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Carotenoid Pigments of Badami Mango Fruit

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The only attempt at the isolation of carotene from mango fruit seems to have been made by Yamamoto Osima & Goma (1932) who obtained 60 mg. of a crystalline preparation from 38 kg. of fresh fruit pulp. From a study of its optical activity and absorption spectrum they concluded that it was a mixture of α - and β -carotenes, which were, however, not separated. The inconclusive nature of their data has led us to re-investigate the carotenoid pigments of the mango fruit by the technique of chromatographic adsorption.

Earlier work from this laboratory (Ramasarma & Banerjee, 1938), as also elsewhere (Crawford & Perry, 1933; De, 1937), has shown that certain varieties of the mango fruit are very rich in carotene. The Badami mango fruit (almost the same as the famous Alfonso variety of Bombay) has been chosen for the present investigation, since it was found to be richer in carotene than any other variety so far studied.

A preliminary experiment of a qualitative nature showed that the mango xanthophylls are mostly in the form of esters and that nearly all of the epiphasic pigment is β -carotene (50 mg. per kg. of fresh pulp).

EXPERIMENTAL

Purification of solvents

Aldehyde-free ethanol was prepared by refluxing rectified spirit with 10 g./l. aluminium turnings and 10 g./l. potassium hydroxide, followed by fractional distillation.

Methanol was purified by the same method as the one used for ethanol. In order to obtain a 92% v/v methanol for the phase separation, 90 ml. of purified alcohol were mixed with 10 ml. of water.

As purified light petroleum was not available, ordinary car petrol was distilled and the fraction boiling between 60 and 75° was collected and purified according to the method of Castille & Henri (1924).

Ethyl ether was freed from peroxides by the procedure described by Brandt (1927). Only freshly distilled ether was used for the extraction of pigments.

Carbon disulphide used for spectrophotometry was purified according to Hammick & Howard (1932). The purified solvent was preserved in brown glass bottles and in the dark for periods not exceeding 1 week, after which it was purified again.

Extraction of pigments

Since the work could not be started immediately, the ripe fruit was preserved in the ice-room at 0° for a week, during which it became slightly soft but remained otherwise in good condition. The minced pulp from 50 Badami mangoes (7.7 kg.) was extracted by steeping overnight in 8 l. of absolute ethanol and the extract was pressed out in a tincture press. The residue was extracted similarly with 3 l. of absolute ethanol and the cake was set aside in cold storage for subsequent extraction. The combined alcoholic extract (17 l.) was concentrated under reduced pressure and in an atmosphere of carbon dioxide to about half the volume. After dilution with water, it was repeatedly extracted with freshly distilled ether till no more colour was taken up.

The dehydrated cake was broken up and thoroughly extracted with freshly distilled ether. The extract (4 l.) was concentrated to 500 ml. when most of the ether was distilled off and only alcohol remained. At this stage, small crystals of pigment appeared, and the solution was left in the refrigerator overnight in order to complete the crystallization. The glistening, dark red crystals were filtered off, washed with 25 ml. of absolute ethanol, dried *in vacuo* and preserved in an evacuated tube in the cold for subsequent work. The yield of crude pigment was 250 mg.

The deeply coloured mother liquor and the pigment solution obtained by the alcoholic extraction of the pulp were mixed and saponified by leaving overnight with 200 ml. of 10% alcoholic potash. The ether solution was next freed of alcohol, potash and soaps by washing it with water, and dried over anhydrous sodium sulphate. The ether was evaporated off under reduced pressure and the residue was dissolved in 200 ml. of light petroleum. On concentration to 50 ml. and cooling in the refrigerator, some colourless impurities came down and were removed by filtration. The filtrate was diluted to 300 ml. and extracted with an equal volume of 92% methanol. The epiphasic layer was further extracted four times with 100 ml. lots of 92% methanol in order to remove all the xanthophylls.

Hypophasic fraction

The combined methanolic solution was concentrated to 200 ml. and extracted repeatedly with ethyl ether till the extracts were colourless. The resulting solution was washed free from alcohol, dried over anhydrous sodium sulphate and evaporated to dryness under reduced pressure. The residue was dissolved in 20 ml. of dichloroethane and chromatographed in a column (30 × 3 cm.) of adsorbent consisting of a mixture of equal parts by weight of magnesia (Micron Brand magnesium oxide No. 2641, manufactured by the California Chemical Co.) and heat-treated siliceous earth (Hyflo Supercel, F.A. 501, manufactured by Johns Manville). The chromatogram was developed by washing the column with fresh portions of dichloroethane (about 200 ml. in all) when the following zones in decreasing intensity of adsorption were obtained:

- Zone 1: Narrow, lemon yellow; no absorption maxima.
- Zone 2: Narrow, greenish yellow; a broad and vague maximum at 450 m μ (445–455 m μ).
- Zone 3: Medium, dark red; maxima at 446 and 472 m μ —pigment 'X'.
- Zone 4: Broad, reddish orange; maxima at 468 and 497 m μ —pigment 'Y'.

The adsorbent column was pushed out and the zones were carefully separated and eluted with dichloroethane containing 2% methanol. Since the separation was not quite sharp, the eluates were purified by chromatography in smaller columns. Solutions in carbon disulphide were examined in a visual spectrophotometer.

The pigments of zones 1 and 2 were obviously destruction products. Much of the pigment of zones 3 and 4 was lost when chromatography was repeated, and therefore it could neither be crystallized nor its identity established. The absorption maxima do not correspond to those of any known xanthophylls. The nature of these two pigments will be discussed later.

Epiphasic fraction

The epiphasic solution was concentrated and chromatographed in three columns of 3 cm. diameter and 15 cm. height, using as adsorbent a 1:1 mixture of magnesia and supercel. On developing the chromatogram with light petroleum (b.p. 60–75°) a very narrow band at the top of the column and a very wide and deeply coloured band at the bottom were formed. The top band was separated by scooping out the adsorbent with a spatula. The eluates from the two bands were purified by chromatography in similar columns.

Top band. A solution in carbon disulphide showed ill-defined absorption maxima at 461 and 491 m μ . It was, perhaps, a decomposition product.

Bottom band. On adding absolute ethanol to a concentrated solution and cooling in the refrigerator, glistening dark red crystals were formed. They were separated by filtration and purified by recrystallization; yield 3 mg. The pigment showed absorption maxima at 482 and 512 m μ in carbon disulphide, and 450 and 480 m μ in ethanol and was thereby identified as β -carotene. The crystals melted at a low temperature, 170°, perhaps due to incomplete drying, or the presence of traces of impurities.

Crystalline fraction

The crude crystals obtained directly from the ether extract of the dehydrated mango pulp were dissolved in a small volume of chloroform and the solution was passed over five columns (3 × 20 cm.) of a 1:1 mixture of magnesia and supercel. Light petroleum (b.p. 60–75°) was used for developing the chromatogram. In each case some ill-defined bands at the top and a broad clear-cut band at the bottom were obtained. The top zones were all scooped out with a spatula and their eluates were combined (extract A). The eluates from the major band were mixed (extract B).

Extract A was rechromatographed when the following zones were obtained:

- Zone 1: Very narrow, dark red; no absorption maxima.
- Zone 2: Very narrow, deep red; no absorption maxima.
- Zone 3: Narrow, pink; no absorption maxima.
- Zone 4: Broad, pink; absorption maximum at 445 m μ and an inflexion at 475 m μ in carbon disulphide.

It is probable that all these four fractions were decomposition products. They represented 3.2% of the original crude crystals.

Extract B gave on rechromatography a single band, the middle portion of which was carefully separated and eluted with light petroleum (b.p. 60–75°) containing 5% ethanol. On concentration to a small volume and addition of absolute ethanol, glistening red crystals separated out. The mother liquor was further concentrated and another crop of crystals was obtained. The total yield of crystalline material was 230 mg. with a melting-point of 180°. The crystals were purified by a fourfold fractional crystallization from carbon disulphide and light petroleum (b.p. 40–60°), and dried *in vacuo* over active alumina at 75°. The purified sample showed the following properties, which agree fairly closely with those reported in the literature for β -carotene (Gillam, 1935; Miller, McKinney & Zscheile, 1935; Seaber, 1940; Hunter & Scott, 1941; Zscheile, White, Beadle & Roach, 1942): m.p. = 181.5° (uncorr.); absorption maxima at 482 and 512 m μ in carbon disulphide, at 451.5 and 482 m μ in light petroleum (b.p. 60–75°); extinction coefficients ($E_{1\%}^{1\text{cm.}}$) in light petroleum (b.p. 60–75°), at 450 m μ —2440; at 463 m μ —2225; at 470 m μ —2020; at 480 m μ —2140; optical activity (at 578 m μ)—Nil. (Analysis (mean of two determinations): Found: C, 88.85; H, 10.43. Calc. for C₄₀H₅₆: C, 89.48; H, 10.52%.)

DISCUSSION

The xanthophyll of the mango fruit studied qualitatively by Yamamoto *et al.* (1932) had absorption maxima at 446, 473 and 505 m μ in carbon disulphide. These maxima correspond closely with those of our xanthophyll pigment 'X' (see above) except that in the latter, the third maximum was not evident. In the present investigation no lutein

(absorption maxima 445, 475 and 508 $m\mu$ in carbon disulphide—Kuhn & Smakula, 1931; Zubrys & Morf, 1933) was found.

Strain (1938, 1941) reported that when lutein was boiled in methanolic solution for four hours or treated with iodine solution, two isomers, one having absorption maxima at 447, 474 and 505 $m\mu$ in carbon disulphide and the other at 469 and 497 $m\mu$ were formed. The absorption maxima of our xanthophyll pigments 'X' and 'Y' are close to these. Apart from the two isomers of lutein, no pigments are reported in the literature with maxima corresponding to those obtained in the course of this investigation.

In view of the great ease with which lutein is known to undergo isomerization, the possibility of these two pigments having been derived from it in this way cannot be altogether excluded (Zechmeister, 1944). However, a qualitative experiment, in which special precautions were taken by neutralizing the fresh fruit pulp with sodium carbonate and extracting the pigments in the cold, gave essentially the same results.

It is of interest to note that the epiphasic pigment isolated from the Badami mango fruit consisted almost entirely of β -carotene; α -carotene seems to have been absent. The fact that the carotene consists entirely of the β -isomer is of particular significance from the point of view of nutrition, since biologically β -carotene is twice as active as any of the other pro-vitamins A (Kuhn & Brockmann, 1933; Hunter & Scott, 1941).

Yamamoto *et al.* (1932), however, thought that their mango carotene was a mixture of α - and β -carotenes. By fractional crystallization of the crude carotene from carbon disulphide and absolute ethanol, they obtained two fractions. The main one, melting at 179° and showing absorption maxima at 449, 475 and 514 $m\mu$ in carbon disulphide and 435, 462 and 487 $m\mu$ in chloroform and an optical rotation in carbon disulphide $[\alpha]_D^{25} = 135^\circ$, was

considered by them to be a mixture of α - and β -carotenes, while the minor fraction, melting at 173–174° and showing absorption bands at 452~487 $m\mu$ and 495~507 $m\mu$ in chloroform was taken to be α -carotene. Considering the fact that they had applied the petrol-methanol phase separation prior to saponification and that the pigments were not separated by chromatography, it seems probable that their carotene sample was contaminated with xanthophyll. While the main fraction (m.p. 179°) might have contained traces of xanthophyll, the wide spectral bands suggest that the other fraction separated from the mother liquor might have contained a considerable amount of it.

There is also another possibility, viz. that different varieties of mangoes may differ in their carotenoid composition, a question that can be settled only after more extensive work.

SUMMARY

Carotene has been isolated from Badami mango fruit in a yield of 230 mg. from 7.7 kg. of the fresh pulp. Chromatographic analysis and spectroscopic data indicate that the carotene of this variety of mango fruit consists entirely of the β -isomer.

Extinction coefficients for β -carotene in light petroleum (b.p. 60–75°) at four different wavelengths are reported.

Analysis of the hypophasic fraction of the mango pigments showed the presence of two unidentified xanthophylls, with absorption maxima at 446 and 473 $m\mu$ and 468 and 497 $m\mu$ respectively in carbon disulphide.

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