tan and Oh (1981).

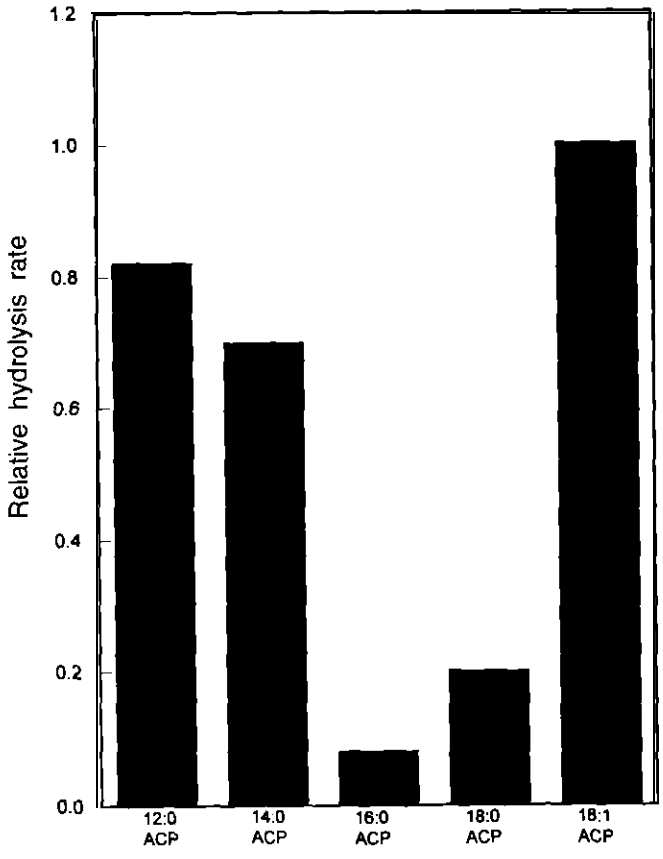

Figure 6. Thioesterase activity in oil palm kernel.

two major peaks were resolved by chromatography (Figure 6). Thioesterase activity towards palmitoyl ACP and oleoyl ACP was thus confirmed to be on two separate peaks (Abrizah, 1995; Abrizah et *al.* 1994), i.e. they are two separate proteins and would be amenable to genetic manipulation independent of each other. Palmitoyl ACP thioesterase clones have been isolated from mesocarp cDNA library. Preliminary investigation was also carried out on oil palm kernel, 12 WAA. *Table 4* shows the fatty acid composition of palm kernel oil. Palm kernel comprises 48% lauric, 16% myristic and 15% oleic acids. High thioesterase activity was observed with lauroyl ACP, myristoyl ACP and oleoyl ACP as substrates *(Figure 7)* (Sambanthamurthi *et* al., 1996).

These results suggest the presence of specific medium chain thioesterase(s) in palm kernel in addition to oleoyl ACP thioesterase. These results are in contrast to those reported by Ohlrogge et al. (1978). Coconut oil contains mainly lauric (32%) and myristic acids (25%) with oleic acid accounting for only 10% of the total fatty acid composition. Yet, it was reported to contain high thioesterase activity with little activity towards lauroyl or myristoyl ACP. Thus, although both palm kernel and coconut are rich in lauric and myristic acids., the mechanism of accumulation of these

fatty acids appears to be different in the two crops. In the oil palm kernel, medium chain thioesterases appear to be responsible for accumulation of lauric and myristic acids.

Stearoyl ACP Desaturase

Oleic acid is formed by the aerobic desaturation of stearic acid by the action of A9 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 A9 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 the palmitoyl ACP is a poor substrate for A9 stearoyl ACP desaturase.

However, under certain conditions, the desaturase changes its preference dramatically. It was shown that protoplasts isolated from both mesocarp and embryogenic cultures of oil palm synthesized palmitoleic acid in excess of 30% of the total fatty acid composition (Sambanthamurthi et al., 1987; 1996). The stearic acid content also increased, showing that the desaturase had changed its preference for stearoyl ACP and was instead acting on palmitoyl ACP. The C18:1 content was about 30%. However, almost all of this was *cis*-vaccenic acid which must have been formed by the 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.

ACYLTRANSFERASES

Various fatty acid biosynthetic enzymes can be manipulated in order to increase or decrease specific fatty acids. However, the final product of interest is the oil. Unless the modified fatty acids are esterified to form triglycerides, they will probably be broken down. Acyltransferases are enzymes that catalyze the esterification of fatty acids. The active fatty acids are in the acyl CoA form. Different acyltransferases catalyze esterification at different positions on the glycerol backbone. The first enzyme is glycerol-3-P acyltransferase (GPAT) which adds an acyl group to the first position on the glycerol-3-P molecule resulting in the formation of 1-monoacylglycerol-3-phosphate (lysophosphatidate). Studies have indicated that this enzyme prefers saturated fatty acyl-CoA (Harwood and Page, 1994).

However, although glycerol 3-phosphate acyl transferase prefers saturated moieties, its specificity is much less than the second acyltransferase, 1-acylglycerol-3-phosphate acyltransferase or lysophosphatidate acyltransferase (LPAT) (Ichihara et al., 1987; Harwood and Page, 1994). This enzyme exhibits strong substrate specificity in most oilseed species. In safflower, this enzyme prefers linoleate to oleate and discriminates almost totally against saturated fatty acids (Griffiths et al., 1985). In rapeseed, erucate was an extremely poor substrate compared to oleate (Sun et al., 1988; Berneth and Frentzen, 1990). Together, the first two acyltransferases determine the non-random distribution of fatty acids at the Sn-1 and Sn-2 positions of the glycerol backbone and hence, the triacylglycerols subsequently formed. Phosphatidate phosphohydrolase hydrolyses phosphate from phosphatidate to yield diglyceride.

The final step in triglyceride synthesis is catalyzed by diacylglycerol acyltransferase (DGAT) which adds the final fatty acid ta position Sn-3. This enzyme has some level of selectivity but in general, it has a broad specificity (Ichihara and Noda, 1982). It is thus obvious that the triglyceride species of a particular oil are determined largely by the action of the three aclytransferases. In. crude palm oil, the C50 and C52 triglyceride species account for about 43% and 41% respectively of the total triglyceride composition (Tan and Oh, 1981). The major components of C50 are PPC and POP while POO and OOP are the major constituents of C52. It is thus obvious that all three positions of the glycerol molecule can

accommodate either palmitic or oleic acid in palm oil. The Sn-2 position which is usually quite specific for unsaturated fatty acids does not appear to have such specificity in palm oil. Oil palm mesocarp LPAT thus does not exhibit high specificity. This information is useful as it indicates that increasing oleic acid (by genetic manipulation) will result in its incorporation into triglycerides.

In palm kernel oil, the major triglycerides are those of carbon numbers 36.38 and 40. C36 comprise mainly lauric acids, while C38 and C40 is a combination of lauric and myristic acids. Here again, it is obvious that the Sn-2 position and hence, kernel LPAT does not exhibit high specificity for unsaturated fatty acids.

CONCLUSION

Several biochemical factors work in concert to regulate the composition of oil in the mesocarp and kernel. In the mesocarp, rate limiting KAS II activity impedes the conversion of palmitoyl ACP to stearoyl ACP thus, resulting in high palmitoyl ACP which is very efficiently converted to palmitic acid by a palmitoyl ACP thioesterase. A more active KAS II would be able to convert more of the palmitoyl ACP to stearoyl ACP which would subsequently be converted to oleoyl ACP and hence, oleic acid. Similarly, a less active palmitoyl ACP thioesterase would allow more of the palmitoyl ACP to be elongated by the albeit inactive KAS II. These are targets of current genetic manipulation efforts.

The oil palm mesocarp contains an active stearoyl ACP desaturase. This accounts for the high oleic acid content of palm oil. It is unlikely that increasing the activity of this enzyme will increase oleic acid levels further in oil palm mesocarp as the stearic acid content of oil palm mesocarp is less than 5%. It may however, play a positive role if KAS II activity is increased. Decreasing the activity of this enzyme by antisense technology for example, could increase stearic acid levels in palm oil. This may be useful in the production of cocoa butter substitute.

In the kernel, medium chain thioesterase(s) appears to play a role in accumulating medium chain fatty acids. The kernel also has an active oleoyl-ACP thioesterase and stearoyl ACP desaturase based on its high oleic acid content (15%).

Biotechnology applied to oils and fats has made rapid progress in creating oil crops with dramatically altered lipid composition (Murphy, 1994). It is imperative that the oil palm industry exploits the opportunities presented by modem biotechnology to maintain a competitive edge. An understanding of the biochemical factors and metabolic pathways regulating oil composition is necessary for exploiting these opportunities and tailoring the oil palm to manufacture value-added products.

REFERENCES

ABRIZAH, 0; OTHMAN, 0; EMBI, M N and SAMBANTHAMURTHI, R (1992). Partial purification and characterization of acyl ACP thioesterase from oil palm (*Elaeis* guineensis) mesocarp. Presented at 18th Malaysian Biochemical Society Conference, 23-24 September 1992.

ABRIZAH, 0; OTHMAN, 0; EMBI, M N and SAMBANTHAMURTHI, R (1994). Purification and characterization of acyl ACP thioesterase from the oil palm mesocarp. Presented at the 11th FAOBMB Symposium on Biopolymers and Bioproducts in Bangkok, 15-18 November, 1994.

ABRIZAH, 0 (1995). Penulenan separa dan pencirian enzim asil ACP tioesterase daripada mesokarpa buah sawit (*E.* guineensis). M.Sc thesis submitted to Universiti Kebangsaan Malaysia.

ABRIZAH, 0 and SAMBANTHAMURTHI, R (1995). Acyl-ACP thioesterase(s) in the oil palm mesocarp (*Elaeis* guineensis). Symposium on Biochemistry and Molecular Biology of Plant Lipids, California, 1-4 June, 1995.

BERNETH, R and FRENTZEN, M (1990). Utilization of erucoyl-CoA by acyl transferases from developing seeds of *Brassica napus* (L) involved in triacylglycerol biosynthesis. Plant *Sci.*, 67: 21-28.

HARWOOD, J L (1988). Fatty acid biosynthesis. *Annu. Rev. Plant Physiol. Mol. Biol., 39:* 101-138.

HARWOOD, J L and PAGE, R A (1994). Biochemistry of oil synthesis. In (ed. Murphy, D J). Designer Oil Crops. VCH Weinheim. p. 165-194.

ICHIHARA, K and NODA, M (1982). Some properties of diacylglycerol acyl transferase in a particulate fraction from maturing safflower seeds. *Phytochemistry, 21:* 1895-1901.

ICHIHARA, K; ASAHI, T and FUJII, S (1987). 1-Acyl-sn-glycerol-3 phosphate acyl transferase in **maturing safflower** seeds and its contribution to the non-random fatty acid distribution of triacylglycerol. Eur. *J. Biochem., 167:* 339-347.

GRIFFITHS, G; STOBART, A K and STYMNE, S (1985). The acylation of sn-glycerol-3-phosphate in the metabolism kof phosphatidate in microsomal preparations from the developing cotyledons of safflower (*Carthamus tinctorius* L.) seed. ***Biochem J., 230: 379-388.***

KRIDL, J C; KNAUF, V C and THOMPSON, G A (1992). In (ed.Verma, D P S). Control of *Plant* Gene Expression. CRC Press, Boca Raton, FL. p. 481-498.

MURPHY, D J (1994). Biotechnology of oil crops. In (ed. Murphy, D J). Designer Oil Crops. VCH Weinheim. p. 165-194.

OHLROGGE, J B; SHINE, W E and STUMPF, P K (1978). Fat metabolism in higher plants. Characterization of plant acyl-ACP and acyl-CoA hydrolases. ***Archives of Biochem. and Biophys., 189:*** 382-391.

00, K C; TEH, S K; KHOR, H T and ONG, A S H (1985). Fatty acid synthesis in the oil palm (*Elaeis* guineensis): incorporation of acetate by tissue slices of the developing fruits. Lipids, *20:* 205-210.

SAMBANTHAMURTHI, R, ABRIZAH, 0 and UMI SALAMAH, R (1996). Towards understanding the biochemical factors that affect oil composition and quality in the oil palm. PIPOC-ISOPB International Conference on Oil and Kernel Production in Oil Palm, 27-28 September 1996, Kuala Lumpur.

SAMBANTHAMURTHI, R; 00, KC and ONG, S H (1987). Lipid metabolism in oil palm (*Elaeis* guineensis and *Elaeis oleifera*) protoplasts. ***Plant*** Science, 51: 97-103

SAMBANTHAMURTHI, Rand 00, KC (1990). Thioesterase activity in the oil palm (***Elaeis guineensis)*** mesocarp. In (eds. Quinn, P J and Harwood, J L). ***Plant Lipid Biochemistry, Structure and*** Utilization. Portland Press Ltd. London. p. 166-168.

SAMBANTHAMURTHI, R, PARMAN, S H and MD NOOR, M R (1996). Oil palm (*Elaeis* guineensis) protoplasts: isolation, culture and microcallus formation. ***Plant Cell, Tissue and Organ Culture, 46:*** 35-41.

SUN, C; CAO, Y Z and HUANG, AH C (1988). Acyl coenzyme-A preference of the glycerol phosphate pathway in the microsomes from the maturing seeds of palm, maize and rapeseed. ***Plant Physiol., 88:*** 56-60.

TAN, B K and OH, F C H (1981). Malaysian palm oil, chemical and physical characteristics. ***PORIM Technology, 3:*** 1-5.

UMI SALAMAH, R (1995). Kajian aktiviti dan penulenan enzim β-ketoasil ACP Sintase II di dalam mesokarpa kelapa sawit. M.Sc thesis submitted to Universiti Kebangsaan Malaysia.

UMI SALAMAH, R and SAMBANTHAMURTHI, R (1996a). Progress towards elucidating the role of β-ketoacyl ACP synthase II in fatty acid biosynthesis of oil palm (*Elaeis guineensis* Jacq.) mesocarp. Presented at the 7th National Biotechnology Meeting in Langkawi, 20-22 November 1995.

UMI SALAMAH, R and SAMBANTHAMURTHI, R (1996b). β-ketoacyl ACP synthase II in the oil palm (*Elaeis* guineensis) mesocarp. In (eds. Williams, J P; Khan, M U and Lem, N W). ***Physiology, Biochemistry and Molecular Biology of Plant Lipids: 570.***