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CHLOROPHYLL AND CAROTENOID CHANGES IN RIPENING PALM FRUIT, *ELAEIS GUINEËNSIS*

J IKEMEFUNA and I ADAMSON

Department of Biochemistry, University of Benin, Benin City, Nigeria

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Abstract—The major pigment changes associated with ripening in two cultivars of the palm fruit were a decrease in the content of chlorophyll and a massive accumulation of carotenes The green pigment did not disappear completely in the ripe fruit However, the relative concentrations of chlorophylls *a* and *b* in the green fruit were reversed in the ripe fruit β-Carotene was the major pigment of the ripe fruit with an unusually high concentration of α-carotene and traces of acyclic carotenes The oils and fats derived from the palm fruit mesocarp are thus excellent sources of provitamin A

INTRODUCTION

The natural habitat of the African palm is in forest outliers It does not grow in primaeval forest but flourishes wherever man has cleared a part of the forest [1] The carotenoids are mainly responsible for the brilliant yellow and red colours of fruits found in the tropical rain forest where the plant predominates The fruit of the oil palm is a drupe with an outer mesocarp or pulp and an inner endocarp or shell Two diffused pigmented areas exist in the individual ripe fruit. The tail which is attached to the bunch is covered by spikelets and is usually light yellow or green while the head which is fully exposed to light is bright yellow or red

To date, no systematic studies have been undertaken on the carotenoids of the palm fruit although it is the most important source of provitamin A in Nigeria At the turn of the century some reports on the occurence of carotenes in the oils of the palm were published [2, 3] and a preliminary observation on ripening of oil palm fruits speculated on the fate of the pigment [4] Recently, commercial plantation gave impetus to genetic manipulations of the palm so that now several forms of the species of different colours ranging from bright yellow to red are available The carotene forms and the processes leading to the accumulation of pigments are unknown

In the present report two cultivars, Dura and Tenera, of the palm fruit have been analysed for chlorophyll and carotenes at different developmental stages

RESULTS

The chlorophyll and carotene contents in the green (1–2 months), matured green (3–4 months) and ripe (5–6 months) fruits are presented in Table 1 The chlorophyll content was highest in the green and decreased as the fruit matured to ripeness A considerable amount of chlorophyll was still present in the ripe, red (Tenera) and yellow (Dura) pigmented fruits Chlorophyll *a* was more abundant than chlorophyll *b* in the early stages of growth by a ratio of about 1 5 1 but this pattern was reversed in the ripe forms where the amount of chlorophyll *b* was twice that of chlorophyll *a*

There was massive accumulation of carotenes during the last stage (5–6 months) of growth, however, small amounts of cyclic carotenes were also detected at the green stages The acyclic carotenes were only found in the ripe fruits β-Carotene was the major type with an unusually high amount of α-carotene (up to 35 % of total carotenes) Generally, more of the coloured carotenes were synthesized by Tenera than Dura but the amounts of the intermediate compounds found in both forms were similar

Total carotenoids and chlorophylls extracted from the two regions found in individual fruits are given in Table 2 Some indication that these two regions may represent progressive stages of carotenogenesis in the same fruit was shown, the total carotenoids and chlorophylls present in the head area were greater than the tail

DISCUSSION

The inverse relationship in the two forms of chlorophyll between the green and the ripe fruit may serve a compensatory function as it becomes necessary to trap energy of suitable wavelength due to the masking of the chlorophylls by carotenes Similar differential rate of disappearance of chlorophylls was shown in some studies of pigment changes in deciduous trees during autumn necrosis [5] In most of the carotenogenic fruits, chlorophyll disappears as the yellow colours of carotenoids are unmasked [6, 7] In the palm fruit a substantial amount of chlorophyll is retained even as carotenes accumulate maximally Pepper and tomato mutants also retain substantial amounts of chlorophyll when fully ripe [8]

Carotenogenesis begins at initiation of the palm fruit but synthesis at this stage is very small compared to the ripe fruits This is contrary to the grape fruit where active carotenogenesis occurs before chlorophyll begins to disappear [9] The smaller quantities of precursor carotenes are usual [5, 10, 11] although they do not conclusively show the sequence of carotenogenesis in the palm fruit They may, however, indicate the extent of synthesis of the final products It could be argued that the precursor carotenes are not found in the green fruits because they

Table 1 Carotenes and chlorophylls of two fruit forms of *E guineensis* at three stages of development

Pigment (mg/100 g)	Tenera Green	Tenera Mature green	Tenera Ripe	Dura Green	Dura Mature green	Dura Ripe
Carotenes						
α-Carotene	0 02	0 06	14 1	0 02	0 5	9 6
	(0 01–0 02)	(0 05–0 08)	(6 3–36 4)	(0 02–0 02)	(0 05–0 1)	(1 0–22 8)
β-Carotene	0 02	0 09	37 2	0 02	0 11	14 7
	(0 02–0 02)	(0 07–0 1)	(14 0–93 0)	(0 02–0 03)	(0 02–0 23)	(2 7–30 0)
γ-Carotene	—	—	0 36	—	—	0 29
	—	—	(0 0–0 38)	—	—	(0 0–0 39)
β-Zea carotene	—	—	0 38	—	—	0 31
	—	—	(0 0–0 48)	—	—	(0 0–0 40)
Lycopene	—	—	0 24	—	—	0 14
	—	—	(0 15–0 34)	—	—	(0–0 23)
Neurosporene	—	—	0 42	—	—	0 40
	—	—	(0 21–0 54)	—	—	(0 23–0 56)
Phytofluene	—	—	0 24	—	—	0 33
	—	—	(0 0–0 27)	—	—	(0 0–0 35)
Chlorophylls						
Chlorophyll *a*	2 89	2.07	0 43	2 65	2 27	0 24
	(1 2–4 8)	(0 3–3 4)	(0.03–0 73)	(1 3–5 4)	(1 6–3 4)	(0 07–0 37)
Chlorophyll *b*	1 86	1 53	0 73	1 9	1 18	0 46
	(0 8–3 3)	(0 3–2 0)	(0 03–1 3)	(1 4–3 5)	(0 5–1 7)	(0 13–0 72)

Figures in parentheses represent the range while the first figures represent the mean of five determinations

Table 2 Regional distribution of pigments in ripe palm fruit

Pigment (mg/100 g)	Tenera Head	Tenera Tail	Dura Head	Dura Tail
Total carotenoids	0 91 ± 0 09	0 62 ± 0 01	0 54 ± 0 08	0 48 ± 0 11
Total chlorophyll	1 45 ± 0 20	0 60 ± 0 02	3 22 ± 0 90	0 50 ± 0 03

Mean ± s e of five samples

are rapidly consumed to form the cyclic carotenes found in such fruits or alternatively new carotenogenic enzyme assemblies have been synthesized in the developing chromoplasts during ripening to account for the massive accumulation of the pigment [G Britton, personal communication] The natural progression of pigmentation of the palm fruit may suggest different rates of carotenogenesis for more of the precursor carotenes are found in the growing tail than the fully ripe head of the fruit

Carotenoid biosynthesis in higher plants is an important part of chloroplast and chromoplast development [12] The oil palm fruit can be used as a model in the study of the development of these organelles The unripe-green fruit contains functional, photosynthetically active chloroplasts, containing chlorophyll, carotenes and xanthophylls On ripening, an increasing chloroplast degeneration occurs leading to loss of chlorophyll However, not all chlorophyll is lost although it is not clear whether the remaining chlorophyll is photosynthetically active As ripening proceeds, chromoplasts are formed, possibly from degenerating chloroplasts rather than *de novo* from proplastids The *de novo* chromoplasts make new carotenoids (new enzyme assemblies may be required) Under these conditions of greater carotene synthesis in chromoplasts, small amounts of intermediates would be detected which are not found in the chloroplasts Furthermore, the progressive carotenogenesis within the ripe fruit will offer excellent possibilities for studying the synthesis and/or transformation of the organelles

Nutritionally, because of the very high concentration of β-carotene in the mesocarp of the fruit, the oils and fats derived from it are the best sources of provitamin A in Nigeria

EXPERIMENTAL

Two cultivars (Tenera and Dura) of *E guineënsis* Jacq were collected from the Oil Palm Research Institute (NIFOR), Benin City, Nigeria The Agronomy department selected the fruits from the following three stages of development immature green (1–2 months), mature green (3–4 months) and ripe (5–6 months) fruits The fruits at different stages of growth were removed from the bunch at random and kept in black plastic bags flushed with N_2 and frozen pending analysis All solvent purification methods and analytical procedures used were based upon those described by Davies [13] and Britton and Goodwin [14]

Extraction of carotenoids Palm fruit mesocarp (20 g) was homogenized in Me_2CO and filtered by suction The debris was re-extracted several times until all pigments were removed An equal vol of Et_2O was added to the extract and later H_2O was added until two layers were formed The aq phase was re-extracted with Et_2O The combined ethereal solns were washed with H_2O to remove traces of Me_2CO For saponification, the Et_2O was removed by rotary evaporation under red pres, the wet residue was dissolved in 5 ml EtOH and KOH (60% w/v) was added to 10% The mixture was allowed to stand under N_2 overnight Et_2O (20 ml) was then added followed by H_2O until two layers were formed The aq phase was re-extracted with Et_2O and the combined ethereal extracts were washed with H_2O until free of alkali The saponified extract was dried over Na_2SO_4 and rotary evaporated to small vol under red pres and finally in a stream of N_2 The column chromatographic elution and separations on TLC of the different carotenes was as described [14]

The pure carotenes obtained were quantitatively estimated by a spectrophotometric method [14] using $E^{1\%}_{cm}$ values tabulated by Davies [13] Chlorophylls were extracted from representative samples (1 g) with Me_2CO and estimated according to the method of Arnon [15] with the specific absorption coefficient given by Mackinney [16]

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