Zones 8 and 9 furnished carotenoids showing absorption maxima at 485 and 465 $m\mu$ and at 455 and 429 $m\mu$ in carbon disulphide respectively.

The carotene zone furnished a mixture of α - and β -carotenes whose dextrorotation in benzene indicated the presence of 28% of the former isomer (see Hunter & Scott, 1944).

The ether-soluble unsaponifiable matter was dissolved in light petroleum and the solution was extracted ten times with 90% methanol. The residue obtained from the united extracts was chromatographed on 'Birlec' alumina and thereafter on calcium oxide. The broad orange-yellow upper zone furnished a carotenoid which showed absorption maxima at 500 and 473–474 $m\mu$ in carbon disulphide.

SUMMARY

1. Examination of the unsaponifiable matter of a 'Malay' palm oil showed the presence of α -, β -, and γ -carotenes, lycopene, the carotenoid intermediate between γ - and β -carotene on the chromatogram, and xanthophylls having absorption spectra similar to those of lutein and neolutein.

2. Examination of a sample of 'Bissao' palm oil showed the presence of lycopene, neolycopene, and γ -carotene in addition to the α - and β -carotenes which are mainly responsible for the deep red colour of this oil.

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Palm Oil Carotenoids

4. THE PROPORTION OF α - TO β -CAROTENE IN UNRIPE AND RIPE PALM OILS AND ATTEMPTED INTERCONVERSION OF α - AND β -CAROTENES

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It was suggested in our first paper (Hunter & Scott, 1941) that the α -carotene present in palm oil may arise from asymmetric isomerization of β -carotene by way of a three-carbon prototropic change of the type analogous to the racemization of mobile azomethine derivatives (Hsu, Ingold & Wilson, 1935). This isomerization is depicted by the partial formulae (I) and (II) in which [H] represents the mobile hydrogen atom:

On this basis, the proportion of α -isomer might be expected to vary with the degree of ripeness of the palm fruit. We have now examined a number of samples of oil obtained from unripe and ripe bunches of fruit collected from the same trees of a West African palm plantation. The amounts of the samples available were necessarily small and permitted only a single determination of the proportion of α - to β -carotene present in them. A qualitative examination was also made of the other carotenoids

present in the petrol-soluble and ether-soluble unsaponifiable matter obtained from the samples.

In the first investigation, an examination was made of three pairs of samples of palm oil obtained from unripe and ripe bunches of fruit, two pairs being from fruits of the *Afia Oku* variety and one pair from the *Lisombe* variety. The results, which are summarized in Table 1, indicate that there is little difference between the proportion of α - to

β -carotene in oil obtained from unripe and ripe fruits from the same palm, although there is quite a wide variation from tree to tree.

It appeared possible that the unripe fruit which had been pressed to obtain these samples might have been too 'ripe' for the purpose in view. A second investigation was therefore carried out in which particularly immature fruit, furnishing less than 10% of oil, was used to provide the samples of unripe oil. The results are summarized in Table 2.

Table 1. *Proportion of α - to β -carotene in oil from ripe and unripe fruits*

Sample	Carotene ($\alpha + \beta$) (%)	α -Carotene (% of total)	Lycopene	γ -Carotene	Lutein (or xanthophyll with similar absorption)	Oil content of fruit (%)	Oil content of pericarp (%)	Free fatty acid (%)
Unripe <i>Afia Oku</i> (a)	0.0508	35	—	Present	Present	28.5	37.3	1.3
Ripe <i>Afia Oku</i> (a)	0.058	35	Present	Present	Present	34.3	43.2	1.6
Unripe <i>Afia Oku</i> (b)	0.0685	25	Not detected	Not detected	—	30.8	37.9	0.75
Over-ripe <i>Afia Oku</i> (b)	0.0785	27	Not detected	Not detected	Present	45.9	55.8	7.4
Unripe <i>Lisombe</i>	0.0495	43	Present	Present	Present	23.5	27.5	0.65
Ripe <i>Lisombe</i>	0.0825	39	Present	Present	Present	41.6	48.5	0.88

Table 2. *Proportion of α - to β -carotene in oil from very immature and ripe fruits*

Sample	Carotene ($\alpha + \beta$) (%)	α -Carotene (% of total)	Lycopene	γ -Carotene	Lutein (or xanthophyll with similar absorption)	Oil content of fruit (%)	Oil content of pericarp (%)	Free fatty acid (%)
Unripe <i>Afia Oku</i>	0.0473	43	Not detected	Not detected	Present	7.3	15.8	2.0
Ripe <i>Afia Oku</i>	0.0688	47	Not detected	Present	Present	17.8	31.8	0.7
Unripe <i>Lisombe</i>	0.0165	42	Not detected	Not detected	Present	7.7	22.6	—
Ripe <i>Lisombe</i>	0.0627	40	Not detected	Not detected	Present	24.1	63.9	2.66

As in the case of the first investigation, the proportion of α - to β -carotene in the oils obtained from ripe fruit did not differ substantially from that in the corresponding oil from unripe fruit.

The prototropic triad system involved in the hypothetical isomerization of β -carotene to α -carotene is of the true three-carbon type such as is present in the methoxydiphenylpropenes (III) and (IV) which were first thought to be static (Ingold & Piggott, 1922) and later found to undergo interconversion under the catalytic influence of ethoxyl ions (Ingold & Shoppee, 1929):

An attempt to convert β -carotene into α -carotene by treatment with sodium isopropoxide in isopropanol, which is a stronger catalyst than sodium ethoxide for prototropic change (Kon & Linstead, 1929), proved unsuccessful. A similar attempt to convert α -carotene into the β -isomer, in which the entry of the double bond into the conjugated system might be expected to lower the activation energy, gave a similarly negative result.

EXPERIMENTAL

First investigation. The oil content is regarded as a criterion of the degree of ripeness of palm fruit, and the figures for this (which were determined for us in West Africa) are shown in Table 1 along with our own determinations of the free fatty acid content of the samples.

The method of examination used may be illustrated by the case of the first sample of unripe *Afia Oku* (a).

The oil (350 g.) was saponified with KOH (105 g.) in water (175 ml.), ethanol (550 ml.), and light petroleum

(350 ml.), the temperature being kept below 50°. The mixture was heated to boiling, allowed to cool, kept overnight at laboratory temperature and thereafter heated under reflux for an hour. The solution was cooled, diluted with ethanol (2 l.), then with water to produce a final dilution of 5 l. and twice extracted with light petroleum. The united extracts were washed, dried with MgSO_4 , and evaporated in an all-glass apparatus with the usual precautions. The residual soap solution was diluted with water to 7 l. and extracted with ether.

The petrol-soluble unsaponifiable matter was chromatographed on 'Birlec' alumina under slight CO_2 pressure, a 20% solution of benzene in light petroleum being used for development of the chromatogram. This formed a series of

seven zones: (1) olive green, (2) narrow brown, (3) narrow dark red, (4) red, (5) yellow, (6) broad orange red typical carotene, (7) pale yellow, in order of decreasing adsorption. The mixture of α - and β -carotenes obtained from zone (6) was dissolved in benzene and the solution was made up to 50 ml. and used for the optical measurements.

1 ml. of the solution was diluted with chloroform to 50 ml. and this was used for spectroscopic measurements. The most intense maximum in the carotene absorption spectrum in chloroform occurs near $460\text{m}\mu$ (458 for α -carotene and $463\text{m}\mu$ for β -carotene) and this point was therefore used for the intensity measurements. The mean of ten observations gave a $\log I_0/\log I$ value of 1.661, corresponding to a concentration of 0.356% of carotene in the original solution.

1 ml. of the original benzene solution was then diluted with benzene to 20 ml. and this was used for the polarimetric estimation of α -carotene. The concentration must lie between 0.016 g./100 ml. and 0.022 g./100 ml., so that the observed rotation shall be as high as possible, and at the same time the light transmitted by the solution shall be sufficient for making the determination. This gave a value of

$[\alpha]_D = +225^\circ$, corresponding to the presence of 35% of α -isomer on the basis of α -carotene having $[\alpha]_D = +640^\circ$ (Hunter & Scott, 1941).

The upper zones of the chromatogram were combined and eluted, and the residue obtained by evaporation was dissolved in light petroleum and rechromatographed on magnesium sulphate (100–120 mesh, previously heated at 250° for $\frac{1}{2}$ hr.) when a chromatogram similar to the original, but more dispersed, was obtained.

Zones 1 and 2 gave substances having indefinite spectroscopic characteristics. The material eluted from zone 3 showed an inflexion at $505m\mu$ in carbon disulphide and an absorption band at $590m\mu$ in the antimony trichloride reaction. The ultra-violet absorption spectrum in cyclohexane showed a band at $295m\mu$, presumably attributable to tocopherol (cf. Emerson, Emerson, Ali Mohammad & Evans, 1937). The material eluted from zone 4 showed absorption maxima at 502 and $474m\mu$ in carbon disulphide. The ultra-violet absorption spectrum of this also showed a band at $295m\mu$ in cyclohexane. Zone 5 contained γ -carotene or neo- γ -carotene (maxima at 523 and $489m\mu$ in carbon disulphide). The material washed out from zone 7 showed general absorption in the ultra-violet in cyclohexane with slight maxima at 335 and $405m\mu$.

The ether-soluble unsaponifiable matter was chromatographed on magnesium sulphate, when an upper yellow zone and a lower orange-yellow zone were obtained. The former of these furnished lutein, or a mixture of this with neolutein (maxima at 504 and $474m\mu$ in carbon disulphide and a transient band at $620m\mu$ in the antimony trichloride reaction; cf. Hunter, Scott & Edisbury, 1942). The substance obtained from the lower zone showed maxima at 503 and $474m\mu$ in carbon disulphide, and gave an olive green colour changing to blue-green in the antimony trichloride reaction.

The oil from ripe *Afia Oku* corresponding to the foregoing (200 g.) was saponified as in the previous case. The petrol-soluble unsaponifiable matter, on being rechromatographed on magnesium sulphate, furnished a zone containing lycopene (maxima at 545 and $506m\mu$ and an inflexion at $475m\mu$ in carbon disulphide) in addition to γ -carotene (maxima at 527 and $493m\mu$ and an inflexion at $455m\mu$ in carbon disulphide).

Second investigation. The oil contents of the fruit used in preparing the samples and the free fatty acid contents are shown in Table 2. With the exception of unripe *Lisombe* of which only 60 g. were available, 200 g. of oil were saponified in each case.

Attempted interconversion of α - and β -carotenes. (1) Sodium (2.3 g.) was dissolved in isopropanol (50 ml. previously treated with a small amount of sodium and redistilled) to give a 2N solution of sodium isopropoxide which was heated under reflux during the addition of α -carotene (10 mg., m.p. 186°) in benzene (5 ml.). Heating was continued for 5 hr. and the mixture was kept overnight at laboratory temperature. Water (100 ml.) was added together with light petroleum and a small quantity of benzene, and the hydrocarbon layer was washed free from alkali and evaporated under reduced pressure in CO_2 atmosphere. The residue was dissolved in benzene (5 ml.) and the solution diluted with light petroleum and chromatographed on 'Mayfair' (Savory and Moore, Ltd.) alumina. The chromatogram formed an upper orange zone and a lower pale yellow zone, containing unchanged α -carotene (maxima at 508 and $477m\mu$ in carbon disulphide) and neo- α -carotene (maxima at 498 and $468m\mu$; cf. Gillam, El Ridi & Kon, 1937) respectively.

(2) A similar experiment was carried out in which β -carotene (m.p. 181 – 182°) in benzene was added to 2N-sodium isopropoxide in isopropanol and the mixture was heated under reflux for 5 hr. Chromatographic adsorption of the product on 'Mayfair' alumina gave an upper orange-red zone and a lower orange-yellow zone, containing unchanged β -carotene (maxima at 516 and $485m\mu$ in carbon disulphide) and ψ - α -carotene (maxima at 505 and $478m\mu$ in carbon disulphide and a band at $590m\mu$ in the antimony trichloride reaction which faded with development of maxima at 660 and $590m\mu$) respectively.

SUMMARY

1. A number of samples of oil was prepared from specially selected bunches of unripe and ripe palm fruit. While the total carotene content varied considerably, the proportion of α - to β -carotene in oils obtained from unripe and ripe fruit of the same tree remained constant. The proportion of isomers varied, however, from tree to tree.

2. A qualitative investigation was also made with regard to the presence of lycopene, γ -carotene and xanthophylls of the lutein type present in the samples.

3. Attempts to effect the interconversion of α - and β -carotene by heating with sodium isopropoxide in isopropanol proved unsuccessful.

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