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FRUIT PIGMENTS

The Carotenoids of *Diospyros Kaki* (Japanese Persimmons)

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A survey has been made of carotenoid distribution in 40 varieties of persimmon or kaki, *Diospyros Kaki.* The major constituent is cryptoxanthin which amounts to 30 to 35% of the total. The hydrocarbon fraction is highly variable, particularly the lycopene, 0 to 30% of the total. The low lycopene varieties show marked increases in the mono- and di-epoxy diol fractions.

Persimmons belong to the genus *Diospyros*, 190 species of which are distributed throughout the world. The Chinese-Japanese species *D. Kaki* has been extensively improved in Japan, so that it is commonly known as the Japanese persimmon. Colors vary from yellow-orange to red, and according to Bailey (*1*) the same variation is found in *D. Virginiana*, native to the United States.

High sugar, low astringency, and red color are attributes of some of the best Japanese varieties, whereas those of Chinese origin tend to be more astringent and yellow-orange in color. The pigments responsible for these colors are carotenoid in nature. Black blotches which may cover as much as 20% of the area are frequently seen on the surface of Hachiya in markets. The pigment involved is not anthocyanin as it is insoluble in methanol-HCl. Simple tests suggest the presence in kaki of leucoanthocyanin, proved recently by Ito and Oshima (*4*). There is, however, no evidence that the color of different varieties is directly influenced by anthocyanins.

The carotenoids of kaki were first examined by Karrer *et al.* (*5*), who isolated and crystallized lycopene and zeaxanthin from the fruit. They noted that the zeaxanthin was esterified, and they also detected carotene and presumably observed other components: "In den roten Kaki-Fruchten . . . sind verschiedene Carotinoide enthalten." Schön (*6*) crystallized cryptoxanthin, lycopene, and β-carotene, epiphase, and zeaxanthin and a small quantity of violaxanthin from the hypophase. He detected α-carotene spectroscopically and observed little if any unesterified xanthophyll before saponification. He obtained values, colorimetrically determined, in mg. per kg. of fresh fruit as follows: α-carotene, 0.3; β-carotene, 1.0; lycopene, 0.3; cryptoxanthin, 5; zeaxanthin, 12.

Tsumaki *et al.* (*7*) studied variation in carotenoid contents of two varieties, Fuyugaki and Kagoshimasaijo-gaki. They noted that the rapid increases in the quantities of lycopene and of esterified xanthophyll were responsible for the striking color in the Fall. Both varieties were reddish in color and had substantially higher proportions of lycopene (and neolycopene) relative to the xanthophylls than were found by Schön.

Using his combination of countercurrent distribution and chromatographic techniques, Curl (*2*) made a detailed study of the Hachiya variety, and separated 26 components not counting cis- isomers. The mixture was comprised of hydrocarbons 20.6%, (major components—lycopene 7.7% and β-carotene 6.8%); monols 39.7% (cryptoxanthin 38%); diols 21.9% (zeaxanthin 18%, lutein 3.9%); monoepoxide diols 11.7% (antheraxanthin 10%); diepoxide diols 3.5% (violaxanthin 2.7%); polyols 2.7%.

Experimental

In 1959, the carotenoids of two varieties, Hachiya and Fuyu (Fuyugaki) were compared, and as a result of major differences, a systematic study was made of 26 varieties maintained by the University of California at Winters, Calif. Estimates were made of total carotenoids and lycopene in all varieties, and three were selected for detailed study: Fuyu, high in lycopene, deep orange-red in color throughout, typical weight 160 grams; Honan Red, intermediate in color, although the peel was bright red, typical weight 85 grams; Tamopan, yellow-orange throughout, with little or no lycopene, weight 350 grams, described, however, by Bailey (*1*) as bright orange-red in color.

In 1961, twelve varieties from Okitsu, Japan, were examined, as well as fruit of the small mamegaki (pea-Kaki) and of black lotus, *D. Lotus*.

Portions of 50 to 100 grams from fully ripe fruit were blended and exhaustively extracted with acetone and petroleum ether. The residue was rewetted, if necessary, and the extraction repeated until the residue was colorless. The pigments were transferred to petroleum ether, and the solution was dried with Na_2SO_4. Absorbances were determined from 350 mμ to 530 mμ at 5-mμ intervals. Fruits with high lycopene content gave extracts with prominent maxima at 500, 470, and 445 mμ. As the proportion of lycopene diminished, the maximum at 500 mμ became progressively

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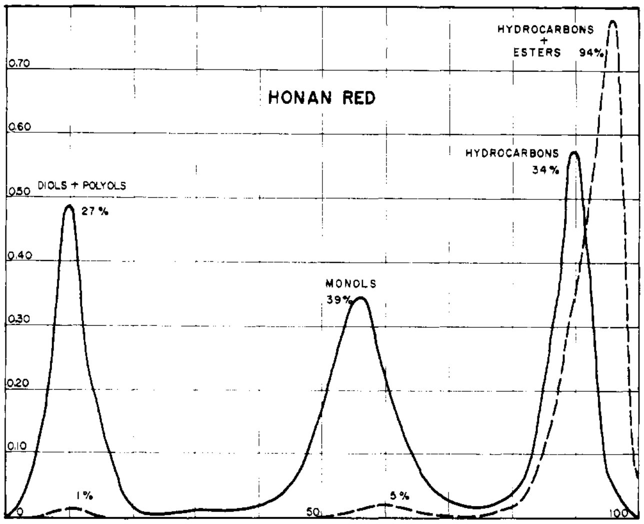

Figure 1. Distribution of pigments in Honan Red

Solvent system: petroleum ether–methanol–water; abscissa, tube number; ordinate, absorbance; saponified: solid line; unsaponified: broken line

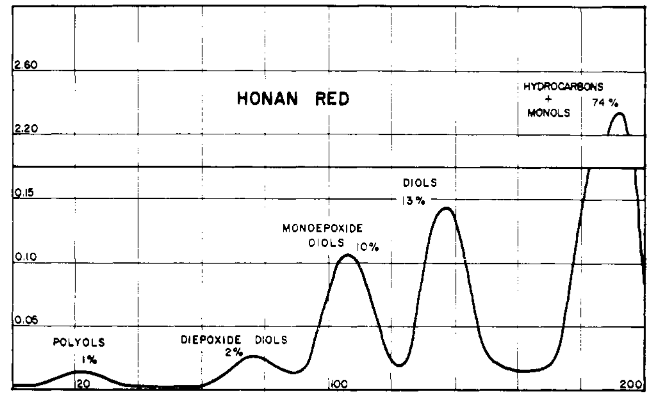

Figure 2. Distribution of pigments in Honan Red

Solvent system: benzene–petroleum ether–methanol–water; abscissa, tube number; ordinate, absorbance

weaker and finally disappeared. Varieties with little or no lycopene gave a xanthophyll-like spectrum with maxima at 470 to 475 and 445 mμ and a shelf at 420 mμ.

When the data were plotted, there was a virtually continuous array from fruit containing little or no lycopene to those in which lycopene comprised 30 to 40% of the pigment mixture. Since 26 or more components were present, the assessment of data on 40 varieties had to be simplified. One could not ignore all except the five or six most abundant components, because what might be negligible in one variety might be abundant in another. For example, antheraxanthin and violaxanthin represented 5.0 and 1.6%, respectively, of the pigment complex in Fuyugaki. Corresponding figures in Tamopan were 21.0 and 14.3.

The first simplification was to make an estimate of total carotenoid using an arbitrary coefficient of 250 (liters per gram cm.) at the 445 mμ maximum of the crude extract. Since phytoene, phytofluene, and ζ-carotene are minor components, this introduces no major error.

A second simplification was made to enable a quick reliable estimate for lycopene. The extracts were treated as a two-component mixture of lycopene and component x. The spectrum of x was determined for several extracts from which lycopene had been removed by chromatography. At wave lengths 500, 475, and 445 mμ, absorbance ratios showed some variability, e.g., Okame—0.205:1:1.17; Mishirazu—0.194:1:1.18.

The lycopene contained traces of neolycopene in some cases, and the following ratios were ultimately fixed: lycopene—1.1:1:0.73; x—0.2:1:1.17.

Absorptivities (in liters per gram cm.) were chosen, 300 for lycopene at 500 mμ, 250 for x at 445 mμ, and the foregoing ratios were maintained.

The absorbances at these three wavelengths were calculated for hypothetical mixtures of lycopene and x, from 0 to 40% lycopene. If ratios are plotted—e.g., D_{475}/D_{500} versus D_{445}/D_{475}, the points must fall on a hyperbola. Experimental data on the 40 varieties fit the curve with very little scatter until low lycopene levels are reached. Here, in some specimens, the x fraction changes in character, primarily due to increases in mono- and di-epoxy derivatives.

The procedure therefore yields reasonably accurate estimates of lycopene in red-fruited specimens (high in lycopene), but only limiting values in yellow-orange fruit (low in, or devoid of lycopene).

It is easy to segregate the extremes, those which have 20 to 40% lycopene in the pigment mixture, and those which have from 5% to traces less than 0.1%, but a large intermediate group exists, with no clear dividing lines. By use of arbitrary boundaries, the following classification can be made:

Group 1: Dai Dai Maru, Fuyu, Tribble, Jumbu, Yeddo, Jivo, Maru Pi32886, Okame (California); Fukurogosho, Fuyu, Okugosho, and hybrids between the two, Zenji-Maru (Japan).
Group 2: Honan Red, Saijo, Kishimoto, Tishitzu, Hyakume, Tsuru, Gosho Lotus (California); Yokono, Yotzumizo (Japan).
Group 3: Ruby, Mishirazu, Pi59343, Hachiya, Mikado, Tamopan, Chienting, Niu Nai, Fuji, Hiragaki (California); Hanagosho, hybrids between Hanagosho and Okurogosho or Fukurogosho (Japan).

In addition, the mamegaki and black lotus belong in group 3. The latter, so far as could be ascertained, was devoid of lycopene. A few of the above names are ambiguous. Hachiya is regarded as a group name in Japan. Saijo carries the connotation best (*4*), Kagoshima-saijo. Results are summarized in Table I.

Detailed Study of Fuyu, Honan Red, and Tamopan Varieties

Fuyu, Honan Red, and Tamopan were chosen to represent each group, and extracts from about 1 kg. of fruit were prepared in each case.

Aliquots containing 5 to 10 mg. of carotenoids were submitted to countercurrent distribution with 100 transfers in an all-glass Post-Craig apparatus, solvent system: Skellysolve B, 1640 ml.; absolute methanol, 900 ml.; and water, 9 ml. (*3*). The carotenoids were thus fractionated into three groups—hydrocarbons plus esters, monols, and diols plus polyols. Results are shown in Table II.

A portion of each extract, containing about 30 mg. of carotenoid, was then saponified and the run was repeated. A third run, with the system benzene 1200 ml., Skellysolve B 1200 ml., absolute methanol 1200 ml., and water 180 ml., was used for 230 to 240 transfers. By means of these two solvent systems used by Curl (*2*, *3*), the saponified extract was fractionated into six groups—hydrocarbons, monols, diols, mono- and di-epoxy diols, and polyols. The individual components in each group were then isolated and estimated, as described by Curl, with the results shown in Tables III and IV, and in Figures 1 and 2.

Distribution of Pigment within the Fruit

When the fruit is over-ripe, the skin or epidermal layer is not unlike that of a tomato. The ripe peel appears visually to have a greater concentration of pig-

Table I. Estimates for Total Carotenoid and Lycopene (Mg. per Kg.) in 40 Varieties of Persimmon

Group	Number of Varieties	Total Carotenoids Av.	Total Carotenoids Range	Lycopene Av.	Lycopene Range
1	13	49.1	28–83	17.2	7.5–41.0
2	12	61.1	28–115	8.5	3.0–15.3
3	13	46.7	20–78	1.2	0.1–2.4
Mamegaki	1	97.9	...	1.0	...
D. Lotus	1	21.6	...	0.0	...

Table II. Effect of Saponification on Distribution (as a Percentage of the Total Pigment)

Variety	Fraction[a]	Saponification Before	Saponification After	Percent Esterification
Fuyu	1	91	46.5	...
	2	2	33.5	94
	3	7	20	74
Honan Red	1	95	35	...
	2	4	39	91
	3	1	26	96
Tamopan	1	93	9	...
	2	5.6	30	84
	3	1.4	61	98

[a] Fraction No. 1, hydrocarbons and esters; No. 2, monols; No. 3, di- and polyols.

Table III. Percentage Distribution of Fractions in Three Varieties

(Figures in parentheses are on a hydrocarbon-free basis)

Variety	Hydro-carbons	Monols	Diols	Mono-epoxy Diols	Di-epoxy Diols	Polyols
Fuyu	46.5	33.5	9.0	7.0	3.0	1.0
	...	(62.6)	(16.8)	(13.1)	(5.6)	(1.9)
Honan Red	35.0	39.0	13.0	10.0	2.0	1.0
	...	(60.0)	(20.0)	(15.4)	(3.1)	(1.5)
Tamopan	9.0	30.0	16.5	23.5	16.0	5.0
	...	(33.0)	(18.1)	(25.8)	(17.6)	(5.5)

Table IV. Percentage Distribution of Carotenoids in Three Varieties[a]

Fraction	Component	Fuyu	Honan Red	Tamopan
Hydrocarbon	Phytofluene	4.2	2.6	0.5
	α-Carotene	0.6	1.5	0.8
	β-Carotene	4.6	6.4	7.5
	ζ-Carotene	7.6	3.8	trace
	γ-Carotene	0.5	1.2	trace
	Lycopene	29.0	19.0	0.2
Monols[b]	Hydroxy α-Carotene	2.3	0.1	2.0
	Cryptoxanthin	31.0	38.0	28.0
Diols	Lutein	2.2	2.0	4.5
	Zeaxanthin	6.8	11.0	12.0
Mono-epoxy diols[b]	Antheraxanthin	5.0	9.2	21.0
	Mutatoxanthin	2.0	0.5	1.2
Di-epoxy diols[b]	Violaxanthin	1.6	1.8	14.3
	Luteoxanthin	...	...	1.7
Polyols[b]	Neoxanthin	1.0	1.0	2.0

[a] Fuyu, Honan Red, and Tamopan contained, respectively, 65, 80, and 61 mg. of carotenoid per kg.
[b] Miscellaneous unidentified components have not been included in this tabulation.

ment than the interior flesh in many cases; this is particularly noticeable in Honan Red, which explains its surface redness.

Extracts of peel and flesh were examined spectrophotometrically for Fuyu, Honan Red, and Tamopan. For all three varieties, 50 to 60% of the carotenoids were found in peel, which amounted to 17 to 23% of the total weight (without seeds). Lycopene was more evenly distributed in Fuyu; in Honan Red, however, 70% of the total was located in the external layers.

Table I shows that the presence or absence of lycopene is unrelated to the total carotenoid present. Regardless of variety, the bulk of the xanthophyll is esterified in the fruit (Table II). When the data on the xanthophyll fractions are placed on a hydrocarbon-free basis (Table III), the similarity between Fuyu and Honan Red and the dissimilarity between these and Tamopan becomes very apparent.

The major change in Tamopan is the marked increase in the contents of antheraxanthin and violaxanthin.

Finally, with regard to the hybrids, it cannot be supposed that the parent stocks are homozygous since they are propagated by cuttings and grafts. All that can be said is that Hanagosho in its crosses with members of group I yields an F_1 whose fruit resembles its low-lycopene parent.

The Chinese varieties are associated with a high degree of astringency and in general a yellower color. Japanese varieties are less astringent, sweeter, and redder, characteristics which may be the result of recessive mutations.

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