Phytochemistry, Vol. 25, No. 2, pp. 405–407, 1986.
Printed in Great Britain.

0031–9422/86 $3.00 + 0.00

CHANGES IN FATTY ACID COMPOSITION OF THE LIPID CLASSES IN DEVELOPING OIL PALM MESOCARP

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(*Revised received* 20 *June* 1985)

Key Word Index—*Elaeis guineensis*; Palmae; mesocarp oil; lipid biosynthesis; fatty acid composition; lipid classes.

Abstract—During fruit development of oil palm (*Elaeis guineensis*) oil deposition in the mesocarp started *ca* 12–13 weeks after flowering (WAF) and continued until the fruit ripened at 20 WAF. Over the next 1–2 weeks oil continued to be deposited but the fruit became loose and readily detached from the bunch. The lipids extracted at this stage contained over 50% free fatty acids and *ca* 6% polar lipids. The major fatty acids in the storage triacylglycerols were 16:0, 18:1 and 18:2. The fatty acid composition of the neutral lipid classes and polar lipids during oil deposition were similar except that the latter also contained a high proportion of 18:3. Longer chain acids (20:3 and 22:0) were detected in certain lipid classes at 8 and 12 WAF.

INTRODUCTION

In recent years the oil palm (*Elaeis guineensis*) has become a major source of vegetable oil. The commercial processing and end-uses of palm oil depend on the fatty acid composition and molecular species of its triacylglycerols (TG). Consequently it is of great interest to know how the fatty acids are synthesized and assembled into the various TG species. We recently studied the biosynthesis of fatty acids from radioactive acetate using fresh mesocarp tissue at different stages of development [1]. In 1958 Crombie and Hardman [2] reported the total fatty acid composition of palm mesocarp during fruit development. We felt it was also important to study the fatty acid composition of the lipid classes using modern analytical techniques. The results of the study are reported in the present paper.

RESULTS AND DISCUSSION

Development of the oil palm fruit has been described in an earlier report [1]. As with the earlier experiments, the flowers were not hand-pollinated and estimated fruit age was no more accurate than ± 1 week of the given values. With fruit development the mesocarp filled with oil at the expense of moisture content. The non-lipid dry wt, largely fibrous material, was fairly constant between 8 and 16 weeks after flowering (WAF) and showed only a slight increase at 20 WAF. Oil deposition started by 16 WAF [1] unlike the situation in African palms [2] and it was clear that the oil content continued to increase until the fruit became detached from the bunch.

The chloroform–methanol solvent used also extracted plant pigments and sterol esters which could be separated from the neutral lipid classes on TLC; they were not estimated as they were minor components and metabolically unrelated to the storage lipids. Table 1 shows the proportion and weight of each neutral lipid class and the polar lipids (PL) at each stage of development, measured by H_2SO_4 charring of the separated lipids. The main storage lipid was TG which increased from 0.02% of the mesocarp fr. wt at 12 WAF to 36.75% at 20 WAF. A surprisingly high proportion of free fatty acids (FA) was seen in 16 and 20 WAF samples. These values were similar to those obtained earlier from incubation of radioactive acetate with mesocarp slices. Of the radioactive lipid products formed using 16 and 20 WAF mesocarp tissue slices, 7.0% and 17.9% were FA [1]. FA levels are generally low in intact tissues and it would appear that some lipolytic activity was still present despite the use in this study of *iso*-propanol during lipid extraction. Of the lipids extracted from the overripe fruits, 58.6% were present as FA. This high FA level could have been caused by the lipolytic activity of exogenous microorganisms which have been shown to commonly infect overripe, loose fruits on palm bunches [3–5].

The polar lipids were not separated into their individual components but they are known to contain glycolipids as well as phospholipids [6]. The proportion of polar lipids (PL) in young fruits fell from 57.8% to 5.3% when oil deposition started (16 WAF) (Table 1). In ripe 20 WAF fruits it was only 0.9%. Nevertheless the actual wt of PL per g mesocarp tissue showed a slight increase between 12 and 16 WAF. This can be attributed to a period of cellular proliferation at this growth stage [1]. Between 20 WAF and the overripe stage an increase in both the proportion and absolute amount of PL was observed. Monoacylglycerols (MG) are not involved in TG biosynthesis and their presence in tissue extracts is strictly due to lipolytic activity on TG or phosphoglycerides [7]. Thus where such activity is controlled the MG level is low (Table 1). It is also low if lipolytic activity is excessive and fairly complete as evidenced by very high FA levels (overripe sample, Table 1). 1,2-*sn*-Diacylglycerol (1,2-DG) is the immediate precursor for the biosynthesis of TG. Its

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Table 1. Changes in lipid content of developing oil palm mesocarp

WAF	Moisture content (g/100 g fr. wt)	Lipid content (g/100 g fr. wt)						
		TL	TG	FA	1,3-DG	1,2-DG	MG	PL
8	87.85	0.09	0.01	0.01	0.01	0.01	tr	0.05
			(7.1)	(9.3)	(8.1)	(13.6)	(1.6)	(60.3)
12	88.42	0.14	0.02	0.01	0.01	0.02	tr	0.08
			(13.9)	(4.8)	(8.4)	(13.2)	(1.8)	(57.8)
16	81.46	6.40	5.08	0.51	0.11	0.28	0.08	0.34
			(79.3)	(8.0)	(1.7)	(4.4)	(1.2)	(5.3)
20	37.45	47.77	36.75	6.17	0.39	2.30	1.76	0.41
			(76.9)	(12.9)	(0.8)	(4.8)	(3.7)	(0.9)
Overripe	22.82	66.69	19.32	39.06	2.29	1.66	0.21	4.15
			(29.0)	(58.8)	(3.4)	(2.5)	(0.3)	(6.2)

Figures in parentheses give the % of each lipid class in the sample. WAF = Weeks after flowering; TL = total lipds; FA = fatty acids; MG, DG, TG = mono-, di-, tri-acylglycerols; PL = polar lipids.

Table 2. Changes in fatty acid composition of lipid classes at different stages of development of oil palm mesocarp

WAF	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	4.2	3.1	1.0	27.5	4.4	22.2	24.0	13.6		
12		1.4	1.5	1.0	27.0	4.5	22.7	23.9	18.0		
16		0.1	1.7	0.4	35.2	5.4	42.6	13.9	0.8		
20		0.4	5.5	1.1	40.8	5.0	35.9	11.3	0.0		
Overripe		0.0	0.8	1.5	44.2	5.4	38.7	9.4	0.0		
8	Triacylglycerols	0.0	0.7	1.4	43.2	3.2	18.9	20.6	10.8	1.2	
12		0.1	0.7	1.3	25.8	1.4	24.5	25.7	20.6	0.0	
16		0.4	0.8	0.6	38.9	4.8	42.6	11.9	0.0	0.0	
20		0.0	0.1	1.4	45.4	5.2	36.7	11.1	0.0	0.0	
Overripe		0.0	0.3	1.4	56.3	3.2	33.3	5.6	0.0	0.0	
8	Fatty acids	0.0	1.4	2.3	49.8	2.6	20.3	7.0	8.7	7.8	
12		0.0	3.5	3.0	25.4	0.4	45.0	9.5	1.4	11.9	
16		0.0	0.6	0.0	28.3	0.8	54.9	15.4	0.0	0.0	
20		0.2	0.2	2.5	58.8	6.0	24.7	7.6	0.0	0.0	
Overripe		0.0	0.2	1.5	35.4	0.5	45.2	17.2	0.0	0.0	
8	1,3-Diacylglycerols	0.0	33.3	7.3	52.4	3.5	3.4	0.0			
12		1.0	18.0	8.2	42.4	5.2	25.5	0.0			
16		0.1	2.1	0.6	68.1	5.8	21.9	1.8			
20		1.1	0.9	2.8	51.3	5.9	29.7	8.3			
Overripe		0.0	0.2	1.9	55.8	4.6	31.2	6.2			
8	1,2-Diacylglycerols	0.2	1.4	2.2	74.2	4.1	7.5	0.0	0.0	10.4	
12		0.1	1.3	1.7	62.5	3.6	12.8	13.6	3.0	1.3	
16		0.0	0.2	0.1	35.7	1.9	45.4	16.7	0.0	0.0	
20		0.3	0.0	1.3	41.8	4.6	37.5	14.5	0.0	0.0	
Overripe		0.0	0.4	1.6	55.5	3.4	31.4	7.8	0.0	0.0	
8	Monoacylglycerols	0.2	0.4	3.9	60.0	3.8	14.0	7.6	2.3	3.4	4.3
12		0.1	1.7	1.8	44.3	2.9	14.7	22.9	11.7	0.0	0.0
16		0.0	0.6	0.8	66.3	8.5	18.5	5.3	0.0	0.0	0.0
20		0.7	1.8	1.4	67.0	9.7	14.8	4.7	0.0	0.0	0.0
Overripe		0.1	2.2	2.0	59.1	5.6	23.8	7.2	0.0	0.0	0.0
8	Polar lipids	0.0	0.3	0.5	40.9	2.0	14.0	28.6	13.7	0.0	
12		0.0	0.3	0.6	32.5	2.1	17.2	24.8	22.3	0.0	
16		0.2	1.0	0.3	33.4	1.9	24.5	28.0	10.8	0.0	
20		1.3	0.3	1.2	45.6	4.3	18.9	14.8	13.6	0.0	
Overripe		0.5	2.0	1.4	40.8	0.7	33.4	14.6	5.4	1.1	

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

concentration in mesocarp extracts therefore represented its steady state concentration in the biosynthetic pathway. With the progressive accumulation of TG during fruit development the percentage of 1,2-DG also decreased (Table 1).

Most natural TG possess a stereospecific distribution of fatty acyl residues characteristic of their source. The control of this distribution is unknown. However, analyses of FA composition of lipid classes during development of a variety of oil seeds have been reported [8–11]. In maturing mustard seed such studies showed that gadoleic (20:1) and erucic (22:1) acids were directly esterified to preformed DG and MG [11]. The major fatty acids in the total lipids were 16:0, 18:1 and 18:2 (Table 2). Large amounts of 18:3 were only found in 8 and 12 WAF mesocarp and medium chain FA (10:0, 12:0 and 14:0) were minor components at all stages of development. Longer chain FA (20:3, 22:0) were present in very small amounts in 8 and 12 WAF mesocarp but were only detected in TG, FA, 1,2-DG and MG classes. It is obvious that these acids were not involved in the synthesis and metabolism of storage lipids since synthesis of storage lipids in the mesocarp occurred later than 12 WAF [1]. Similarly linolenic acid (18:3) was present in PL at all stages but only found in other lipid classes at 8 and 12 WAF. The FA composition of TG and 1,2-DG classes from 16 WAF to the overripe stage were similar. While this might suggest that there was no particular specificity in the acylation of 1,2-DG to TG, the possibility of modification or turnover of pre-formed TG was not excluded. Indeed, if acyl exchange reactions achieved rapid equilibrium, their occurrence would be difficult to demonstrate from examining the composition of the products in the steady state. Nevertheless it might be worthwhile to analyse the substrates for acylation reactions, viz. the acylthioesters, acyl-CoAs and acyl-ACPs [12, 13] in mesocarp tissue.

EXPERIMENTAL

Fresh fruit bunches from the Palm Oil Research Institute of Malaysia were harvested at 8, 12, 16 and 20 WAF. Outer fruits from ripe bunches which became detached with a slight manipulation were collected and classified as overripe (21–22 WAF).

From each stage of development, *ca* 20 fruits were removed from the outer region of the spikelets. After surface washing with Chlorox, the mesocarp tissues were removed and pooled. A sample was taken for measurement of dry wt (48 hr at 70°) and oil content. The remainder was immediately used for lipid extraction. It was first stirred with *iso*-PrOH at 60° for 30 min to minimize lipolytic activity and then extracted with $CHCl_3$–MeOH (2:1) and washed according to established procedures [14]. Neutral lipids (*ca* 200 μg) were separated by TLC on silica gel G using hexane–Et_2O–HOAc (40:10:1) and quantitated by charring with conc. H_2SO_4 [15]. Linear responses were obtained by charring standard lipids from each class each ranging between 10–500 μg.

Individual lipid classes were isolated by prep. TLC using the above solvent system. For lipid samples from 8 and 12 WAF mesocarp, a clearer separation between MG and PL was obtained using hexane–Et_2O–HOAc (25:25:1). FA Me esters of the isolated lipid classes were prepared [1] and analysed by FID-GC using a stainless steel column (2 m × 2 mm) packed with 15% EGSS-X on Gas Chrom Q. The analyses were carried out isothermally at 170° and FA Me esters identified against authentic standards and RR_t values. Peak areas were quantitated by electronic integration. The figures given in Table 2 are mean values of four separate GC analyses.

Acknowledgements—This work was supported by a grant from the Palm Oil Research Institute of Malaysia (PORIM). We thank the Director-General of PORIM for permission to publish the results.

REFERENCES

1. Oo, K. C., Teh, S. K., Khor, H. T. and Ong, A. S. H. (1985) *Lipids* **20**, 205.
2. Crombie, W. M. and Hardman, E. E. (1958) *J. Exp. Botany* **9**, 247.
3. Hartley, C. W. S. (1977) in *The Oil Palm*, 2nd edn, pp. 704–705. Longman, London.
4. Wuidart, W. (1973) *Oleagineux* **28**, 551.
5. Tombs, M. P. and Stubbs, J. M. (1982) *J. Sci. Food Agric.* **33**, 892.
6. Goh, S. H., Khor, H. T. and Gee, P. T. (1982) *J. Am. Oil Chem. Soc.* **59**, 296.
7. Gurr, M. I. (1980) in *The Biochemistry of Plants—A Comprehensive Treatise* (Stumpf, P. K. and Conn, E. E., eds) pp. 205–248. Academic Press, London.
8. Wilson, R. F. and Rinne, R. W. (1978) *Plant Physiol.* **61**, 830.
9. Cherif, A., Drira, A. and Marzouk, B. (1979) in *Advances in the Biochemistry and Physiology of Plant Lipids* (Appelqvist, L. A. and Liljenberg, C., eds) pp. 399–402. Elsevier, Amsterdam.
10. Osagie, A. U. and Kates, M. (1984) *Lipids* **19**, 958.
11. Mukherjee, K. D. and Kiewitt, I. (1984) *Phytochemistry* **23**, 349.
12. Sanchez, J. and Mancha, M. (1980) *Phytochemistry* **19**, 817.
13. Soll, J. and Roughan, G. (1982) *FEBS Letters* **146**, 189.
14. Khor, H. T. and Oo, K. C. (1984) *Phytochemistry* **23**, 1579.
15. Marsh, J. B. and Weinstein, D. B. (1966) *J. Lipid Res.* **7**, 574.