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Carotenes in Typical and Dark Orange Carrots

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Carotenes from a genetically diverse collection of carrots were separated and quantified with reversed-phase high-performance liquid chromatography and their identities verified with column chromatography and thin-layer chromatography. Extraction of carotenes from lyophilized samples stored less than 1 month was comparable to that from raw or frozen samples, and saponification was not necessary. Six carotenes (α-, β-, γ-, ζ-carotene; β-zeacarotene; lycopene) were routinely detected. β-Carotene accounted for 44–79% of the total carotenes whereas β-, α-, and ζ-carotene accounted for 94–97% of the total carotenes. Total carotene content ranged from 63 to 548 ppm over lines and location/years. The very dark orange line HCM contained more than twice as much total carotene as any other line tested in all location/years.

Much of the dietary vitamin A, especially in developing countries, is derived from carotenes in vegetables and fruits (Simpson, 1983). Carotenes have also been implicated as anticancer compounds in numerous studies (Moon and Itri, 1984). Carrots are the major single source of provitamin A carotenoids in the American diet, contributing 14% of the total vitamin A consumption (Senti and Rizek, 1975). Although an "average" carrot in the United States contains 66 ppm total carotenes (Adams, 1975), carrots from different genetic sources have been reported to contain 0–370 ppm carotenes, with β-carotene usually accounting for approximately half of this total (Umiel and Gabelman, 1971).

With the potential for genetically increasing the carotene content of carrots, selection for dark orange, high provitamin A carrot roots was initiated in 1977 (Simon et al., 1985). To increase carotene concentration over successive generations, large populations must be analyzed. The evaluation of large numbers of carrot roots for carotene concentration is time consuming and made difficult by analytical methods that can result in carotene breakdown or isomerization. Important factors to consider in carotene analysis include method of sample preparation, extraction conditions, saponification, and method of chromatographic separation and quantification (Davies, 1976; DeRitter and Purcell, 1981).

Carotenes from carrots of diverse genetic background have been quantified with thin-layer chromatography (Umiel and Gabelman, 1971; Buishand and Gabelman, 1979). Six colored pigments (α-, β-, γ-, ζ-carotene; lycopene; one unknown) were able to be separated with that system. Reversed-phase, high-performance liquid chromatographic determination of carotenes lends itself to a more rapid, quantitative analysis of carotenes, and systems for measuring lycopene and α- and β-carotene in higher plants, including carrots, are reported (Zakaria et al., 1979; Bushway and Wilson, 1982).

This paper details a method to analyze carotenes in typical and dark orange carrots. The method utilizes lyophilized samples, direct extraction of carotenes into hexane, and quantification with a reversed-phase HPLC solvent system modified from that developed by Nells and DeLeenheer (1983) and used by Bieri et al. (1985) for analysis of human plasma carotenoids. Time needed for this method is less than that for other methods used with plant samples, carotene breakdown is minimal, and six carotenes (α-, β-, γ-, ζ-carotene; β-zeacarotene; lycopene)

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are able to be routinely measured.

EXPERIMENTAL SECTION

Plant Materials. Six carrot inbreds representing a diverse range of fresh market carrot germplasm (F524, B2566, B3080, B6274, B6439, B9692) and one deep orange selection (HCM) from the USDA carrot improvement program were grown in El Centro, CA, Zellwood, FL, and Palmyra, WI. Carrot roots were washed and stored at 4 °C until sampling.

"Total Carotenoid" Measurement. The absorbance at 450 nm of carotene extracts in hexane was measured on a Gilford 300N spectrophotometer. An estimate of "total carotenoid" concentration was obtained by comparing sample absorbance to a standard curve of β-carotene (Sigma), 0–10.0 ppm (Umiel and Gabelman, 1971).

Carotene Chromatography. Individual carotenes of B6274 and HCM from El Centro, CA, and Palmyra, WI, were separated with column chromatography, thin-layer chromatography (TLC), and high-performance liquid chromatography (HPLC). Carotene separation with column chromatography utilized a 10 × 300 mm column with 60–200-mesh silica gel followed by magnesia-HyFlo Super Cel (1:2) (Purcell, 1958). Chromatography was performed at room temperature under N_2 with opaque covering over the column and continuous supply of wash solution to minimize isomerization. From a 1.0-mL sample of carrot extract in hexane (as described below) were eluted three pigmented fractions on the silica column with 15 mL of hexane (fraction I), 20 mL of hexane–ethyl ether (1:1) (fraction II), and 25 mL of methanol (fraction III). Four pigmented fractions were eluted from fraction I (concentrated to 3 mL in a rotary evaporator) when developed on the magnesia-HyFlo Super Cel column with 60, 40, and 20 mL of 2%, 5%, and 10% acetone in hexane, respectively, followed by 50 mL of 2% methanol plus 10% acetone in hexane.

Carotene separation with TLC was performed on 20.5 cm × 20.5 cm glass plates coated with 0.25-mm magnesium oxide (heavy powder)–Kieselguhr (Baker's TLC grade) 4:1 mixture and activated at 100 °C for 45 min. Plates were developed in either chromatography tanks or S-chambers with a toluene–heptane (2:3) mixture until α- and β-carotene were separated (approximately 15 min) followed by 100% toluene [modified from Buishand and Gabelman (1979)].

Carotene separation with HPLC employed a Whatman Partisil 5 ODS-3 column and PC Pellosil guard column. The solvent used was acetonitrile–methylene chloride–methanol (82:12:6) [modified from Nells and DeLeenheer (1983)]. Solvent delivery was with a Waters 6000A pump, flow rate 2.0 mL/min. Sample injection volume was 25 μL (15 μL for line HCM) with a Waters WISP 710A. Peak detection utilized a Waters 440 absorbance detector set at 0.05 AUFS with 436-nm and 0.01 AUFS with 340-nm fixed wavelengths. Peak area was measured with a Waters 730 Data Module.

Apo-8′-carotenal (Fluka) was used as an internal standard for HPLC analysis. Immediately after dilution of carotene extract to 100.0 mL, 9.00 mL of extract was mixed with 1.00 mL of 5 ppm internal standard in hexane. Before HPLC analysis, 2.0 mL of hexane extract with internal standard was dried in a rotary evaporator, redissolved in 2.0 mL of acetonitrile, and filtered through a Millipore FH (0.5-μm) filter. Solvents were all HPLC grade (Burdick and Jackson) and degassed under vacuum before use. In addition to the use of an internal standard in HPLC samples, a standard curve was established every day using 0, 2, 4, 6, 8, and 10 ppm each of α- and β-carotene (Sigma). Each standard contained 5 ppm β-apo-8′-carotenal standard. Since the purity of the α- and β-carotene and β-apo-8′-carotenal used was 92%, 81%, and 98%, respectively, as determined by Beer's law, (Davis, 1976; Bushway, 1986), the quantity of crystalline carotene used to prepare standards was increased appropriately to arrive at the desired concentrations. The standard series was prepared in hexane, concentrated with a rotary evaporator, and reeluted in acetonitrile as described for carrot extracts.

Bands purified by magnesia column chromatography and TLC were concentrated in a rotary evaporator and reeluted in hexane. The four bands from column chromatography of carrot extract were chromatographed individually with TLC and HPLC to test for cochromatography of several compounds. Likewise, the six bands from TLC were chromatographed individually with column chromatography and HPLC. Visible spectra from 300 to 520 mm were taken for each band (in hexane) on a Hitachi Model 100-60 spectrophotometer. Standards used were α- and β-carotene (Sigma), lycopene (Sigma), and γ-carotene (Hoffmann-LaRoche). Purity of lycopene and α-carotene were 84% and 60%, respectively, as determined by Beer's law.

Sample Preparation and Carotene Extraction. For the sample preparation method of comparison in this work (i.e. the "standard method"), 2 g of cross-sectional slices of carrot from midroot were weighed and lyophilized (frozen overnight at −22 °C, dried under 80 μmHg vacuum, −50 °C condenser). Roots were stored at 4 °C for up to 1 week before sample preparation. Lyophilized samples were stored at −22 °C in air-tight bags with air evacuated from bags before closing. Within 1 month, carotenes were extracted.

To extract carotenes, lyophilized samples were comminuted for 5 min with 15 mL of hexane in an air atmosphere at 50K rpm with a VirTis 60K homogenizer (grinding cup surrounded by ice water) and filtered under suction through Whatman No. 2 paper. Residue was washed with hexane until colorless. Extracts were filtered through a column containing 25–30 g of anhydrous granular Na_2SO_4, diluted to 100.0 mL, and stored in an air atmosphere at −22 °C in amber bottles for less than 5 days before analysis. Hexane was passed through 60–200-mesh silica gel before use. All comminution and extract washing was done in an air atmosphere under dim fluorescent light. For HPLC analysis, hexane extracts were mixed with apo-8-carotenal internal standard, concentrated, and redissolved in acetonitrile as described earlier. One sample of each carrot inbred from each growing location was prepared without addition of apo-8-carotenal and separated with HPLC to determine whether any carrot carotenoids eluted with the internal standard.

In one experiment, the sample preparation and carotene extraction method for lyophilized samples was compared to raw and to frozen (5–7 days at −22 °C) samples from B6274, B9692, and HCM roots grown in El Centro, CA. Carotenes in raw and in frozen samples were extracted as described by Zscheile and Porter (1947) and Umiel and Gabelman (1971). Briefly, samples were comminuted for 5 min with 30 mL of acetone, 20 mL of hexane, and 10 mL of water (plus 100–200 mg of $MgCO_3$) at high speed in a chilled Waring Blendor and filtered under suction through Whatman No. 2 paper, the residue was washed with hexane and acetone until colorless, and acetone was removed from extract by washing with water for $2^1/_2$ h at 21 °C in a separatory funnel (protected from light with opaque paper cover).

Table I. Major Colored Carotenes of Typical and Dark Orange Carrots from Magnesia Column Chromatography and Thin-Layer Chromatography

Column Chromatography

fraction	elution vol, mL	hexane–acetone–methanol ratio	color	abs max, nm	HPLC ret time, min	identifcn
1	0–60	98:2:0	yellow	420, 442, 470	7.37	α-carotene
2	60–100	95:5:0	orange	426, 448, 475	7.75	β-carotene
3	100–120	90:10:0	pale yellow-green	377, 398, 424	6.56	ζ-carotene
4	120–170	88:10:2	pale yellow	399, 422, 449	6.98	β-zeacarotene

Thin-Layer Chromatography

band	R_f	color	abs max, nm	HPLC ret time, min	identifcn
1	0.83	yellow	420, 441, 469	7.37	α-carotene
2	0.60	orange	424, 448 479	7.75	β-carotene
3	0.36	yellow-green	379, 398, 423	6.56	ζ-carotene
4	0.20	pale orange	400, 424, 450	6.98	β-zeacarotene
5	0.13	yellow	440, 465, 488	6.13	γ-carotene
6	0.02	red	444, 475, 502	4.86	lycopene

To assess the effects of sampling conditions upon carrot carotenes, lyophilized samples of B6274 and HCM from El Centro, CA, were handled in the following "nonstandard" ways: Samples were grated to produce about 20 small fragments instead of using one slice; samples were saponified as described by DeRitter and Purcell (1981) before Na_2SO_4 filtration; comminution was performed with a N_2 (not air) atmosphere above sample; carotenes were extracted 7 months (not less than 1 month) after lyophilization; carotene extracts were stored under N_2 (not air) atmosphere in amber bottles; and carotene extracts were stored 2 weeks (not 5 days) before analysis.

RESULTS AND DISCUSSION

Identification and Quantification of Carotenoids. Separation of carotenes from both typical and dark orange carrots with silica column chromatography yielded very little mono- or polyhydroxylated carotenoids (fractions II or III). The maximum absorbance found in scanning these fractions from 350 to 520 nm was at most 0.5% of the maximum absorbance found in scanning fraction I.

Separation of fraction I from silica column chromatography on magnesia columns produced four distinct colored fractions (Table I). Fractions 1 and 2 cochromatographed with purchased α- and β-carotene samples available and had typical absorption spectra for these compounds (Davies, 1976). Fraction 3 had the absorption spectrum and yellow-green color of ζ-carotene (Davies, 1976). Fraction 4 had spectral maxima similar to that of β-zeacarotene, although the 449-nm maximum was lower than that of published spectra (Petzold et al., 1959). β-Zeacarotene might be expected in carrots since it is a precursor to β-carotene in biosynthetic schemes (Bauernfeind, 1972), and it has been reported before (Ogunlesi and Lee, 1979). Presuming fraction 4 to be β-zeacarotene and correcting for the specific extinction coefficient (Davies, 1976), the relative quantities of β-, α-, ζ-, and β-zeacarotene in HCM grown in California (1984) were 54:39:5:2 from magnesia column chromatography. The relative quantities of these carotenes in B6274 were 58:36:4:2 by this method. Cochromatography of carrot carotenes with lycopene increased absorbance of fraction I from the silica column, but upon separation on a magnesia column, no distinct band appeared.

Separation of carotenes from both typical and dark orange carrots with TLC yielded six bands visible in white light (Table I). Bands 1–4 proved to have all of the characteristics of fractions 1–4 separated by column chromatography, as determined by absorption spectra, HPLC retention, and cochromatography of magnesia column fractions and TLC bands on both systems. Cochromatography of TLC bands 5 and 6 with purchased γ-carotene and lycopene, respectively, using TLC and HPLC and absorption spectra of these bands verified their identification. From HCM and B6274 (California, 1984) the relative quantities of α-, β-, ζ-, β-zea-, and γ-carotene and lycopene bands from TLC were 51:40:4:2:2:1 and 57:36:3:2:1:1, respectively, which was very similar to values obtained with column chromatography. A sample of δ-carotene was available (Hoffmann-LaRoche), but this compound was not detected in carrot samples analyzed with TLC or HPLC.

Cis–trans carotene isomers were not separated by any of the systems used in this study. However, inspection of absorption spectra suggested the presence of little or no cis isomers since the characteristic 300–360-nm "cis peak" (Sweeney and Marsh, 1971; Schwartz and Patroni-Killam, 1985) was not evident in samples from either column chromatography fractions or TLC bands. This is a relatively insensitive method for detecting *cis*-carotene isomers in this study since the absorbance of the "cis peak" is only 10% that of the absorption maxima for pure *cis*-carotene and typically 91–98% of α- and β-carotene are trans isomers (Schwartz and Patroni-Killam, 1985; Jensen et al., 1987). Yet we were able to note a distinct "cis peak" (~3% the height of the absorption maxima) in the α- and β-carotene absorption spectra from a canned carrot sample (data not presented). This observation agrees with the reports of Schwartz and Patroni-Killam (1985) where processing increased the quantity of isomerized carotene severalfold.

Identification of carotenes separated with HPLC and detected at 436 nm (Figure 1) was established by cochromatography with purchased standards and with bands isolated from TLC. No peak was found in carrot samples with a retention time similar to that of the internal standard (none closer than 0.3 min). Quantification of α-, β-, and γ-carotene and lycopene was accomplished by using standard curves of compounds available, with correction made for impurity based on application of Beer's law. The β-zea- and ζ-carotene quantities from HPLC were estimated by multiplying observed peak areas by 0.85 and 3.7, respectively, since A_{436} of β-carotene is approximately 85% that of β-zeacarotene (Petzold et al., 1959) and approximately 3.7 times that of β-carotene (Zscheile and Porter, 1947). The corrected peak areas were then used to obtain values from the β-carotene standard curve. With this procedure to estimate individual carotenes separated by HPLC, the relative quantities of α-, β-, ζ-, β-zea-, and γ-carotene and lycopene in HCM and B6274 (California,

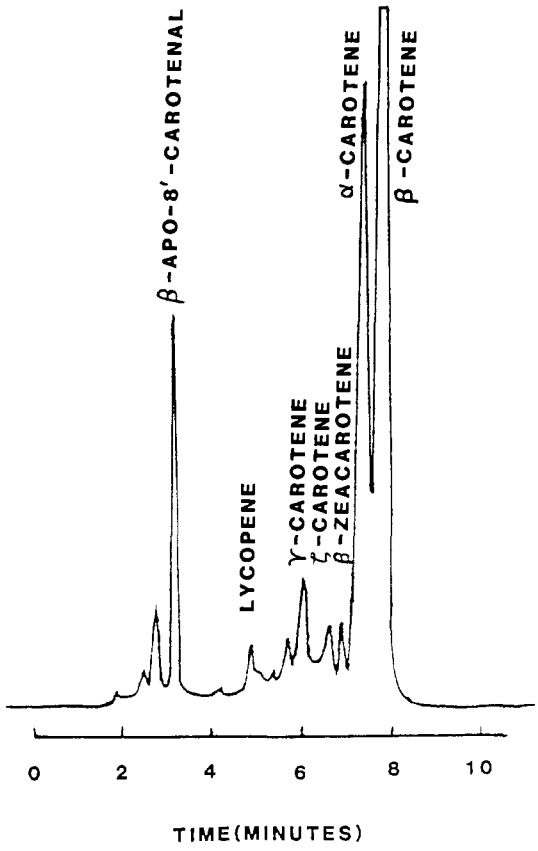

Figure 1. Chromatogram of carrot carotenoids (line HCM) separated on a Partisil-5 ODS-3 column and detected at 436 nm as described in the text.

1984) were 53:38:4:2:2:1 and 59:34:3:2:1:1, respectively. Thus, carrot carotenes separated with column chromatography, TLC, and HPLC yielded comparable results.

The HPLC eluants from α- and β-carotene fractions from column chromatography and TLC and from carrot extracts were monitored at both 436 and 360 nm, and little or no isomerization was indicated since the 340 nm/436 nm ratios were 0.074 and 0.077, respectively. These values are less than the values Schwartz and Patroni-Killam (1985) indicated as suggestive of isomerization. Interestingly, these ratios were 0.088 and 0.097 for the purchased α- and β-carotene standards. The canned carrot sample yielded values of 0.143 and 0.151 for α- and β-carotene, respectively, to suggest isomerization. This supported earlier reports and our column chromatography and TLC results, which indicated that isomerization of α- and β-carotene was minimal in raw carrot extracts and in fractions from column chromatography and TLC but did occur in canned carrots. Chromatographic separation of isomers would be necessary to quantify this conversion.

Sample Preparation and Carotene Extraction. The β-carotene standard curve used to estimate total carotenoids closely approximated linearity ($R^2 > 0.97$) as did the α- and β-carotene standard curves used to quantify HPLC peaks ($R^2 > 0.95$ without correction for the apo-8′-carotenal internal standard, $R^2 > 0.99$ with correction). The coefficient of variability for all carrot samples (each run in three or four replicates) was always less than 8%, which compares well with the accuracy of serum and plant carotenoid quantification by HPLC from other reports (Zakaria et al., 1979; Bushway and Wilson, 1982; Katrangi et al., 1984; Tangney, 1984; Bieri et al., 1985; Miller and Yang, 1985; Nierenberg, 1985; Bureau and Bushway, 1986; Bushway, 1986).

Although carotenoids in carrot are susceptible to breakdown when heated during drying and stored at room temperature (Arya et al., 1979), lyophilization yielded slightly more total carotenoids and more α- and β-carotene than frozen or raw carrot samples (significant difference at the 5% confidence level for the lyophilized–raw comparison by Waller–Duncan LSD; Table II). In one experiment, lyophilized and frozen samples of B9692 were comminuted with the Virtis homogenizer (VH) or Waring Blendor (WB) using hexane or AHW (acetone–hexane–water, 3:2:1). Comparing all other combinations of sample preparation, grinder, and solvent to lyophilized samples comminuted with the Virtis homogenizer in hexane as 100%, the following results were obtained: frozen/VH/AHW, 98%; frozen/WB/AHW, 95%; lyophilized/VH/AHW, 94%; lyophilized/WB/hexane, 67%; lyophilized/WB/AHW, 62%; frozen/WB/hexane, 52%; frozen/VH/hexane, 47%. Although samples were colorless upon filtration (with one exception noted below), use of the high-speed Virtis homogenizer contributed to somewhat higher carotene levels for frozen samples. For lyophilized samples, use of the Waring Blendor did not completely extract carotene from carrot root fragments even after 10–15 min of grinding. When frozen samples were comminuted in hexane, the water available in the carrot root slices clouded the hexane and greatly reduced the efficiency of carotene extraction. Perhaps factors such as the reduced exposure to room temperature, dim light, acetone and/or water during extraction concomitant with the penetration of hexane and elimination of $2^1/_2$-h phase separation account for the superior extraction of carrot carotenes with hexane from lyophilized samples. Schadle et al. (1983) also found significantly more carotene recovery in lyophilized samples than in raw samples. In addition to the apparent improved efficiency of carotene extraction from lyophilized samples, lyophilization also yielded time savings for extraction. Although the time required for comminuting samples by each method was the same, the opportunity to delete $2^1/_2$ h for phase separation allowed approximately 15 more lyophilized samples to be prepared per day.

Table II. Carotenes[a] from Lyophilized, Frozen, and Raw Samples of Three Carrot Lines

carrot line	sample	total carotenoids	HPLC α-carotene	HPLC β-carotene	HPLC total carotene
B6274	lyophilized	92	34	60	101
	raw	86	32	52	94
	frozen	84	31	51	92
B9692	lyophilized	186	61	138	209
	raw	175	66	117	194
	frozen	176	66	116	192
HCM	lyophilized	520	203	282	533
	raw	490	206	251	504
	frozen	483	204	255	507

[a] In ppm, fresh-weight basis, average of five replicates.

A tendency to underestimate total carotenoids by the evaluating A_{450} of the hexane extract as compared to summing individual carotenes separated with HPLC was observed throughout this research (Table II). Part of this underestimation, which ranged from 3% to 11%, may be due to the higher absorbance of β-carotene than all others in carrot, at A_{450} (Zscheile and Porter, 1947). Since total carotenoid estimation at A_{450} was based upon a standard curve of pure β-carotene, all other carotenes were therefore less efficiently estimated.

Six variations in the standard method were evaluated (Table III). Slices of carrot roots are convenient for an-

Table III. Carotenes[a] Extracted from Two Carrot Lines with Several Sampling Treatments

	line B6274				line HCM			
treatment	total carotenoids	α-carotene	β-carotene	total HPLC carotene	total carotenoids	α-carotene	β-carotene	total HPLC carotene
standard method[b]	92	34	60	101	520	203	282	533
grated	90	34	59	100	524	202	280	530
saponified extract	94	33	63	103	528	205	284	537
carotenes extracted under N_2	94	33	62	102	527	203	285	536
carotene extract stored under N_2	96	37	62	106	523	207	284	539
carotenes extracted 7 months after lyophilization	68	35	33	74	369	169	226	403
carotene extract stored 2 weeks	85	42	48	97	502	229	256	513

[a] In ppm, fresh-weight, average of five replicates. [b] Standard method: single carrot slice from midroot, lyophilized, and carotenes extracted in air atmosphere within 1 month of lyophilization; carrot extract stored in air atmosphere at −22 °C and analyzed within 5 days; total carotenoids estimated from A_{450} of hexane extract; α- and β-carotene quantified with HPLC.

Table IV. Carotenes in Seven Carrot Lines Grown in Several Years and Locations

	location and year				
line	CA 1984	CA 1985	WI 1985	FL 1986	CA 1986
HCM	533 (53:38:4)[a]	548 (65:25:5)	452 (44:40:10)	251 (68:20:7)	517 (60:28:7)
B6274	101 (59:34:3)	132 (74:21:2)	90 (57:35:4)	63 (67:27:2)	104 (73:21:2)
B2566	98 (69:25:2)	144 (74:16:4)	111 (64:29:3)		108 (71:23:2)
F524		113 (76:18:2)	142 (53:40:3)		176 (61:32:2)
B3080	104 (65:27:4)	156 (79:13:3)	137 (60:33:3)		145 (72:20:3)
B6439		173 (74:21:2)	137 (57:36:4)		186 (61:30:3)
B9692	209 (66:29:2)	213 (78:15:2)			

[a] Sum of carotenes from HPLC, ppm, fresh-weight basis (% β-carotene:% α-carotene:% ζ-carotene), average of five replicates.

alyzing individual roots, but grated samples are desirable for assessing population means since the gratings from different roots can be readily mixed to yield "average" samples for analysis. Grating increases surface area and cell breakage and therefore may be expected to increase carotene degradation, but no effect of grating was reflected in individual or total carotene quantities. As Bushway and Wilson (1982) also found in their work, saponification did not alter carrot carotene levels measured in this research. Neither extraction of carotenes from lyophilized carrot under N_2 nor storage of the extract under N_2 altered carotene content.

Long-term storage of lyophilized carrot samples for 7 months at −22 °C reduced the carotenoid content by approximately 27% for both the low-carotene inbred carrot line B6274 and the high-carotene line HCM (Table III). The ratios of β-carotene to α-carotene in standard samples of these two carrot lines from this California planting were 71:29 and 60:40, respectively, but 7-month storage reduced relative content of β-carotene to make these ratios 59:41 and 56:44, respectively. Storage of carotene extracts also affected both total carotenoid levels and individual carotene content. Two-week storage of extracts reduced total carotenoid content by approximately 6%, most of which was reflected by β-carotene loss as the β-carotene to α-carotene ratios dropped to 62:38 and 58:42 for B6274 and HCM, respectively. Thus, it was demonstrated that the storage of lyophilized carrot roots and of extracted carotenes at −22 °C should be kept at a minimum.

Genetic and Environmental Variation in Carrot Carotenes. Both total carotene content and relative amount of individual carotenes varied significantly over genotypes and location/years (Table IV). The dark orange line HCM always contained at least twice as much carotene as any other line tested and has the highest carotene content for carrot thus far reported. Lines B6274 or B2566 tended to contain the least of those tested except for California-grown carrots in 1985 when line F524 had the lowest carotene content. Lines F524 and B3080 tended to vary greatly between location/years. No general trends were observed between location/years except that carotene content was very low for the two lines included in Florida, 1986. This is probably attributable to cold weather, which yielded smaller and less physiologically mature roots. Less mature carrots have previously been reported to contain less carotene (Werner, 1941; Fritz and Habben, 1977).

The predominant carotenoid in all samples was β-carotene, accounting for 44–79% of all carotenes quantified (Table IV). The high-carotene line HCM had the lowest relative amount of β-carotene and tended to have the highest relative amounts of all other carotenoids in all location/years. Among the other lines tested there was no general relationship between total carotene content and relative β-carotene amount. ζ-Carotene accounted for 2–4% of the total carotenes in all lines except HCM where it ranged from 4% to 10%. Carrots from Wisconsin, 1985, tended to have higher relative amounts of α-carotene. From 94% to 97% of the total carotenes was accounted for by β-, α-, and ζ-carotene in all samples. The remaining 3–6% was distributed between β-zeacarotene, γ-carotene, and lycopene in a ratio of 1–3%:1–2%:1%.

The variation in individual carotene quantities of carrot due to genotype and environment reported here plus the advantages of HPLC quantification of carotenes, as compared to other techniques (Simpson, 1983), support the use of HPLC in the genetic selection of high provitamin A carotene carrots. Sample preparation need not include saponification or protection from air atmosphere, but storage of lyophilized samples and hexane extracts should be kept to a minimum. Six carrot carotenes can be readily separated and quantified with HPLC using isocratic, reversed-phase HPLC. Verification of the high provitamin A carotene content in line HCM grown in several years and locations suggests the possibility of developing carrot genetic stocks to aid in improving human vitamin A status.

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Registry No. α-Carotene, 432-70-2; β-carotene, 7235-40-7;

γ-carotene, 472-93-5; ε-carotene, 472-92-4; β-zeacarotene, 514-90-9; lycopene, 502-65-8.

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