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Effect of Fruit Ripening on Content and Chemical Composition of Oil from Three Oil Palm Cultivars (*Elaeis guineensis* Jacq.) Grown in Colombia

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ABSTRACT: A series of physical and chemical changes occur as oil palm fruits ripen in the bunch. We evaluated changes in lipid content in the mesocarp and fruits, and the chemical composition of fatty acids (FA), triacylglycerol (TAG), tocols, and carotenes of the lipids extracted from fruits of three commercial *tenera* cultivars, namely, Deli × La Mé, Deli × Ekona, and Deli × Avros, planted in two different geographical regions in Colombia, during the ripening process 12, 14, 16, 18, 20, 22, and 24 weeks after anthesis (WAA). It was found that 12 WAA the mesocarp contained less than 6% of total lipids. Oil content increased rapidly after 16 WAA, reaching the maximum oil content of 55% in fresh mesocarp and 47% in fresh fruits at 22 WAA, which was found the optimal time for harvesting. Changes in FA and TAG showed that total polyunsaturated fatty acids (PUFA) and triunsaturated triacylglycerols (TUTAG) decreased, while total saturated fatty acids (SFA) and disaturated triacylglycerols (DSTAG) increased, over the ripening period. Changes in FA were mainly observed in palmitic, oleic, linoleic, and linolenic acids, and in POP, POO, POL, and OLL for the TAGs evaluated. Levels of tocols changed depending on whether they were tocopherols or tocotrienols. In the earliest stages tocopherols were predominant but decreased rapidly from 6600 mg kg^{-1} of oil at 14 WAA to 93 mg kg^{-1} of oil at 22 WAA. Tocotrienols appeared at the same time as oil synthesis started, and became the main source of total tocols, equivalent to 87% in total lipids extracted.

KEYWORDS: HPLC, lipids, triacylglycerol, fatty acids, tocols, carotenoids, palm oil

INTRODUCTION

Oil palm is a monocot of the genus *Elaeis*. It is one of the most productive oleaginous plants, yielding an average of 4.4 tons per hectare per year.[1] It is the primary source of vegetable oil worldwide, with more than 45 million tons produced in 2009.[1] The genus *Elaeis* comprises two species: *Elaeis guineensis* and *Elaeis oleifera*. *E. guineensis* originated from the tropical rain forest region of West Africa. The cross between *Dura* × *Pisifera* known as *Tenera* is the most planted variety around the world. *E. oleifera* is native to central and northern South America. Its oil contains higher amounts of oleic acid than the *tenera* variety and it has shown resistance to some diseases affecting *E. guineensis*.[2]

Fruit consists of three parts: the exocarp or skin; the mesocarp containing red crude palm oil, used for human consumption and feedstock for many industrial processes; and the endocarp or shell that encloses the endosperm or kernel, from which palm kernel oil is obtained. This oil is used in various forms in the industry. The formation of oil in the fruit (mesocarp) and the knowledge of its composition during fruit development has been a subject of interest for physiological and breeding research in the oil palm industry. Some reports have been written on the process of accumulation of various types of lipids, in the mesocarp of *Elaeis guineensis* Dura, pisifera and tenera[3−8] and recently in other species.[9−14] However, there are few reports of changes in the individual content of FAs and TAGs, which represent between 90 and 95% of the total lipid composition of palm oil,[5,7] and the variation of carotenoids and tocols during oil accumulation in the mesocarp and fruit ripening. Carotenoids and tocols confer special features on crude palm oil because of their biological properties that play an essential role in nutrition and human health.[15] Additionally, its occurrence in plants has become important because of its relation to many cellular interactions necessary for survival.[16] On the other hand, the FA and TAG content in the mesocarp can change considerably during the ripening process, both in quantity and in quality for different climatic conditions such as those in the tropics. Understanding the physical and chemical changes in the formation of lipids and the composition of FA, TAGs, carotenoids, and tocols during their biosynthesis is necessary for setting the optimal harvest time. In addition, it could be the first step to develop strategies leading to production of cultivars with higher oil content in fruits, modified composition of fatty acids, and higher concentrations of tocols in *E. guineensis* or *E. oleifera* species.

The aim of this study was to evaluate the changes in fruit weight, total lipid content, and FA, TAG, carotene, and tocol composition of oil during fruit ripening of three *Elaeis guineensis* cultivars grown in two different regions of Colombia in order to increase the understanding of fruit development, which may be useful for optimal harvest and physiological and breeding purposes.

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MATERIALS AND METHODS

Reagents. Individual triacylglycerols OLL, PLL, MLP, OOL, POL, PLP, OOO, POO, POP, PPP, SOO, and POS (O, Oleic; P, Palmitic; M, Myristic; L, Linoleic; S, Stearic) of purity >98% were from Sigma (Steinheim, Germany). A mixture of fatty acid methyl esters (FAMEs; 37 component FAME mix, 10 mg/mL Cat. 47885-U) was purchased from Supelco (Bellefonte, PA). Methanol, acetonitrile, 2-propanol, and hexane were HPLC grade. Reagent-grade chloroform, potassium hydroxide, and BF_3/MeOH 20% were from Merck (Darmstadt, Germany); the HPLC water grade was obtained using a Millipore Milli-Q Academic purification system (São Paulo, Brazil); the β-carotene (99%), α-, β-, γ-, and δ-tocopherol (>95%) and α-, β-, γ-, and δ-tocotrienol (95%) standards were from Calbiochem (Darmstadt, Germany); the cellulose paper was purchased from Whatman (Maidstone, England), and nitrogen gas was purchased from Linde (Bucaramanga, Colombia).

Plant Material. The research was carried out using 5-year-old *Elaeis guineensis* palms planted at two different locations in Colombia. The palms corresponded to three morphologically and genetically contrasting groups of cultivars. The first two cultivars, Deli × Ekona and Deli × La Mé, produced in ASD (Costa Rica), were planted in the municipality of Puerto Wilches (location 1). The third cultivar, Deli × Avros, produced in Colombia, was planted at the Oil Palm Research Center—Cenipalma (location 2); both locations are in Santander, Colombia. Deli × Ekona is a robust type cultivar, with higher leaf area, longer petioles (over 5 m in the adult stage), and greater vertical growth of the stem than Deli × La Mé. Deli × La Mé is shorter and produces a large number of bunches with low weight. Finally, Deli × Avros is a fast growth cultivar, with abundant emission of leaves, and big bunches. To determine the variation in mean fruit weight and content of lipids, FA, TAG, and tocols during fruit ripening in the bunch, female inflorescences were tagged at the anthesis stage at the beginning of the experiment. The inflorescences were pollinated naturally. At least 42 inflorescences were selected for each cultivar to evaluate 6 bunches in each stage at 12, 14, 16, 18, 20, 22, and 24 WAA. Variation in carotenoid content was determined only in bunches of Deli × Ekona and Deli × La Mé plants planted at the Puerto Wilches location.

Physical Analysis and Total Lipid Content Determination. The analysis of bunches was performed using the methodology described by García and Yañez.[17] Bunches from each stage were harvested, the spikes of the peduncle were removed, and a sample of defruited spikes was taken. These fruits were divided into two groups, those from the outer part of the spikes and those from the inner part of the spikes. In each case mean fruit weight was determined. The mesocarp of each sample of fruits was removed. For moisture determination and total lipid content, each sample of fresh mesocarp was placed in oven overnight at 105 °C. Dry mesocarp was ground; 5 g was placed in closed thimbles for Soxhlet extraction during 24 h using hexane as the extraction solvent. Many samples were analyzed at the same time and in the same Soxhlet system. Total oil content was quantitatively determined gravimetrically. Oil for chromatographic analysis was obtained as follows: a sample of mesocarp of fruits from the inner and outer parts of the spikes was placed in an oven at 70 °C during 6 h. Mesocarp was placed in a thimble for individual Soxhlet extraction using hexane for a period of 8 h. The hexane was removed using a rotoevaporator (Heidolph, Schwabach, Germany) and a stream of nitrogen to preserve the tocols. The oil was recovered for further analysis. However, for the analysis of carotenoids, the oil of all bunches at each ripening stage was mixed to obtain a single sample and perform the respective analysis.

Table 1. Changes in the Mean Fruit Weight[a] during Ripening of Fruits from Deli × La Mé, Deli × Ekona, and Deli × Avros Cultivars

	fruit wt, g		
WAA[b]	Deli × La Mé	Deli × Ekona	Deli × Avros
12	7.8 ± 2.1	5.5 ± 0.6	
14	7.5 ± 0.3	6.8 ± 1.4	16.8 ± 1.7
16	8.1 ± 1.0	6.4 ± 1.2	16.0 ± 1.7
18	9.3 ± 2.2	5.8 ± 1.4	15.6 ± 1.6
20	8.1 ± 1.1	6.9 ± 1.0	16.8 ± 1.8
22	7.6 ± 2.4	7.7 ± 1.7	19.6 ± 2.1
24	10.9 ± 0.6	8.7 ± 1.1	21.4 ± 2.8

[a] Mean ± standard deviation (n = 6). [b] WAA, weeks after anthesis.

Table 3. Changes in Oil Content[a] in Fresh Fruits during Ripening of Fruits from Deli × La Me, Deli × Ekona, and Deli × Avros Cultivars

	g of oil/100 g of fresh fruit		
WAA[b]	Deli × La Mé	Deli × Ekona	Deli × Avros
12	4.7 ± 2.8	3.9 ± 2.3	
14	6.1 ± 3.1	7.9 ± 2.6	1.0 ± 1.7
16	4.6 ± 3.3	3.3 ± 1.1	1.1 ± 1.2
18	15.8 ± 5.8	13.2 ± 2.6	8.4 ± 5.7
20	29.6 ± 5.1	28.1 ± 6.7	22.9 ± 9.2
22	46.6 ± 2.8	41.9 ± 3.0	37.9 ± 7.7
24	40.7 ± 2.5	39.4 ± 4.8	43.2 ± 6.0

[a] Mean ± standard deviation (n = 6). [b] WAA, weeks after anthesis.

Table 2. Changes in Total Lipids (Oil) and Humidity Content[a] in Mesocarp during Ripening of Fruits from Deli × La Mé, Deli × Ekona, and Deli × Avros cultivars

	total lipids (g/100 g of fresh mesocarp)			humidity (g/100 g of fresh mesocarp)		
WAA[b]	Deli × La Mé	Deli × Ekona	Deli × Avros	Deli × La Mé	Deli × Ekona	Deli × Avros
12	6.2 ± 3.6	5.3 ± 2.8		73.9 ± 6.0	77.1 ± 3.6	
14	7.8 ± 4.2	10.9 ± 3.7	1.2 ± 1.9	74.5 ± 4.4	71.1 ± 4.6	80.8 ± 4.2
16	5.9 ± 4.3	4.3 ± 1.7	1.2 ± 1.4	75.4 ± 5.7	77.2 ± 2.0	73.7 ± 1.9
18	20.3 ± 7.3	17.6 ± 3.2	9.8 ± 6.5	61.6 ± 7.1	60.0 ± 4.6	72.9 ± 5.4
20	36.3 ± 5.3	37.0 ± 11.0	26.2 ± 10.3	44.6 ± 4.8	43.7 ± 12.0	57.1 ± 9.3
22	54.7 ± 2.7	49.5 ± 3.5	42.8 ± 8.1	29.7 ± 3.2	33.4 ± 4.3	39.4 ± 8.3
24	49.3 ± 3.0	47.1 ± 5.6	48.4 ± 6.4	28.6 ± 4.4	25.3 ± 2.2	34.3 ± 6.9

[a] Mean ± standard deviation (n = 6). [b] WAA, weeks after anthesis.

Table 4. Changes in Main Fatty Acid Composition[a] during Ripening of Fruits from Deli × Avros Cultivar

WAA[b]	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3
14	0.6 ± 0.2	29.9 ± 4.0	4.5 ± 0.1	3.3 ± 0.7	19.1 ± 2.7	33.3 ± 3.4	10.2 ± 2.6
16	0.4 ± 0.1	31.6 ± 3.1	2.8 ± 0.1	3.2 ± 0.6	29.9 ± 5.5	28.1 ± 5.1	4.6 ± 2.4
18	0.4 ± 0.1	38.3 ± 1.2	1.5 ± 0.0	4.4 ± 1.1	37.4 ± 2.0	18.2 ± 2.5	0.8 ± 0.2
20	0.6 ± 0.1	40.7 ± 1.9	1.2 ± 0.1	4.7 ± 1.0	39.4 ± 2.2	13.7 ± 1.2	0.4 ± 0.0
22	0.9 ± 0.1	44.2 ± 3.9	1.3 ± 0.0	5.0 ± 1.1	37.3 ± 4.0	11.6 ± 0.8	0.3 ± 0.1
24	1.1 ± 0.1	44.9 ± 2.0	1.3 ± 0.0	4.8 ± 0.9	36.8 ± 2.7	11.3 ± 1.2	0.3 ± 0.0

[a] Mean ± standard deviation (n = 6). [b] WAA, weeks after anthesis.

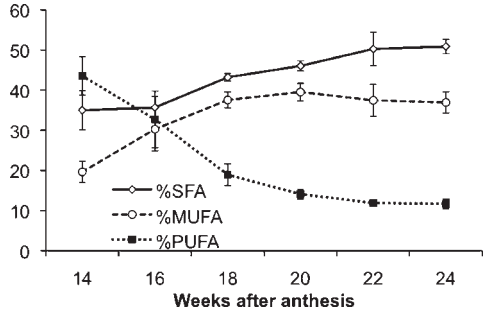

Figure 1. Changes in SFA, MUFA, and PUFA during ripening of fruits from Deli × Avros cultivar. (Abbreviations:SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids). The bars represent the standard deviation of the mean (n = 6).

Chromatographic Analysis of Fatty Acids, Triacylglycerol, Tocols, and Carotenoids. *Fatty Acid Analysis.* FAMEs were prepared for FA analysis as described in AOCS Official Method Ce 2-66 and Ce 1-62.[18] ~100 mg of oil was saponified with 2 mL of KOH/MeOH 2 N (70 °C, 10 min, stirring), and free fatty acids were derived to FAMEs using 3 mL of BF_3/MeOH (70 °C, 5 min, stirring). FAMEs were extracted with 2 mL of n-hexane. One microliter of the FAMEs was analyzed with a 7890A gas chromatograph (GC) (Agilent Technologies, Wilmington, DE) equipped with a flame ionization detector (FID) and a 7683B automatic liquid injector. A fused silica DB23 capillary column (60 m × 0.25 mm i.d., 0.25 μm film thickness; J&W Scientific, Folsom, CA) was used. The oven temperature was programmed as follows: 50 °C for 1 min, increased to 175 at 25 °C min^{-1}, then increased to 230 at 4 °C min^{-1}, and kept at 230 °C for 7 min. The injection and detector temperatures were 250 and 280 °C, respectively. The carrier gas was hydrogen at 33 cm s^{-1}. The split ratio was 1:50. FAME identification was based on retention times as compared with those of the standard FAME mixture. Results were expressed as percentage of peak area without any corrections.

Triacylglycerol and Tocol Analysis. TAG was analyzed according to Official Method AOCS Ce 5c-93[18] and Chen et al.[19] with some modifications. Tocols (α-, β/γ-, and δ-tocopherols and α-, β/γ-, and δ-tocotrienols) were analyzed as described in Rocha et al.[20] using a Merck-Hitachi liquid chromatograph with line UV and fluorescence detection. Lipids were separated on a Merck Chromolith RP-18e column (100 mm × 4.6 mm). For determination of TAG, 25 mg of oil was dissolved in 1 mL of solvent A (acetronitrile:2-propanol:hexane, 55:45:5, v/v). An aliquot (20 μL) was injected into the chromatograph and eluted with 96% solvent A and 4% water at 0.8 mL min^{-1} for 60 min. TAGs were detected at 220 nm. Peaks were identified by comparison with standards, and quantification was by peak area normalization.

For determination of tocols, 25 mg of oil was dissolved in 1 mL of chloroform. An aliquot (20 μL) was injected into the chromatograph. The separation of tocol isomers was performed in gradient mode. Two solvents were used, water (A) and methanol (B). The gradient run conditions were programmed as follows: 80−100% B in 7.5 min, an isocratic step with 100% B for 7.5 min, and return to initial conditions over 2 min. Total run time was 17 min. Flow rate was 2.5 mL min^{-1}. Tocol isomers were detected at $\lambda_{excitation}$ = 290 nm and $\lambda_{emission}$ = 330 nm. Peaks were identified by comparison with standards. Compounds were quantified based on peak areas compared with external standards.

α-and β-Carotene Analysis. These were analyzed according to the methodologies described by Rodriguez-Amaya.[21] The determination and quantification of analytes was performed on lipids extracted from the mesocarp using a liquid chromatograph (described before). For HPLC determination of carotenoids, the same column, solvents, and gradient were used as in tocol determination. The UV−vis detector was set at 450 nm for the α- and β-carotene determination. The quantification of the compounds was done by measuring the areas and using response factors of the calibration curves of pure compounds.

RESULTS AND DISCUSSION

The average weight of fruits, during the ripening process 12, 14, 16, 18, 20, 22, and 24 WAA for 5-year-old palms, ranged between 7.5 and 10.9 g for Deli × La Mé cultivar, 5.5−8.7 g for Deli × Ekona cultivar, and 16.0−21.4 for Deli × Avros cultivar (Table 1). Changes in average fruit weight during the ripening process showed no definite trends in any of the cultivars evaluated. Consequently, it was observed that, from the earliest week of evaluation up to the optimal harvest time, these cultivars reached the highest accumulation of biomass in the fruit, and during this time, there were only biochemical transformations inside the mesocarp.

Changes in the total lipid (oil) accumulation and moisture in the mesocarp of fruits from 12 to 24 WAA are shown in Table 2. Unlike the average fruit weight that remains almost constant in all stages (Table 1), clear changes occur in the mesocarp that were reflected in the synthesis of lipids. Lipid synthesis started 16 WAA and rapidly increased within the next 6 weeks. The highest content of oil was reached 22 or 24 WAA, according to the cultivar. Deli × La Mé and Deli × Ekona cultivars planted in location 1 showed 54.7 and 49.5% oil in fresh mesocarp 22 WAA, respectively, while Deli × Avros planted in location 2 showed 48.4% of oil in fresh mesocarp 24 WAA. On the contrary, moisture in mesocarp decreased at the same time fruits ripened, and ranged from 80.8 to 25.3% for all cultivars evaluated. This feature was used to calculate the oil content in wet mesocarp using a simple equation.[17] For cultivars Deli × Avros and Deli × Ekona, moisture in mesocarp continued decreasing after reaching maximum oil content at 22 WAA; this occurred due to fruit dehydration. Despite the differences in weeks to reach the highest value of oil in mesocarp according to type of cultivar or location, it was concluded that the optimal harvest time was 22 WAA for all

Table 5. Changes in the Main Triacylglycerol during Ripening of Fruits from Deli × La Mé, Deli × Ekona, and Deli × Avros Cultivars[a]

WAA	OLL	PLL	MLP	OOL	POL	PLP	OOO	POO	POP	PPP	SOO	POS
						Deli × La Mé						
12	30.3 ± 12.0	25.7 ± 9.8	3.9 ± 0.3	6.9 ± 6.1	5.2 ± 4.1	12.8 ± 7.2	1.5 ± 1.0	5.8 ± 1.8	0.9 ± 0.5	5.3 ± 3.6	1.7 ± 0.9	0
14	5.1 ± 0.5	6.4 ± 7.8	1.3 ± 0.1	6.9 ± 1.5	22.0 ± 1.0	13.6 ± 0.5	5.6 ± 1.1	16.5 ± 3.9	18.0 ± 0.7	2.1 ± 0.0	0.8 ± 0.0	1.2 ± 0.6
16	4.9 ± 2.7	7.1 ± 3.5	1.5 ± 0.1	4.3 ± 0.2	18.4 ± 2.1	16.2 ± 4.9	3.5 ± 0.2	15.5 ± 2.9	21.3 ± 2.3	3.1 ± 0.1	1.2 ± 0.4	2.6 ± 0.5
18	1.0 ± 0.6	5.7 ± 0.1	0.8 ± 0.6	3.6 ± 0.7	16.3 ± 1.3	15.0 ± 0.5	4.2 ± 0.5	21.5 ± 0.5	25.3 ± 1.5	1.6 ± 0.2	0.9 ± 0.2	3.5 ± 0.7
20	0.6 ± 0.3	5.1 ± 0.7	0.7 ± 0.6	3.2 ± 0.3	16.2 ± 0.3	14.7 ± 0.7	3.6 ± 0.1	22.0 ± 0.4	26.4 ± 1.4	0.8 ± 0.2	2.1 ± 0.5	4.4 ± 0.9
22	1.2 ± 0.5	5.7 ± 1.6	1.1 ± 1.0	3.3 ± 0.0	17.4 ± 0.4	15.8 ± 0.2	3.1 ± 0.1	19.5 ± 1.5	24.4 ± 1.6	1.4 ± 0.6	2.2 ± 0.7	3.8 ± 0.3
24	0.9 ± 0.4	5.3 ± 1.0	0.8 ± 0.7	2.9 ± 0.2	16.6 ± 0.4	16.7 ± 0.8	2.6 ± 0.1	19.0 ± 0.1	27.4 ± 1.3	1.0 ± 0.5	1.5 ± 0.4	2.6 ± 0.5
						Deli × Ekona						
12	36.2 ± 13.0	9.1 ± 7.5	7.5 ± 4.8	10.9 ± 7.5	6.5 ± 4.8	10.5 ± 9.5	1.8 ± 1.4	6.3 ± 5.0	4.3 ± 2.3	3.6 ± 1.6	1.0 ± 0.2	0.7 ± 0.3
14	27.8 ± 10.5	6.1 ± 5.4	2.3 ± 1.2	9.0 ± 4.2	14.8 ± 7.5	11.4 ± 1.9	2.4 ± 1.8	9.2 ± 4.8	13.3 ± 4.7	1.0 ± 0.3	1.0 ± 0.2	1.2 ± 0.6
16	4.4 ± 2.8	7.1 ± 2.4	0.8 ± 0.7	3.3 ± 0.5	15.2 ± 3.8	14.9 ± 4.3	3.7 ± 1.1	19.5 ± 5.1	23.5 ± 3.9	2.6 ± 0.1	1.2 ± 0.6	3.6 ± 1.2
18	2.0 ± 0.9	5.7 ± 0.1	0.5 ± 0.2	4.1 ± 0.1	16.2 ± 1.4	12.8 ± 3.1	5.0 ± 0.7	22.8 ± 1.4	23.2 ± 1.4	1.3 ± 0.2	1.6 ± 0.6	4.0 ± 1.4
20	1.2 ± 0.6	5.7 ± 0.1	1.0 ± 0.1	3.7 ± 0.4	16.2 ± 0.4	13.7 ± 0.7	3.8 ± 0.2	21.6 ± 0.2	24.6 ± 1.1	1.1 ± 0.6	2.5 ± 0.1	4.6 ± 0.5
22	1.6 ± 0.1	6.4 ± 0.8	1.6 ± 0.2	4.0 ± 1.0	16.5 ± 1.1	13.3 ± 3.5	3.9 ± 1.3	20.4 ± 2.7	22.6 ± 1.1	1.4 ± 0.6	3.1 ± 0.5	4.7 ± 0.9
24	1.1 ± 0.1	5.2 ± 1.3	0.7 ± 0.7	3.6 ± 0.6	17.1 ± 0.3	15.1 ± 2.0	3.3 ± 0.8	21.1 ± 2.3	26.2 ± 1.3	1.5 ± 1.1	1.8 ± 0.8	2.9 ± 1.4
						Deli × Avros						
18	5.6 ± 2.8	6.6 ± 1.0	0.5 ± 0.5	5.2 ± 2.5	16.7 ± 2.6	14.2 ± 2.9	3.7 ± 0.6	16.8 ± 1.0	19.6 ± 1.8	3.9 ± 3.3	2.1 ± 1.4	3.5 ± 1.5
20	1.6 ± 1.0	6.4 ± 1.6	0	4.5 ± 0.7	17.7 ± 0.8	15.5 ± 3.1	3.8 ± 0.3	18.5 ± 0.3	20.8 ± 2.3	2.6 ± 0.9	2.1 ± 0.9	4.9 ± 2.8
22	1.9 ± 0.4	9.1 ± 1.5	0	3.8 ± 1.3	16.5 ± 0.5	16.7 ± 1.5	2.8 ± 0.6	16.3 ± 1.9	23.8 ± 0.5	3.6 ± 0.8	1.1 ± 0.3	3.5 ± 0.5
24	1.6 ± 1.5	8.1 ± 1.6	0	3.4 ± 1.6	15.6 ± 0.7	16.6 ± 2.8	2.0 ± 0.8	15.3 ± 2.5	24.8 ± 1.5	4.9 ± 1.3	2.1 ± 1.0	4.4 ± 1.9

[a] Abbreviations: M, myristic; P, palmitic; S, stearic; O, oleic; L, linoleic Mean ± standard deviation ($n = 6$).

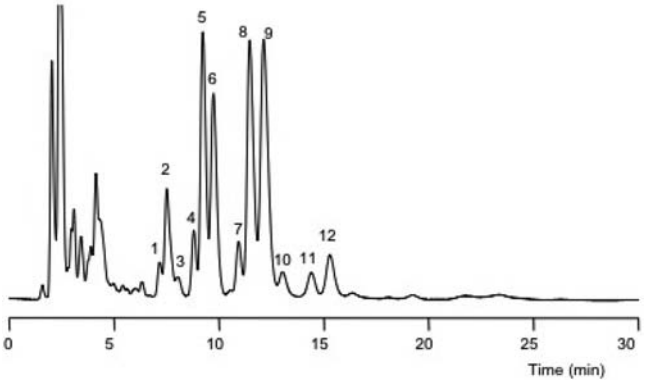

Figure 2. TAG profile of a sample of lipids extracted from mesocarp at 22 WAA: 1, OLL; 2, PLL; 3, MLP; 4, OOL; 5, POL; 6, PLP; 7, OOO; 8, POO; 9, POP; 10, PPP; 11, SOO; 12, POS.

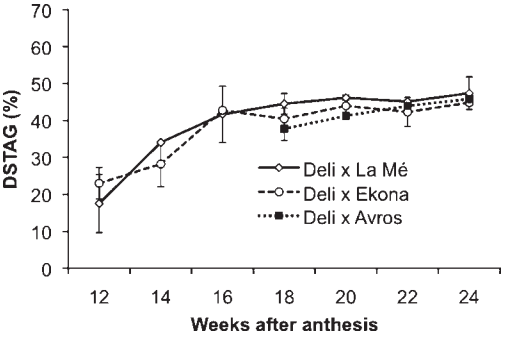

Figure 3. Changes in disaturated triacylglycerols (DSTAG) during ripening of fruits from Deli × La Mé, Deli × Ekona, and Deli × Avros cultivars. The bars represent the standard deviation of the mean ($n = 6$).

cultivars evaluated, because we also observed that, at 24 WAA, many fruits had fallen down from the bunch, and many others showed a high degree of deterioration or over-ripening, which could represent economical losses for the plantation. The results of this study are consistent with previous studies carried out by Ruiz.[22] He reported that the maximum lipid accumulation in fresh fruits of *Elaeis guineensis* occurred after 20 WAA.

In relation to the total lipid content in fresh fruits (Table 3), the same trend was found for fresh mesocarp. In the earliest stages (12 to 16 WAA), there were only 1 to 8% of total lipids present in the fruit; after 16 WAA a rapid increase in lipid accumulation occurred, reaching a maximum of 41.9, 46.6, and 43.2% oil in fresh fruit for Deli × La Mé and Deli × Ekona, and Deli × Avros cultivars, respectively. Similar trends in oil accumulation have been reported for other species.[12−14] Deli × La Mé and Deli × Ekona cultivars showed a slight decrease in the average of lipid content in the fruit at 24 WAA. It was found that this decrease in oil content at 24 WAA was concordant with the over-ripening and low quality appearance of fruits and bunches. These results are consistent with data reported by Bafor and Osagie[3] and Oo et al.,[4] who independently measured the accumulation of different classes of lipids in oil palm mesocarp, including total lipids, during fruit development in *Elaeis guineensis* palms. In both cases, the accumulation of oil peaked at 22 WAA. However, Sambanthamurti et al.[23] found that commercial *tenera* palms from Malaysia reached the maximum accumulation of total lipids 20 WAA, while in Nigerian palms the accumulation occurs

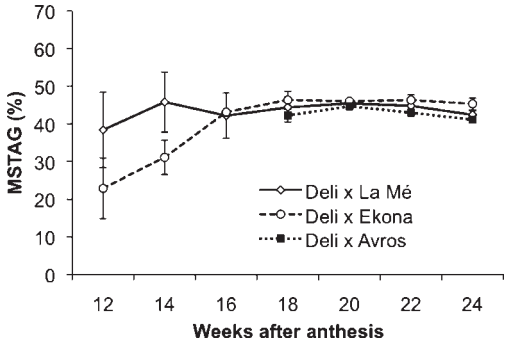

Figure 4. Changes in monosaturated triacylglycerols (MSTAG) during ripening of fruits from Deli × La Mé, Deli × Ekona, and Deli × Avros cultivars. The bars represent the standard deviation of the mean ($n = 6$).

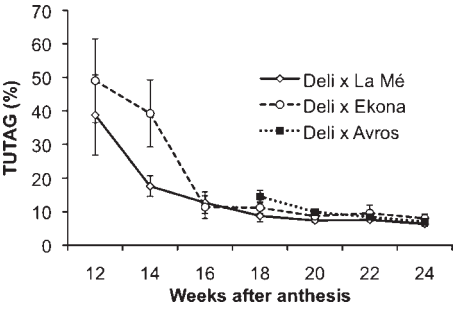

Figure 5. Changes in triunsaturated triacylglycerols (TUTAG) during ripening of fruits from Deli × La Mé, Deli × Ekona, and Deli × Avros cultivars. The bars represent the standard deviation of the mean ($n = 6$).

between 18 and 22 WAA. Fluctuations in oil content shown in this and other reports could be attributed to differences in age, cultivar, and environmental conditions[3] and should be evaluated in oil palm, which could be used to select adapted and higher-yielding cultivars.[24]

There were considerable variations among the fatty acid profiles for the six stages evaluated for the Deli × Avros cultivar (Table 4). A total of seven main FA were evaluated for all stages. FA were grouped according to the number of double bonds present in the structure as saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) (Figure 1). In the earliest stages evaluated (14 WAA), the SFA represented 35.0% of the total FA in which palmitic (C16:0), stearic (C18:0), and myristic (C14:0) contributions were 29.9, 3.3, and 0.6%, respectively. MUFA represented 19.6% of total FA with oleic (C18:1) and palmitoleic (C16:1) contributions of 19.1 and 4.5%, respectively. PUFA represented 43.6% of the total FA in which linoleic (C18:2) and linolenic (C18:3) contributions were 33.3 and 10.2%, respectively. During fruit development, PUFA values decreased, whereas MUFA and SFA increased (Figure 1). At optimal harvesting stage (22 WAA), values of PUFA, MUFA, and SFA were 11.9, 37.5, and 50.3%, respectively. Changes in FA occurred mainly in linoleic and linolenic acids, which decreased from 33.3 to 11.6, and 10.2 to 0.3; and oleic and palmitic acids, which increased from 19.1 to 37.3, and 29.9 to 44.2, respectively. Concentrations of major fatty acids in the optimal harvest time (22 WAA) were 44.2, 37.3, 11.6, and 5.0% for palmitic, oleic, linoleic, and stearic, respectively; minor FAs were palmitoleic, myristic, and linolenic with 1.3, 0.9, and 0.3%, respectively. This data were consistent with previous reports for crude palm oil fatty acid composition: 39−47% for palmitic, 36−44% for oleic, and 9−12% for linoleic.[25] Bafor and Osagie[3] and Oo et al.[4] also reported the individual composition of fatty acids at different stages of fruit development and showed that the main changes occurred in palmitic, oleic, and linoleic acids. Results found by Oo et al.[4] showed that for the early stages of fruit formation (8 WAA) the concentrations of linoleic and oleic acids were of 20.6 and 18.9%, compared to the composition of 11.1 and 36.7% 20 WAA, respectively.

The FA composition could be used to evaluate the nutritional quality of fats and oils; however, this study also includes changes in composition of TAG during the fruit ripening process (Table 5). The main TAG evaluated in all stages were OLL, PLL, MLP, OOL, POL, PLP, OOO, POO, POP, PPP, SOO, and POS. A typical HPLC profile for TAG is shown in Figure 2.

TAGs were grouped according to the type of FA bonded to the glycerol structure as disaturated triacylglycerols (DSTAG), monosaturated triacylglycerols (MSTAG), and triunsaturated triacylglycerols (TUTAG). It was detected that the trends in changes of TAG were similar for all cultivars considered in this study. To analyze this part, Deli × La Mé cultivar was taken as the example. In the earliest stages evaluated (12 WAA), the DSTAG represented 17.6% of the total TAG (Figure 3) in which PLP, MLP, and POP contributions were 12.8, 3.9, and 0.9%, respectively. MSTAG represented 38.4% of total TAG (Figure 4) with PLL, POO, POL, and SOO contributions of 25.7, 5.8, 5.2, and 1.7%, respectively. TUTAG represented 38.7% of the total TAG (Figure 5) with OLL, OOL, and OOO contributions of 30.3, 6.9, and 1.5%, respectively. During all stages evaluated, the most prominent variation in TAG occurred between 12 and 16 WAA; apparently it is a critical stage in the formation of oil in the mesocarp, and after 18 WAA the TAG proportion remained fairly constant (Figures 3−5). During fruit ripening, total DSTAG increased from 17.6 to 47.4%, and MSTAG increased from 38.4 to 44.8, whereas TUTAG decreased from 38.7 to 6.3%. Changes in TAG were observed mainly for POP, POO, POL, and OLL. It was found that POP increased its proportion from 0.9 to 27.4%, POO from 5.8 to 19.0%, and POL from 5.2 to 16.6%, while OLL decreased from 30.3 to 0.9%. Moreover, it was found that the TAGs that increased in proportion were those that contained palmitic and oleic fatty acids, and those that decreased contained mainly linoleic acid. This observation agrees with the results shown previously in the analysis of fatty acids during the ripening process of fruit.

The typical composition of TAG in crude palm oil was found in the fruits at week 22 (Table 5). The relative amount ratio of TAG reported in this study was slightly different from that reported for Malaysia by Chen et al.[19] and Sambanthamurthi et al.[23] While the oil of *tenera* varieties shown in this paper contained mainly 22.6−24.4% of POP, 16.3−20.4% of POO, 13.3−16.7% of PLP, and 16.5−17.4% of POL, Chen et al.[19] reported 29.9, 25.6, 9.0 and 9.9%, respectively. From these data, it is not possible to find a reasonable explanation for this situation, but it is part of the interesting results to find differences by studying the same species under different environmental conditions.[26]

The last of the aspects evaluated in the lipids extracted from the mesocarp of fruits at different weeks of development were the minor components, such as tocols and carotenoids that add nutritional value to crude palm oil, due to their biological properties attributed to these molecules.[15,27] Tocol accumulation in the fruits of Deli × Avros cultivar, during fruit development, was expressed in mg kg^{-1} of total lipids and in mg kg^{-1} of fresh fruits (Figure 6). In the case

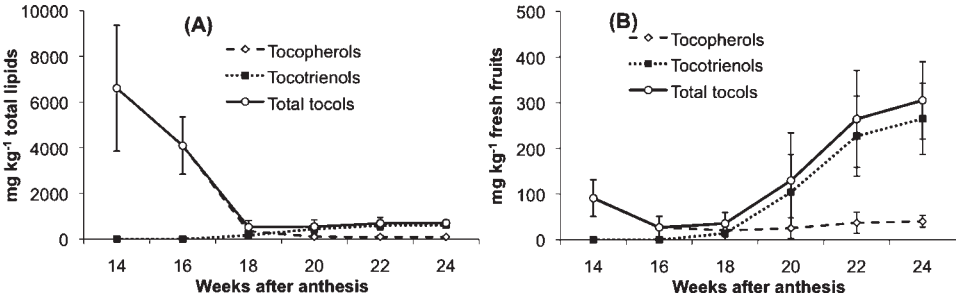

Figure 6. Changes in tocols, tocopherols, and tocotrienols during ripening of fruits from Deli × Avros cultivar expressed as both (A) mg kg^{-1} of total lipids and (B) mg kg^{-1} of fresh fruits. The bars represent the standard deviation of the mean (n = 6).

Table 6. Carotene Content Variation during Lipid Formation in the Fruit Mesocarp in the Cultivars Deli × La Mé and Deli × Ekona between 12 and 24 Weeks after Anthesis (WAA)

	α- and β-carotene	
WAA	mg kg^{-1} of total lipids	mg kg^{-1} of FFB
	Deli × La Mé	
12	7135	252
14	3549	132
16	536	16
18	586	61
20	831	164
22	573	182
24	808	243
	Deli × Ekona	
12	1335	33
14	1775	81
16	424	8
18	756	64
20	514	93
22	905	251
24	1531	412

of tocols, changes of tocopherols and tocotrienols during fruit ripening were evaluated separately. In the first stage of evaluation, 14 WAA, the concentration of total tocols was found to be 6600 mg kg^{-1} of lipids, and then the value rapidly decreased to nearly 500 mg kg^{-1} of lipids at 18 WAA and remained almost constant until 24 WAA (Figure 6A). This apparently stable behavior in the amount of tocols in total lipids means that there was an increase in their concentration at the same time lipids were synthesized. To visualize this effect, Figure 6B shows the change in the amount of tocols synthesized per kg of fresh fruit. From 16 to 22 WAA the amount of total tocols increased from 91 to 265 mg kg^{-1} fresh fruit; this value is equivalent to 700 mg kg^{-1} of total lipids, which is a typical value in crude palm oil.[23,25]

Individual tocopherols and tocotrienols showed a particular behavior. Between 14 and 16 WAA, tocopherols were found in high concentrations (6000 mg kg^{-1} of lipid), representing 100% of the total content of tocols in the fruit. After 18 WAA, at the time lipid synthesis started, a fast decrease in concentration of tocopherols occurred and tocotrienols started to appear and became the main compound of tocols at optimal harvesting stage (Figure 6).

It is known that accumulation of tocopherols occurs mainly in green tissues[16,28,29] and they have been associated with different functions in plants, such as membrane stability, development and growth, and response to different types of stress, not only maintaining the integrity of chloroplasts, but also modulating the expression of genes involved in its synthesis, and the expression of other related genes involved in controlling lipid peroxidation and formation of secondary species such as jasmonic acid.[16] This compound was related to the modulation of plant functions such as fruit ripening, pollen production, root growth, and plant resistance to insects and pathogens.[30] In seeds, the presence of tocols is mainly attributed to antioxidant activity, preventing the peroxidation of fatty acids during seed formation. Their role in the mesocarp of oil palm, as in other species, could be also related to membrane protection and stability in the oil that is synthesized and accumulates in the fruit.[16]

At week 12 after anthesis, the concentration of carotenoids was found to range between 1330 and 7130 mg kg^{-1} of lipids, then fell sharply at week 16 after anthesis to 420−530 mg kg^{-1} of lipids, and remained nearly constant until week 22. This constant behavior in the concentration of carotenoids means that there was an increase in its synthesis concurrent with lipid synthesis. To visualize this effect, Table 6 shows the change in the amount synthesized per kg of fresh fruit bunches (FFB). It was found that the amount of carotenoids synthesized increased from 8−16 mg kg^{-1} at week 16 to 180−250 mg kg^{-1} at week 22, ideal for harvesting, and is equivalent to 570−900 mg kg^{-1} of lipids, a typical value in crude palm oil.

In conclusion, the synthesis of lipids present in the mesocarp of Deli × La Mé, Deli × Ekona, and Deli × Avros cultivars grown in Colombia started 16 WAA and reached maximum values 22 to 24 WAA. Optimal harvest time was established as 22 WAA. At this stage, oil content in the fruits was between 37.9 and 46.6%, major fatty acids were palmitic, oleic, linoleic, and stearic with a contribution of 44.2, 37.3, 11.6, and 5.0%, respectively, and major TAGs were POP, POO, PLP, and POL with a concentration of 22.6−24.4, 16.3−20, 13.3−16.74, and 16.5−17.4%, respectively. Crude palm oil has a good relation of SFA and MUFA + PUFA, and in comparison to other edible oils, it has the advantage of being very useful for deep frying processes. Finally, changes observed in tocotrienols were closely related to lipid synthesis in the fruit; concentration of tocols in lipids decreased rapidly between 14 and 16 WAA, but increased in relation to fruit weight after week 18. In the earliest stages only tocopherols were found in fruits; in optimal harvest time tocotrienols represent 87% of tocols in oil palm fruits.

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