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Major Carotenoids of the Seeds of Three Cultivars of the Tomato, *Lycopersicon esculentum* L.

Carotenoids from the seeds of three tomato cultivars were separated by chromatography and identified by absorption spectroscopy. Lutein, β-carotene, and lycopene were the major pigments of Chico Grande and Rutgers seeds, and lutein, β-carotene, and ζ-carotene were the major pigments of Golden Jubilee seeds. Xanthophyll pigments predominated over carotenes in the seeds of all cultivars. β-Carotene was predominate over lycopene in the seeds of both red fruited cultivars and over ζ-carotene in the seeds of the yellow fruited cultivar.

The carotenoid composition of tomato fruit and the inheritance of fruit color of many species and cultivars of tomatoes have been studied extensively since the early 1930's (Kuhn and Grundmann, 1932). However, the pigment composition of tomato seeds has not been reported. It was the purpose of this study to identify and quantify the seed pigments from seeds of three different tomato cultivars and to compare the results with known pigments of the fruit.

MATERIALS AND METHODS

Dried seeds (4.5 kg) of each of the three cultivars (Chico Grande, Rutgers, and Golden Jubilee) were prepared for pigment analysis. Seeds were cleaned to remove all visible foreign matter and fragments of skin, and the whole seeds were washed three times with methylene chloride solvent. Following solvent washing, seeds were ground and oils were extracted with methylene chloride. After removal of solvents, residues were saponified and partitioned between petroleum ether and 95% methanol (Goodwin, 1965). Epiphase and hypohase pigments were chromatographed separately using increasing amounts of acetone in petroleum ether ranging from 0 to 35% acetone. Epiphase pigments were chromatographed on a column of alumina (Baker) and hypophase pigments were separated on a column of equal parts of magnesium oxide (Baker) and Hyflo-Super-Cel (Johns Manville). The alumina column separations were by gravity flow, while 5–10 psi air pressure was used with the magnesium oxide columns.

Individual pigments were characterized by their absorption spectra in the range of 180–550 nm using both hexane and petroleum ether, by comparison of absorption maxima with literature values (Goodwin, 1965), and by comparison of their spectra with those of pure crystalline carotenoids. Crystalline lycopene and lutein (3,3′-dihydroxy-α-carotene) were isolated from tomato paste and spinach leaves, respectively, synthetic *all-trans*-β-carotene was obtained from Hoffman-La Roche, and the ζ-carotene (7,8,7′,8′-tetrahydrolycopene) spectrum was compared to the spectrum published by Nash and Zscheile (1945). Spectral data were obtained using 1.0-cm matched cells with a Coleman-Perkin-Elmer Model 124 ratio recording spectrophotometer and Model 165 recorder. Quantitative estimations were made in the usual manner using absorbance (Gillam and Stern, 1958).

RESULTS AND DISCUSSION

Solvent washing of the whole tomato seeds was necessary to ensure that subsequent analyses for seed pigments did not include pigments of the fruit that might have adhered to the outside of the seeds. However, when washing residues were examined spectrophotometrically, no materials absorbing visible light were found. There was a considerable amount of waxy material removed from the seeds by solvent washing. Subsequent saponification of this material showed a uniform unsaponifiable fraction among the three cultivars (Table I). The unsaponifiable fractions of the oils were more variable in quantity, rang-

Table I. Solvent Washing Residues,[a] Unsaponifiable Matter,[b] and Seed Oil[c] of Three Tomato Cultivars

Cultivar	Solvent washing residues		Solvent-extracted oil	
	% of seed wt.	% unsapon. matter	% of seed wt.	% unsapon. matter
Chico Grande	0.17	4.57	19.3	1.28
Rutgers	0.26	4.74	19.8	1.05
Golden Jubilee	0.35	4.67	21.4	2.43

[a] Whole tomato seeds were washed three times with 3 parts of methylene chloride, w/w, by rolling in glass jars for 1 hr at 20 rpm. Residues were dark oily liquids. [b] Following saponification of washing residues and oils, unsaponifiable residues were dried 24 hr in a vacuum dessicator. [c] Ground tomato seeds (No. 10 mesh screen) were extracted four times with 3 parts of methylene chloride, w/w, by rolling in glass jars for 1 hr at 20 rpm.

Table II. Total Carotenoids in Seed Oil from Three Tomato Cultivars

Cultivar	Total pigments,[a] ppm	Hypophase (xanthophylls), %	Epiphase (carotenes), %	Xanthophylls: carotenes
Chico Grande	0.6	64.4	35.6	9:5
Rutgers	0.2	90.2	9.8	9:1
Golden Jubilee	1.3	59.7	40.0	3:2

[a] Quantitative estimates of total pigments of the oils were made using absorbance at 450 nm and $E_{1cm}^{1\%}$ = 2505 following saponification and partitioning between petroleum ether (epiphase) and 95% methanol (hypophase).

ing from 1.05% for Rutgers oil to 2.43% for Golden Jubilee oil (Table I). The unsaponifiable fractions of the oils were amorphous orange solids indicating a high percentage of waxy material. Nash and Zscheile (1945) noted that the unsaponifiable material from tomato fruit extractions contained a considerable amount of phytosteroids and waxes which were not readily separated by chromatography and which interfered with crystallization and spectrophotometry. This problem was intensified in the seed analyses since the total amounts of pigments in the seed oils (Table II) were proportionately less than the quantities of pigments normally found in tomato fruit (Mackinney *et al.*, 1956). No column adsorbent or solvent system was found that would effect a complete separation of all these compounds from the carotenoids. Repetitive chromatography reduced them proportionately, and more were removed by precipitating them from petroleum ether at low temperatures. However, enough remained in both epiphase and hypophase separations to interfere with thin layer chromatography and to dominate absorption spectra in the ultraviolet region. For this reason a small amount of phytofluene (7,8,11,12,7′,8′-hexahydrolycopene) which was detected on the column of epiphase extract from Golden Jubilee seed (by blue-green fluorescence under 365-nm ultraviolet light) could not be satisfactorily quantified. No fluorescence was seen on the columns of either of the red-fruited varieties.

Xanthophylls predominated over carotenes in all cultivars (Table II). The ratio was approximately 9:5 for Chico Grande, 9:1 for Rutgers, and 3:2 for Golden Jubilee seed pigments.

The major carotenoids identified are shown in Table III. Lutein was the only xanthophyll found in seeds of all three of the cultivars. The visible absorption maxima agreed with published values (Goodwin, 1965) and the absorption spectrum matched the spectrum of pure crystalline lutein. Similarly, β-carotene was the major carotene found in all cultivars. Lycopene was found in the seeds of the two red-fruited cultivars, Chico Grande and Rutgers, while ζ-carotene was found only in the seeds of the yellow- or golden-fruited cultivar Golden Jubilee along with a small amount of phytofluene. In addition to the spectral data used for the other pigment identifications, iodine catalysis was conducted in the cuvette with the ζ-carotene in hexane. The absorption maxima of 380, 403, and 427 nm were shifted to 378, 400, and 425 nm after catalysis, indicating that the naturallly occurring form of ζ-carotene in tomato seeds was the all-trans form (Zechmeister, 1962).

In general the major pigment of the fruit of red tomatoes is *all-trans*-lycopene with varying proportions of β-carotene and lutein, while ζ-carotene as a major pigment is restricted to the tangerine tomato (Jenkins and Mackinney, 1953). Qualitatively the carotenoid composition of the seeds paralleled that of the fruit. The major differences were in quantitative relationships. In the fruit of most tomato cultivars the ratio of carotene to xanthophyll pigments is about 9:1 (Mallia *et al.*, 1967), while in the seeds, the xanthophyll pigment predominated with ratios of 9:5, 9:1, and 3:2. Furthermore, the ratio of lycopene to β-carotene in fruits of most standard red cultivars is about 11:1 with some cultivars running as high as 26:1 (Thompson *et al.*, 1965), whereas β-carotene was the predominant carotene in the seeds of all three cultivars. The ratio of β-carotene to lycopene was 3:2 and 7:6 in Chico Grande and Rutgers, and that of β-carotene to ζ-carotene was 5:4 in Golden Jubilee.

Table III. Major[a] Carotenoids in Seed Oil from Three Tomato Cultivars

Cultivar	% of total carotenoids[b]				
	Lutein	β-Carotene	Lycopene	ζ-Carotene	Carotene ratio
Chico Grande	64.4	21.5	14.1		$\frac{\beta\text{-Carotene}}{\text{lycopene}} = \frac{3}{2}$
Rutgers	90.2	5.3	4.5		$\frac{\beta\text{-Carotene}}{\text{lycopene}} = \frac{7}{6}$
Golden Jubilee	59.7	22.2		18.1	$\frac{\beta\text{-Carotene}}{\zeta\text{-carotene}} = \frac{5}{4}$

[a] A small amount of phytofluene was detected by fluorescence on the chromatographic column during separation of Golden Jubilee pigments but was not measurable. [b] Quantitative estimates were made using absorbance at the wavelength of maximum absorption.

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Identification of Some Volatile Compounds from Cucumber

Volatile compounds from cucumber (*Cucumis sativus L.*), obtained by vacuum steam distillation–extraction of the fruit at 60–70° in a water recycling apparatus, were separated by gas chromatography and subjected to mass and infrared spectral analyses. Compounds identified for the first time as cucumber components are: 1-nonanol, *trans*-2-nonen-1-ol, *cis*-3-nonen-1-ol, *cis*-6-nonen-1-ol, *trans,cis*-2,6-nonadien-1-ol, *cis,cis*-3,6-nonadien-1-ol, *cis*-6-nonenal, and C_{10} through C_{15} saturated straight chain aldehydes. *cis*-3-Nonenal and *cis,cis*-3,6-nonadienal were tentatively identified.

In previous work on muskmelon and watermelon volatiles (Kemp *et al.*, 1972a,b, 1974) several compounds were identified including a group of C_9 aldehydes and alcohols. Among these are *cis,cis*-3,6-nonadien-1-ol which has a flavor reminiscent of watermelon or watermelon rind and *cis*-6-nonenal which is reminiscent of melon or green melon generally. The former was found to be a major component and the latter a minor component of watermelon volatiles, whereas both compounds are trace constituents of muskmelon volatiles. Forss *et al.* (1962) attributed the flavor of cucumber (same genus as muskmelon) to similar C_9 compounds (*trans,cis*-2,6-nonadienal and *trans*-2-nonenal).

As part of a study of the interrelationships of volatile constituents of the major cucurbits (muskmelon, watermelon, and cucumber) we were interested in learning if the melon constituents were unique to melon or if they also occurred in cucumber. In the course of this work additional higher boiling compounds were isolated from cucumber and identified.

EXPERIMENTAL SECTION

Cucumbers (*Cucumis sativus* L.; cultivar "SMR 58") were grown on the University of Kentucky Experiment Station farm in Lexington. Fresh green fruit approximately 1–1.5 in. in diameter was subjected to steam distillation–extraction in a water recycling apparatus (Kemp *et al.*, 1968) operated under reduced pressure. Charges consisted of 1.6-kg portions (rind removed) pureed with 2 l. of distilled water. Redistilled hexane (4 ml) was placed on top of the water layer in the side arm of the apparatus. The pressure in the system was reduced and the temperature of the puree ranged from 60 to 70° during a 3-hr run. Hexane layers from several runs were combined and dried over Na_2SO_4 and the solvent was removed under a stream of N_2 to give a concentrated essence.

Glc separation of the cucumber essence was carried out initially on a 6 ft × 0.25 in. o.d. stainless steel column packed with 20% SE-30 coated on 60–80 mesh, acid-washed, silanized Chromosorb W. The column temperature was programmed from 100 to 180° at 1°/min. Fractions collected from the SE-30 column were rechromatographed on a 12 ft × 0.25 in. o.d. stainless steel column packed with 5% diethylene glycol succinate (DEGS) or 10% Carbowax 20M coated on 60–80 mesh, acid-washed, silanized Chromosorb W. Resulting subfractions were collected and submitted to spectral analysis.

Mass spectra were recorded on a Hitachi RMU-6E double focusing mass spectrometer operated at 70 eV. Infrared spectra were obtained with the aid of a NaCl microcell and a mirror beam condenser; spectral grade CS_2 was used as solvent. Reference compounds were obtained from commercial suppliers or from other laboratories. Reference samples of *cis*-3-nonen-1-ol and *cis,cis*-3,6-nonadien-1-ol were isolated from watermelon.

RESULTS AND DISCUSSION

The amount of essence obtained from cucumber by vacuum steam distillation–extraction corresponded to a concentration of approximately 10 ppm based on fresh cucumber weight. The essence was initially separated on an SE-30 column and the fractions containing the C_9 aldehydes and alcohols were readily located since the conditions used were similar to those used earlier for the separation of melon volatiles. Fractions were rechromatographed on a DEGS or a Carbowax column and purified compounds were submitted to spectral analysis. A list of compounds identified, evidence for identification, and estimates of glc peak sizes are given in Table I.

The major components of cucumber essence were found to be *trans,cis*-2,6-nonadienal and *trans*-2-nonenal in agreement with results previously reported (Forss *et al.*, 1962). In addition, retention data indicated that the melon constituents, *cis*-6-nonenal and *cis,cis*-3,6-nonadien-1-ol, were present in cucumber essence and their identification was confirmed by means of mass and infrared spectral data. Other C_9 alcohols and an aldehyde obtained