Table 3. Comparison of results obtained by the hypochlorite method and our new method

Results expressed in g./100 g. original material

Material	Norman & Jenkins method			
	Cellulose product	Xylan in cellulose product	Lignin in cellulose product	True cellulose
<i>Tephrosia vogelii</i>	25.00	1.28	2.32	21.40
Grass cuttings	34.40	6.62	2.08	25.70
Tea leaf	18.34	2.37	2.38	13.59
Paddy straw	41.76	7.51	3.00	31.25

Material	Bromination method			
	Cellulose product	Xylan in cellulose product	Lignin in cellulose product	True cellulose
<i>Tephrosia vogelii</i>	23.20	0.98	1.80	20.42
Grass cuttings	33.00	6.00	2.40	24.60
Tea leaf	19.14	2.23	3.02	13.89
Paddy straw	40.66	6.95	2.72	30.99

SUMMARY

1. A new method for the determination of cellulose in plant materials, based on the use of bromine

as a halogenating agent for lignin, is described. Bromine is liberated in the nascent state in contact with the material by acidifying a mixed solution of bromide and bromate. Bromination under alkaline conditions can also be effected by adding an equivalent amount of NaOH to the acidified mixture. The reactions involved in the process are similar to those in the hypochlorite methods. Results obtained by the use of either NaOBr or a mixture of bromide and bromate are comparable to those given by the NaOCl method.

2. The method is distinctly advantageous in the tropics, where it is difficult to obtain and store NaOCl. One or two treatments (depending upon the nature and age of material) in excess of those needed in the NaOCl method are required, since bromine is less reactive than chlorine. This lesser reactivity is an advantage in the case of tender green plant tissues, which are liable to damage by over-chlorination.

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Palm Oil Carotenoids

3. EXAMINATION OF THE LIPOID PIGMENTS PRESENT IN 'MALAY' AND 'BISSAO' PALM OILS

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The new type of palm grown on plantations in the Malay Peninsula was especially selected to furnish an oil of much paler tint than the palm oil produced commercially in West Africa. It was therefore of interest to compare the carotenoids present in Malay oil with those which occur in a deep red palm oil such as that obtained from the Portuguese island of Bissao.

Samples of both types of oil were examined on the lines described in our first paper (Hunter & Scott, 1941). Chromatographic analysis of the petrol-soluble unsaponifiable matter of 'Malay' palm oil furnished crystalline specimens of α - and

β -carotene. Evidence was also obtained of the presence of lycopene, γ -carotene, and the carotenoid which is adsorbed at a position intermediate between γ - and β -carotene on the chromatogram (Hunter & Scott, 1941). The ether-soluble unsaponifiable matter furnished somewhat ill-defined xanthophylls having absorption spectra similar to those of the neoluteins (Zechmeister & Tuzson, 1938).

The amount of 'Bissao' palm oil at our disposal was very limited, but an examination of this indicated 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.

Both palm oils therefore show a close similarity to those previously examined with regard to the carotenoids present (Hunter & Scott, 1941; Hunter, Scott & Edisbury, 1942).

EXPERIMENTAL

Malay palm oil

The sample of 'Malay' palm oil used showed an extinction coefficient of 0.78 at 458 $m\mu$ in chloroform.

The oil (1500 g.) was saponified in two batches of 750 g. as described for 'Sherbro' palm oil (Hunter & Scott, 1941). The soap solution was diluted with ethanol (3 l.), made up to 10 l. with water, and thrice extracted with light petroleum, and the united extracts were washed, dried with Na_2SO_4 , and evaporated under reduced pressure in an atmosphere of hydrogen, with the usual precautions. The extracted soap solution was diluted with water, thrice extracted with methylated ether, and the combined extracts washed, dried, and evaporated.

The petrol-soluble unsaponifiable matter (2.77 g.) was dissolved in a 20% solution of benzene in light petroleum, chromatographed on 'Birlec' (Birmingham Electric Furnaces Ltd.) alumina, and the chromatogram developed with 30% benzene in light petroleum. On elution, the carotene zone furnished 0.516 g. of semi-solid mixture of α - and β -isomers which was rechromatographed on 'Mayfair' (Savory and Moore, Ltd.) alumina. The concentrates of α - and β -carotene thus obtained were again rechromatographed.

α -Carotene separated from acetone in glistening red-brown crystals, m.p. 184–185°, which showed absorption maxima at 511, 479 and 449 $m\mu$ in carbon disulphide, at 490, 460 and 431 $m\mu$ in benzene, and at 490, 458 and 429 $m\mu$ in chloroform. This specimen had $E_{1\text{ cm}}^{1\%} = 2250$ at 460 $m\mu$ in chloroform and showed $[\alpha]_D = +620^\circ$ in benzene. β -Carotene had m.p. 182–183° after several recrystallizations, showed absorption maxima at 516 and 486 $m\mu$ in carbon disulphide, and had $E_{1\text{ cm}}^{1\%} = 2200$ at the highest absorption band in chloroform.

The material eluted from the upper portions of the original chromatogram was rechromatographed on 'Birlec' alumina and the chromatogram developed with 30% benzene in light petroleum. The following series of zones was obtained in order of decreasing adsorption: several very narrow dark orange-red zones treated as zone 1, orange (narrow, zone 2), deep orange (zone 3), purple-brown (broad, zone 4), orange (broad, zone 5), and pale orange (very broad, zone 6).

Zones 1 and 2. The substances eluted from zones 1 and 2 showed very vague absorption spectra and were therefore combined and rechromatographed, and the chromatogram developed with 50% benzene in light petroleum and finally with benzene alone. An intermediate orange zone was obtained which on elution, and further chromatography, gave a carotenoid which showed absorption maxima at 506 and 478 $m\mu$ in carbon disulphide and at 486 and 456 $m\mu$ in chloroform.

Zone 3 furnished lycopene which was rechromatographed on 'Birlec' alumina. The material eluted from the main lower orange zone showed absorption maxima at 543, 503, and 474 $m\mu$ in carbon disulphide.

Zone 4 gave an eluate which appeared to be a mixture of lycopene and γ -carotene.

Zone 5 furnished a carotenoid having the spectroscopic characteristics of γ -carotene. After being rechromatographed this showed absorption maxima at 528, 494, and 463 $m\mu$ in carbon disulphide and a band at 594 $m\mu$ in the antimony trichloride reaction.

Zone 6 furnished a carotenoid which showed absorption maxima at 485 and 454 $m\mu$ and an inflexion at 425 $m\mu$ in carbon disulphide. The zone below this (zone 7) contained similar material.

The ether-soluble unsaponifiable matter (2.66 g.) in a 25% solution of benzene in light petroleum was chromatographed on 'Birlec' alumina. The chromatogram developed by 40% benzene in light petroleum formed a series of zones: (1) light orange, (2) cream, (3) light pink, and (4) dark cream, in order of decreasing adsorption.

Zone 1 furnished an orange-coloured slightly gummy material which showed absorption bands at 503 and 473 $m\mu$ in carbon disulphide but which could not be satisfactorily purified by further chromatography. The zone below this had little colour and was therefore neglected.

Zone 3 furnished yellow gummy material which showed a maximum at 479 $m\mu$ with inflexions at 503 and 451 $m\mu$ in carbon disulphide.

Zone 4. The material from zone 4 was dissolved in a 5% solution of benzene in light petroleum and rechromatographed on alumina which had been 'weakened' by exposure to air. The narrow upper orange-coloured zone of the chromatogram and the yellow zone below this gave substances with closely similar absorption spectra (maxima at 503 and 470 $m\mu$ with an inflexion at 447 $m\mu$ and at 502 and 471 $m\mu$ with an inflexion at 446 $m\mu$ in carbon disulphide respectively) which were combined, dissolved in ethanol and the sterols removed with digitonin. The carotenoid thus obtained showed absorption maxima at 503, 472, and 443 $m\mu$ in carbon disulphide and a band at 584 $m\mu$ in the antimony trichloride reaction.

Bissao palm oil

The sample of oil used showed an extinction coefficient of 5.3 at 458 $m\mu$ in chloroform.

The oil (510 g.) was saponified, and the petrol-soluble unsaponifiable matter in a 20% solution of benzene in light petroleum was chromatographed on 'Birlec' alumina. The chromatogram formed the following series of zones, in order of decreasing adsorption: (1) narrow brown, (2) narrow pink, (3) narrow pink, (4) light red, (5) red-brown, (6) orange, (7) red-brown, (8) orange, (9) yellow, and (10) broad orange red (carotene zone).

The material eluted from zone 1 showed a vague band at 485 $m\mu$ and that from zone 2 absorption maxima at 485 and 450 $m\mu$ in carbon disulphide. Zones 3 and 4 contained lycopene which after recrystallization from acetone-methanol had m.p. 158° and showed maxima at 547, 507, and 478 $m\mu$ in carbon disulphide.

Zone 5 furnished a small amount of material showing absorption maxima at 535, 499, and 465 $m\mu$ in carbon disulphide. Zechmeister & Tuzson (1938) record 536, 498, and 466 $m\mu$ for the absorption maxima of neolycopene in carbon disulphide.

Zone 6 also appeared to contain neolycopene.

Zone 7 furnished a carotenoid having the spectroscopic characteristics of γ -carotene (maxima at 530, 496, and 465 $m\mu$ in carbon disulphide after further chromatography).

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.