Positional Distribution of Fatty Acids in the Triacylglycerols of Developing Oil Palm Fruit

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The pattern of accumulation of triacylglycerols, their fatty acid compositions and the positional distribution of the fatty acids at the *sn*-2- and *sn*-1,3-positions of the triacylglycerol molecules at progressive stages of oil palm fruit development were determined. There was an exponential rate of increase of triacylglycerols and their fatty acids toward the end of fruit development. The fatty acid composition of the triacylglycerols in the early stages of development, prior to active accumulation, was more or less similar, but differed appreciably from the later stages, and the transition of fatty acid composition toward that of normal palm oil occurred at around 16 wk after anthesis (WAA) and stabilized at 20 WAA. All fatty acids increased in terms of absolute quantity. There was an overall consistency in fatty acid positional distribution, irrespective of development stage. More saturated fatty acids were found to be esterified at the *sn*-1,3-positions and more unsaturated fatty acids at the *sn*-2-position of triacylglycerol. Higher rate of incorporation of 16:0 at the 1,3-positions during the active phase of triacylglycerol synthesis was observed, while 18:1 acid exhibited a reverse trend.

KEY WORDS: Fatty acid composition, oil palm fruit, positional distribution, triacylglycerol.

Physical properties (1), oxidative stability (2) and nutritive value (3) of fats and oils are not only functions of their constituent fatty acids, but also are largely influenced by the position of fatty acids in the triacylglycerol molecules. Available studies that correlate positional distribution of fatty acids and properties of fat have shown (i) that the unique properties of cocoa butter, such as its sharp melting point, can be attributed to the symmetrical triacylglycerols, the mono-oleo-disaturates [1,3-*rac*-palmitoyl-stearoyl-2-oleoylglycerol (POS) and 1,3-distearoyl-2-oleoylglycerol (SOS)] (1,4); (ii) that the atherogenicity of certain varieties of peanut oil is due to the predominance of unsaturated fatty acids in the *sn*-2-position of the triacylglycerol molecule (3); and (iii) that oxidative stability of certain fats is related to the positioning of polyunsaturated fatty acids in the *sn*-2-position (2,5). Knowledge of the fatty acid distribution therefore is important. Each fat has a characteristic triacylglycerol structure and composition, which are largely determined by the relative abundance of fatty acids available for biosynthesis in the course of development of the seed or fruit (6–8).

Palm oil is the second most prominent edible oil in the world and has varied applications in the food industry. The fatty acid composition of palm oil has been well established (9) and has been reported in the course of studies related to varieties of oil palm fruit (10), geographic origin (11), fruit development (12), processing, fractionation and refining (13), and food applications (14). However, data on the distribution of fatty acids in palm oil triacylglycerols is limited to a few studies on palm oil from the Congo (14), Sumatra (15) and Malaysia (10). Rossell *et al.* (11) have also reported the fatty acids at the *sn*-2-position of palm oils from different geographic origins. Palm mid-fraction has been investigated as a cocoa butter equivalent, and the positional distribution of fatty acids is comparable to that of cocoa butter (4).

It is known, from studies on the changes in triacylglycerol molecular species in soybean (6,7), corn kernel (16) and crambe seeds (17) during development, that not only the composition of the fatty acids changes but also that of the triacylglycerols. Similar studies on oil palm fruit are confined to only fatty acid composition (12). The present study attempts to correlate the fatty acid profile of the developing oil palm fruit with position in the triacylglycerol molecule.

EXPERIMENTAL PROCEDURES

Collection of samples. Oil palms of the tenera variety (15 years old) were randomly selected from the germ-plasm garden of the Central Plantation Crops Research Institute (Palode, Trivandrum). Female inflorescences were identified. When more than two-thirds of the flowers on the inflorescence became receptive, the inflorescence was tagged. Samples were harvested at four-week intervals. Fruits from an entire bunch were separated from the bunch stalk and spikelets and were mixed well. A random representative sample (500 g) was then removed from the bulk for analysis. Samples at each development stage were collected from three different palms and analyzed in duplicate. Moisture, volatile matter and oil content were estimated according to the International Union of Pure and Applied Chemistry (IUPAC) (18) methods.

Extraction of total lipid. Total lipids were extracted with chloroform/methanol (2:1, vol/vol) from the fresh mesocarp as described by Goh *et al.* for oil palm fruits (19). Solvent was evaporated under vacuum in a rotary evaporator. The total lipids were dissolved in a minimum volume of chloroform.

Separation of triacylglycerol. About 20 mg of total lipid was spotted and separated into the various lipid classes by thin-layer chromatography (TLC) on a 1-mm thick Silica Gel G (Merck, Bombay, India) adsorbent with petroleum ether/diethyl ether/formic acid (60:40:1.6, vol/vol/vol). Bands were detected by brief exposure to iodine vapor. The triacylglycerol band was identified by co-chromatography with standard tripalmitin (Sigma Chemical Co., St. Louis, MO) and eluted from the silica with four 5-mL portions of chloroform. The extraction was found to be complete by monitoring on micro TLC plates.

Quantitation of triacylglycerol. The triacylglycerols separated by TLC were quantitated by conversion of the component fatty acids of the triacylglycerols to methyl esters and analysis of the fatty acid methyl esters by gas–liquid chromatography (GLC) with an internal standard as described by Kates (20). An accurately weighed amount of internal standard, methyl pentadecanoate, 15:0 (Sigma

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Chemical Co.), was added to the triacylglycerol prior to saponification and esterification of the triacylglycerols. The methyl esters were subjected to GLC. The amount of triacylglycerol sample was determined by relating the total area of the fatty acid methyl ester peaks to the area of the internal standard peak. A correction factor calculated from average molecular weight of the fatty acids, determined from the fatty acid composition, was used to convert the total amount of fatty acid to weight of triacylglycerol.

Determination of fatty acid composition. Methyl esters of the fatty acids were prepared by saponification of triacylglycerols with alcoholic potassium hydroxide, followed by esterification with alcoholic sulfuric acid reagent according to IUPAC procedure (18). A Hewlett-Packard 5840 A model gas chromatograph equipped with a flame-ionization detector (FID) (Hewlett-Packard, Palo Alto, CA) was used for GLC analysis. The methyl esters were analyzed on a 2 m × 2 mm i.d., 10% EGSS-X on Chromosorb W 100 metal column. Injector and detector temperatures were 250 and 300°C, respectively. Column temperature was maintained isothermally at 180°C. Carrier gas was nitrogen at a flow rate of 20 mL/min. Methyl esters were identified by reference to authentic standards (Sigma Chemical Co.) and were quantitated by electronic integration (Hewlett-Packard).

Determination of fatty acids at the sn-*2-position.* Approximately 5 mg of triacylglycerols were isolated from the total lipids by TLC as described. Triacylglycerols were subjected to hydrolysis with porcine pancreatic lipase (Sigma Chemical Co.) by the method of Luddy *et al.* (21) as modified by Rossell *et al.* (11) for palm oil triacylglycerol. Triacylglycerol (5 mg) was weighed into a 5-mL screw-cap vial. Tri(hydroxymethyl)methyl amine (TRIS) (1 mL) buffer adjusted to pH 8.0, 0.1 mL 22% calcium chloride solution and 0.25 mL 1% bile salts solution were added. The contents were warmed in a water bath for 1 min at 40°C; then, 5 mg of pancreatin was added. The contents were shaken for 4 min with a vortex cyclo-mixer. At the end of the reaction time, the mixture was immediately transferred to a separatory funnel and extracted three times with 5-mL portions of diethyl ether. The extracts were combined, washed with distilled water, dried over anhydrous sodium sulfate and filtered, and the solvent was evaporated. The monoacylglycerol formed was immediately isolated by preparative TLC on Silica Gel G with petroleum ether/diethyl ether/formic acid (60:40:1.6, vol/vol/vol) as the developing solvent system. The fatty acid composition of the monoacylglycerol thus isolated was determined by GLC of the methyl esters. Fatty acid composition at the combined 1,3-positions of the triacylglycerols was computed according to the method of Coleman and Fulton (22).

RESULTS AND DISCUSSION

Formation of triacylglycerol in the developing oil palm fruit. The biosynthesis of lipids is shown in Table 1. The total fresh mesocarp content showed a gradual increase in lipids from 4 to 20 wk after anthesis (WAA). Lower mesocarp content for 24 WAA fruit could be attributed to variations in the size of the fruits. In the case of total lipids, there was little accumulation up to 16 WAA. This lag phase was followed by a rapid phase, *i.e.*, between 16 and 20 WAA, during which the maximum rate of biosynthesis of lipids occurred. Contribution of triacylglycerols to the total lipid was low until 12 WAA, which could probably be due to the predominance of structural lipids, such as phospholipids and glycolipids, during this period. The actual formation of storage lipids (triacylglycerols) occurred only after 16 WAA, with a rapid rise in the triacylglycerol content, and this was consistent with the rapid formation of total lipid. The results, therefore, indicate that the active lipid biosynthesis in oil palm fruit occurred between 16 and 20 WAA, which was primarily due to formation of the storage lipids, the triacylglycerols. Similar findings have been reported by few authors for developing oil palm fruit (12,23). A short, rapid phase of lipid formation is characteristic of many other oilseeds (6,7,16,17,24–30).

Fatty acid profile of the triacylglycerols. Changes in the fatty acid composition of triacylglycerols from progressive stages of fruit development are presented in Table 2. The major fatty acids were 16:0, 18:1 and 18:2. The relative abundance of these fatty acids exhibited significant changes during fruit development. The 16:0 increased from 20.8 mole % at 4 WAA to 44.5% at 24 WAA. Corresponding values for 18:1 were 16.8 and 39.8%. The 18:2 showed a decrease from 46.5 to 8.6% for the corresponding stages. It is further evident from the data that the fatty acid composition of the triacylglycerols from early stages of fruit development (4 to 12 WAA) was more or less similar, but differed appreciably from the subsequent stages. Similar trends have been reported for other oilseeds (7,16,17,26). Most studies reported so far on fatty acid composition of palm oil are related to total lipids of developing oil palm fruit (31,32). During early stages of fruit development (up to 16 WAA), total lipids are mostly composed of the structural lipids of membranes, which consist of polar lipids with little triacylglycerol (12,23,32), and studies of this nature will not reflect the triacyl-

TABLE 1

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

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

TABLE 2

Distribution of Fatty Acids in the Triacylglycerols, and at the *sn*-2- and Combined 1,3-Positions of the Triacylglycerols of Developing Oil Palm Fruit

Age of fruit in weeks after anthesis	Fatty acid position	Fatty acid (mole %)						
		12:0	14:0	16:0	18:0	18:1	18:2	18:3
4	Total	2.26	1.92	20.80	3.49	16.79	46.51	8.22
	sn-2-	0.40	1.24	11.37	0.31	31.38	50.19	5.09
	1,3-	3.19	2.26	25.52	5.08	9.50	44.67	9.79
8	Total	4.64	5.32	35.62	6.04	28.82	15.14	4.42
	sn-2-	6.49	3.47	24.02	2.96	43.83	17.08	2.15
	1,3-	3.72	6.25	41.42	7.58	21.32	14.17	5.55
12	Total	5.42	1.64	33.54	8.39	21.13	23.62	6.26
	sn-2-	1.55	1.86	23.90	1.81	32.16	36.17	2.55
	1,3-	7.36	1.53	38.36	11.68	15.62	17.34	8.12
16	Total	0.84	1.13	48.75	5.72	34.59	8.49	0.48
	sn-2-	1.74	1.29	19.41	1.76	58.93	16.03	0.84
	1.3-	0.39	1.05	63.42	7.70	22.42	4.72	0.30
20	Total	0.23	1.37	42.03	4.69	43.66	7.49	0.53
	sn-2-	0.41	1.35	16.34	0.87	69.40	10.81	0.81
	1.3-	0.14	1.38	54.88	6.60	30.79	5.83	0.39
24	Total	0.78	2.04	44.53	3.87	39.81	8.62	0.34
	sn-2-	1.20	1.98	22.38	2.59	62.27	9.16	0.42
	1,3-	0.57	2.07	55.61	4.51	28.58	8.35	0.30

glycerol fatty acid profile for this period of fruit development. However, Oo *et al.* (12) reported the fatty acid composition of the triacylglycerols of developing oil palm fruit; the study indicated a similar fatty acid compositional change to that presented here. The differential rates of biosynthesis of fatty acids, particularly after 12 WAA, and the factors responsible for them were not explained by these authors. We showed that the transition of fatty acid composition of the triacylglycerols toward that of mature palm oil occurred at around 16 WAA, stabilizing by 20 WAA. Interestingly, this period was synchronized with the rapid phase of triacylglycerol synthesis in the oil palm fruit. Even though there was a decrease in the relative percent of 18:2, all fatty acids registered an actual increase in terms of absolute quantity (Fig. 1). The decrease in the relative concentration of 18:2 was due to the greater rate of synthesis of 16:0 and 18:1, diluting the concentration of 18:2 and 18:3, which are formed at lower rates. Appelqvist (29), Hitchcock, Nichols (28) and Gurr (30) have also indicated that there is no loss of any fatty acids during development of other oilseeds, but the changes result from a difference in the rate of accumulation for the various fatty acids with the stage of development. The higher turnover rate for 16:0 and 18:1 fatty acids in the oil palm fruit during the latter half of development may be attributed to the activation of enzymes responsible for biosynthesis during this period (28).

Positional distribution of fatty acids. The triacylglycerols isolated from the mesocarp of fruits from 4 to 24 WAA were subjected to pancreatic lipase hydrolysis. Distribution of fatty acids in the triacylglycerol molecule at the *sn*-2-position was determined from the 2-monoacylglycerols isolated and that at the combined 1,3-positions was calculated by the method of Coleman and Fulton (22) (Table 2). The fatty acid profile for the different

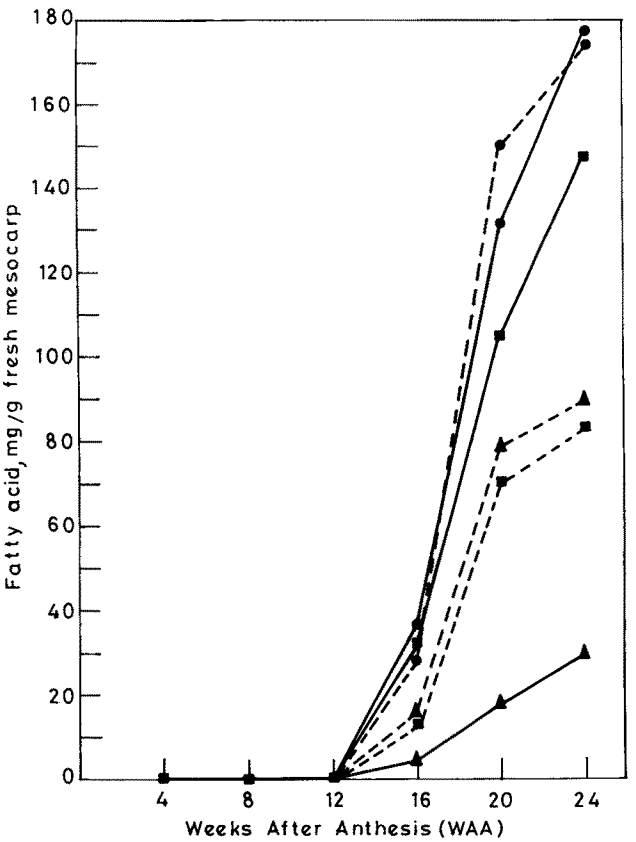

FIG. 1. Changes in 16:0 (—) and 18:1 (— —) fatty acid content of triacylglycerols (●), and at *sn*-2-position (▲) and combined 1,3-positions (■) of the triacylglycerols of developing oil palm fruit.

positions in the triacylglycerol molecules showed a general pattern; *i.e.*, saturated fatty acids preferred 1,3-positions, while unsaturated fatty acids showed an affinity for the *sn*-2-position, irrespective of the stage of development. However, the relative concentration of individual fatty acids in the respective positions was influenced by the abundance of the fatty acid at a given stage. As discussed before, there was a spurt in the total lipid biosynthesis around 16 WAA, with a rapid increase in all fatty acids, particularly 16:0 and 18:1. From the point of high turnover rates, the newly formed 16:0 was found increasingly at the 1,3-positions of the triacylglycerols and less at the *sn*-2-position (Fig. 1). This higher rate of preference for the 1,3-positions by 16:0 was largely compensated for by 18:1 occupying the *sn*-2-position, but not as exclusively as 18:1. The fatty acid composition of the positions also showed a clear distinction between early developmental stages and the latter stages (*i.e.*, 16 to 24 WAA) of triacylglycerol accumulation and was a consequence of and coincidental to the changes in fatty acid profile.

Because of the similarity of palm oil, particularly palm mid-fraction, to cocoa butter (4), there have been attempts to use it as a cocoa butter equivalent, and in this context comparisons have been made with respect to the positional distribution of fatty acids. The overall pattern is that palm mid-fraction with predominantly POP compares well with POS of cocoa butter, with comparable physical properties. Similarly, many other desirable properties of palm oil or its fractions can be attributed to the glyceride structure and, therefore, qualify them for formulations in shortenings, margarines, and confectionery fats (2,4). The distribution of fatty acids in normal palm oil has been carried out by a few authors with respect to geographic origin (1,14,15) and variety (10). The results are comparable to those of the present study for triacylglycerols of 20- and 24-WAA fruits. Positional distribution of fatty acids in the triacylglycerols from other oilseeds also demonstrated preference of the saturated acids for the 1,3-positions and unsaturated fatty acids for the 2-positions (28,30).

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