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Provitamin A and Carotenoid Content of Citrus Juices

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The carotenoid content in juice of seven citrus cultivars was followed during the fruit maturation period. The analyses were performed with high-performance liquid chromatography (HPLC) procedures that are rapid and reproducible. Using these methods, isomers of cryptoxanthin as well as the carotenes were separated and determined for calculating provitamin A values. β-Cryptoxanthin readily separated from α-cryptoxanthin. The β isomer was found to be the main source of provitamin A in citrus juice while the structure of the α form would indicate that it is nonactive. The provitamin A content of citrus juice varies with fruit cultivar. Juice of the sweet orange cultivars had the lowest provitamin A carotenoid content while that of Dancy tangerine and Robinson were intermediate. The provitamin A content of Murcott was found to be the highest among all the cultivars studied.

The provitamin A value and color of citrus juice is due to a complex mixture of carotenoids (Curl, 1953; Curl and Bailey, 1956; Yokoyama and White, 1967; Gross et al., 1971; Subbarayan and Cama, 1965). Numerous papers published over the past 25 years on carotenoids in citrus have been concerned mainly with extraction and identification. There have been only fragmentary reports on the amounts of the various pigments that occur in the juice. The lack of quantitative information of these compounds has been due to their unstable nature and unsatisfactory analytical methods. Previously, separation has been by open column and thin-layer chromatography; and with these procedures, it has been difficult to obtain reproducible results. During the past 8 years, work has been going on in this laboratory using high-performance liquid chromatography (HPLC) for the quantitative determination of carotenoids (Stewart and Wheaton, 1971, 1972, 1973; Leuenberger and Stewart, 1976; Leuenberger et al., 1976). The purpose of this paper is to describe some of the procedures which have been developed and to report on the amounts of the major carotenoids found in the juice of several citrus cultivars.

EXPERIMENTAL SECTION

Samples. Fruit representing seven citrus cultivars, Hamlin, Pineapple, and Valencia orange, Dancy tangerines, and three hybrids, Orlando, Robinson, and Murcott were used. Sampling commenced in October when all varieties except Valencia were starting to mature and continued at approximately 2-week intervals through March. A sample consisting of 24 fruit was selected at random around the outside of the trees. For juice color determinations, 12 fruit were extracted with an electric hand reamer and the juice filtered through a 17 mesh stainless steel screen. A 75-mL aliquot was deaerated for 30 min in a desiccator jar using a water aspirator to draw a vacuum. Duplicate color readings were made with a Hunterlab Model D-45 Citrus Colorimeter (Huggart et al., 1969; Hunter, 1967). For carotenoid analyses, the remaining 12 fruit from the sample were extracted. The juice from the extractor flowed directly into 50 mL of a solution containing 10 g of KOH. The juice was made alkaline to reduce isomerization of the epoxide containing carotenoids. The remainder of the extraction procedure has previously been reported (Stewart, 1977).

Apparatus. The carotenes and cryptoxanthin with the exception of phytofluene were separated on a MgO column (Stewart, 1977) (Figure 1). Phytofluene was chromatographed on the same MgO column but eluted isocratically with 1% acetone in hexane at the rate of 2.5 mL/min. The detector was an Aminco Fluoro-Monitor using a primary filter, Corning 7-51, and a secondary filter Wratten No. 8.

The xanthophylls were chromatographed on a high-capacity silica pellicle, Pellosil (Reeve Angel), a method reported for the first time in this paper (Figure 2). The HPLC was carried out using a Milroyal D high-pressure pump (Milton Roy Co.) and a Technicon No. 1 detector with a 440-nm filter. The hookup was similar to that reported by Stewart and Wheaton (1971). The column was made of stainless steel tubing $^3/_{16}$ in. o.d. × ca. 3 mm i.d. × 13 cm with reducers (Swagelok) $^3/_{16}$ in. to $^1/_{16}$ in. on each end. Reducers were plugged with porous Teflon discs. The column was packed by tap-filling. A water jacket was constructed from $^1/_2$ in. copper tubing and the column maintained at 16 °C with a circulating water bath.

A drip-type gradient was constructed from two beakers (Konte K-42001, Konte Glass Co.) similar to that previously reported (Stewart, 1977). Some changes were necessary to give a satisfactory chromatogram. The polar solvent was dripped into hexane at the rate of 0.5 mL/min. The flow rate was achieved by raising the reservoir 20 cm above the end of the dripping tube. The diameter of the dripping tube also controlled the flow rate of the polar solvent. Teflon tubing, size 18, 0.042 in. o.d. × 9.5 cm (Allied Electronics) was attached to the reservoir. A

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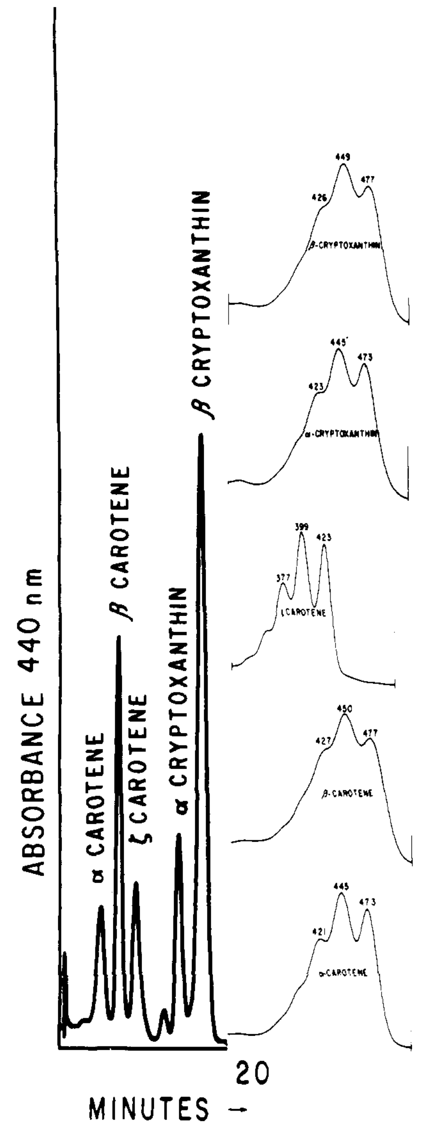

Figure 1. HPLC chromatogram and visible absorption spectra of the principal carotenes and cryptoxanthin in citrus juice.

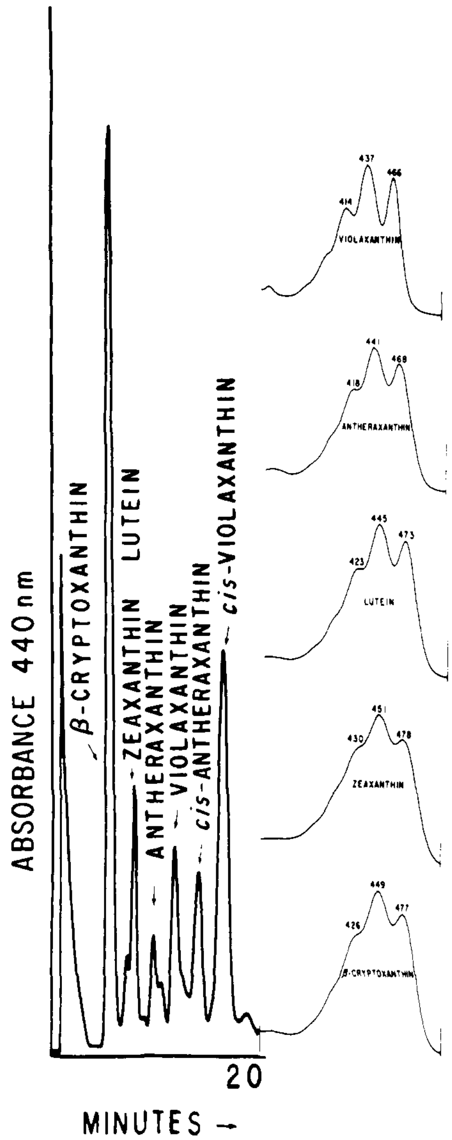

Figure 2. HPLC chromatogram and visible absorption spectra of the principal xanthophylls in citrus juice.

smaller tube $^{1}/_{16}$ in. o.d. × 0.012 in. i.d. × 8 cm (Chromatronix) was inserted into the end of the larger tube to restrict flow. The gradient mixture consisted of the polar solvent, 100 mL 20% *tert*-pentyl alcohol (TPA) (distilled over KOH) in freshly distilled hexane in the reservoir. The gradient was started by dripping this mixture into 35 mL of hexane in the mixing chamber.

Chromatography. For the separation of the xanthophylls, a sample of carotenoids in benzene (5–25 μL) was injected on the column and the solvent pumped at the rate of 1.0 mL/min. After the pigments were eluted, the column was washed for 1 min with acetone to which 1% water had been added. The column was then washed with hexane for 10 min at the rate of 2 mL/min.

Standards. Concentration curves were made for each carotenoid according to previous procedures (Stewart, 1977). In addition, the following extinction coefficients were used: ζ-carotene 2270 at 400 nm in hexane, lutein 2545 at 446 nm in ethanol, antheraxanthin 1908 at 442 nm in ethanol, and violaxanthin 2216 at 454 nm in benzene. Phytofluene was isolated from carrot oil and used for determining a concentration curve.

RESULTS AND DISCUSSION

The citrus color score values obtained are the basis for Florida Department of Citrus and USDA juice standards. Orange juice must have a color score of 36–40 points to be classified as Grade A, 32–35 for Grade B, and a color score of 31 or below is substandard. Sufficient color to make Grade A juice was not generally attained by oranges in this study until January (Figure 3). Valencia is the main orange cultivar grown in Florida and it does not reach marketable maturity until about March. Color determinations we have made in previous years have shown that a color score of 40 for Valencia juice may be reached late in the season. Usually, in Florida, substantial amounts of the early and midseason fruit, Hamlin and Pineapple, respectively, are processed prior to the time that the juice has sufficient color for Grade A. Dancy tangerine and Robinson, Orlando, and Murcott juice attained a high color score early in the season prior to the time they were suitable for consumption. The differences in color between orange juice and that of the other cultivars may be even greater than the color scores would indicate. This is because the instrument was not designed for evaluating color values in the high ranges such as shown by the tangerine and hybrid juice.

The carotenoids determined in this study are not the only ones in citrus juice; however, they are the primary determinates of color. Pigments found in the largest amounts were violaxanthin, antheraxanthin, and cryp-

Table I. Carotenoid Content (in μg/100 mL) of Juice from Seven Citrus Cultivars

Cultivar	Sampling dates 10-7	10-27	11-19	12-19	1-7	1-28	2-23	3-30
				Phytofluene				
Hamlin	0.8 ± 0.1[a]	0.9 ± 0.0	0.7 ± 0.0	2.6 ± 0.1				
Pineapple		0.4 ± 0.0	0.8 ± 0.0	2.5 ± 0.2				
Valencia				1.1 ± 0.1				
Robinson	2.6 ± 0.2	11.4 ± 0.0	16.2 ± 0.4	24 ± 0.4				
Dancy	3.8 ± 0.0	11.8 ± 0.4	11.2 ± 0.4	36 ± 0.2				
Orlando	0.6 ± 0.0	2.1 ± 0.1	2.7 ± 0.0	10.3 ± 0.2				
Murcott		4.1 ± 0.0	12.3 ± 0.4	80 ± 2.0				
				α-Carotene				
Hamlin	0.8 ± 0.0	0.4 ± 0.0	0.7 ± 0.0	0.7 ± 0.0	1.3 ± 0.1	1.7 ± 0.1	2.6 ± 0.3	2.4 ± 0.1
Pineapple	0.6 ± 0.0	0.7 ± 0.0	1.6 ± 0.1	3.0 ± 0.1	3.9 ± 0.5	4.2 ± 0.2	4.0 ± 0.3	6.5 ± 0.1
Valencia	0.8 ± 0.0	0.4 ± 0.0	0.7 ± 0.0	2.0 ± 0.1	2.5 ± 0.0	5.6 ± 0.0	5.1 ± 0.2	7.0 ± 0.0
Robinson	3.4 ± 0.0	9.0 ± 0.5	11.2 ± 0.1	9.0 ± 0.0	13.2 ± 1.2			
Dancy	4.6 ± 0.2	8.0 ± 0.0	6.2 ± 0.4	22.5 ± 1.5	13.8 ± 0.4	17.5 ± 1.5	28.2 ± 0.0	
Orlando	1.7 ± 0.1	3.4 ± 0.1	3.2 ± 0.1	9.0 ± 0.1	9.8 ± 0.7	9.9 ± 0.2	11.7 ± 0.3	
Murcott	1.4	4.0 ± 0.1	6.0 ± 0.8		11.0 ± 1.2	7.5 ± 0.0	18.0 ± 0.0	11.0 ± 0.0
				β-Carotene				
Hamlin	1.6 ± 0.1	1.1 ± 0.0	1.5 ± 0.1	1.8 ± 0.1	3.0 ± 0.1	3.9 ± 0.1	5.2 ± 0.1	5.7 ± 0.1
Pineapple	0.7 ± 0.0	1.1 ± 0	2.3 ± 0.0	6.2 ± 0.1	4.6 ± 0.3	6.1 ± 0.1	8.6 ± 0.0	11.9 ± 0.4
Valencia	0.8 ± 0.1	0.6 ± 0.1	0.6 ± 0.0	1.9 ± 0.0	3.4 ± 0.2	6.2 ± 0.2	8.1 ± 0.1	8.9 ± 0.0
Robinson	6.0 ± 0.3	24.5 ± 0.0	32.8 ± 0.0	51.0 ± 0.0	59.3 ± 0.8			
Dancy	6.6 ± 0.3	18.8 ± 0.4	18.7 ± 0.3	42.5 ± 3.5	56.7 ± 1.4	52.5 ± 2.5	66.9 ± 0.9	
Orlando	1.7 ± 0.0	3.7 ± 0.0	5.8 ± 0.2	15.9 ± 0.0	16.3 ± 1.0	24.6 ± 1.0	32.6 ± 0.1	
Murcott	2.7 ± 0.0	9.4 ± 0.6	9.4 ± 0.6	43.5 ± 0.0	64.1 ± 1.1	66.0 ± 0.8	105.8 ± 1.6	237.5 ± 7.5
				ζ-Carotene				
Hamlin	4.5 ± 0.8	2.0 ± 0.4	2.7 ± 0.2	5.7 ± 0.2	11.0 ± 0.4	9.0 ± 0.5	9.4 ± 0.8	7.7 ± 0.0
Pineapple		1.6 ± 0.1	4.0 ± 0.2	7.9 ± 0.5	8.3 ± 0.0	10.6 ± 1.4	12.9 ± 0.0	16.2 ± 0.9
Valencia				2.6 ± 0.2	6.1 ± 0.0	11.8 ± 1.1	14.9 ± 0.2	13.1 ± 0.6
Robinson	18.4 ± 0.0	55.5 ± 0.0	56.4 ± 0.0	102 ± 9.0	101.0 ± 0.0			
Dancy	24.5 ± 0.0	61.4 ± 0.0	59.0 ± 0.0	147.0 ± 0.0	180.0 ± 0.0	153.0 ± 6.0	202.0 ± 0.0	
Orlando	3.4 ± 0.1	10.8 ± 0.2	15.2 ± 0.1	53.5 ± 1.5	60.7 ± 0.0	70.5 ± 2.5	67.5 ± 1.2	
Murcott	7.6 ± 0.0	27.7 ± 0.5	61.9 ± 6.4	215.0 ± 32.0	215.8 ± 13.8	271.5 ± 0.0	285.0 ± 0.0	437.5 ± 7.5
				α-Cryptoxanthin				
Hamlin	1.2 ± 0.1	0.5 ± 0.1	1.0 ± 0.0	1.0 ± 0.1	1.8 ± 0.1	2.6 ± 0.1	4.0 ± 0.2	3.2 ± 0.0
Pineapple	0.5 ± 0.0	0.5 ± 0.0	1.9 ± 0.1	4.2 ± 0.0	4.0 ± 0.3	7.3 ± 0.3	7.5 ± 0.1	11.0 ± 0.1
Valencia		0.3 ± 0.0	0.5 ± 0.0	2.6 ± 0.0	4.0 ± 0.0	10.5 ± 0.0	10.4 ± 0.1	12.2 ± 0.2
Robinson	11.2 ± 0.5	29.3 ± 2.3	37.6 ± 0.0	29.3 ± 2.3	38.0 ± 2.0			
Dancy	8.3 ± 0.6	15.0 ± 1.2	16.9 ± 1.7	29.5 ± 0.5	32.0 ± 0.0	19.5 ± 1.5	30.0 ± 2.4	
Orlando	1.4 ± 0.1	3.6 ± 0.0	6.2 ± 0.2	12.9 ± 0.3	11.8 ± 0.6	12.0 ± 0.6	20.7 ± 0.4	
Murcott	3.2 ± 0.0	15.0 ± 1.3	10.2 ± 0.2	39.0 ± 0.0	28.2 ± 1.2	28.9 ± 1.2	54.3 ± 0.9	63.3 ± 2.0
				β-Cryptoxanthin				
Hamlin	5.4 ± 0.3	3.5 ± 0.0	3.6 ± 0.1	11.2 ± 0.3	20.6 ± 0.2	20.0 ± 0.0	20.0 ± 0.7	34.6 ± 0.6
Pineapple	1.3 ± 0.1	2.7 ± 0.1	7.7 ± 0.4	11.3 ± 0.2	8.5 ± 0.5	21.8 ± 0.8	25.7 ± 0.2	52.3 ± 1.2
Valencia	0.5 ± 0.0	0.7 ± 0.0	0.6 ± 0.0	8.6 ± 0.6	14.4 ± 0.0	24.3 ± 0.4	37.9 ± 0.0	27.3 ± 0.0
Robinson	223.8 ± 6.4	427.0 ± 3.0	511.0 ± 0.0	565.5 ± 7.5	573.0 ± 15.0			
Dancy	105.5 ± 1.5	170.5 ± 4.1	147.6 ± 6.0	189.0 ± 21.0	242.0 ± 18.0	213.5 ± 2.5	429.9 ± 0.9	
Orlando	2.9 ± 0.1	9.3 ± 0.0	10.4 ± 0.5	24.0 ± 1.1	25.0 ± 1.7	28.9 ± 2.0	72.2 ± 1.9	
Murcott	73.2 ± 0.0	240.5 ± 1.5	194.0 ± 16.8	426.0 ± 9.0	425.3 ± 4.8	472.5 ± 63.5	612.0 ± 9.0	1466.0 ± 40.0

				Lutein				
Hamlin	9.1 ± 0.2	6.4 ± 0.1	3.7 ± 0.2	83.0 ± 1.1	15.0 ± 0.0	20.0 ± 1.6	47.0 ± 1.4	35.8 ± 0.2
Pineapple	16.6 ± 0.2	7.3 ± 2.0	22.0 ± 0.4	32.0 ± 3.1	44.8 ± 0.0	70.8 ± 0.4	67.6 ± 0.0	89.0 ± 2.6
Valencia	9.7 ± 0.4	5.1 ± 0.1	2.7 ± 0.2	26.6 ± 1.3	34.0 ± 1.6	83.5 ± 1.5	82.0 ± 5.6	60.8 ± 0.0
Robinson	37.1 ± 0.5	124.6 ± 0.6	128.8 ± 3.6	144.9 ± 4.5	128.5 ± 1.5			
Dancy	75.5 ± 5.5	111.4 ± 5.0	117.4 ± 4.2	173.8 ± 6.2	225.5 ± 0.5	195.6 ± 6.0	266.0 ± 0.0	
Orlando	27.2 ± 0.6	51.0 ± 1.2	45.6 ± 3.4	76.2 ± 1.7	140.0 ± 0.0	186.8 ± 3.6	194.6 ± 4.0	
Murcott	19.3 ± 0.5	48.0 ± 0.8	86.8 ± 2.8	85.5 ± 4.5	131.0 ± 6.0	85.8 ± 0.6	167.4 ± 12.6	175.2 ± 4.0
				Antheraxanthin				
Hamlin	14.3 ± 0.1	8.1 ± 0.2	11.1 ± 0.0	12.0 ± 0.3	22.0 ± 0.5	33.0 ± 5.0	62.6 ± 1.0	49.8 ± 0.0
Pineapple	11.4 ± 0.2	5.6 ± 0.5	17.0 ± 0.3	20.8 ± 1.3	35.7 ± 0.0	47.4 ± 2.4	65.5 ± 3.9	87.0 ± 1.0
Valencia	5.3 ± 0.1	3.6 ± 0.6	4.5 ± 0.3	33.4 ± 2.1	24.0 ± 2.4	66.0 ± 1.5	84.0 ± 1.0	72.3 ± 3.9
Robinson	45.8 ± 1.0	114.2 ± 2.6	120.0 ± 4.0	131.1 ± 7.5	159.5 ± 10.5			
Dancy	53.0 ± 0.6	91.4 ± 2.2	60.4 ± 1.6	96.6 ± 5.0	174.0 ± 4.0	153.9 ± 4.5	212.7 ± 10.5	
Orlando	18.4 ± 0.1	60.0 ± 0.1	33.0 ± 3.3	70.2 ± 1.3	127.2 ± 0.0	173.8 ± 3.8	234.6 ± 11.8	
Murcott	14.0 ± 0.3	44.8 ± 0.0	61.0 ± 3.0	48.0 ± 3.6	139.5 ± 11.5	106.8 ± 1.2	209.6 ± 10.4	248.4 ± 10.0
				Violaxanthin				
Hamlin	14.1 ± 0.1	8.5 ± 0.4	9.5 ± 0.0	11.7 ± 1.6	18.0 ± 0.0	23.8 ± 2.4	38.7 ± 0.5	33.4 ± 1.6
Pineapple	14.0 ± 0.4	6.2 ± 0.1	10.2 ± 0.1	23.5 ± 0.1	34.9 ± 0.0	50.3 ± 0.5	49.6 ± 3.4	52.9 ± 0.5
Valencia	9.0 ± 0.4	7.6 ± 0.1	12.0 ± 0.4	59.3 ± 4.7	35.8 ± 0.9	78.6 ± 2.6	92.7 ± 3.3	74.6 ± 0.0
Robinson	63.0 ± 0.0	138.0 ± 0.8	140.0 ± 4.4	198.9 ± 9.9	183.5 ± 3.5			
Dancy	17.9 ± 0.7	29.0 ± 1.8	21.8 ± 1.8	34.2 ± 1.4	57.6 ± 0.0	60.6 ± 0.6	52.2 ± 3.0	
Orlando	12.3 ± 0.9	34.1 ± 1.1	32.0 ± 1.0	37.2 ± 1.3	67.4 ± 0.6	97.1 ± 11.9	83.4 ± 1.0	
Murcott	21.8 ± 0.4	47.4 ± 1.0	67.2 ± 3.2	44.1 ± 3.3	114.5 ± 1.5	90.0 ± 5.4	155.4 ± 0.0	165.0 ± 9.0
				cis-Antheraxanthin				
Hamlin	34.2 ± 1.9	27.7 ± 0.2	37.7 ± 1.7	33.7 ± 1.9	44.5 ± 1.0	53.3 ± 1.5	97.4 ± 2.2	110.8 ± 4.0
Pineapple	33.0 ± 0.5	13.8 ± 0.3	58.3 ± 1.8	80.1 ± 3.7	90.1 ± 3.0	138.3 ± 1.5	100.2 ± 3.6	240.0 ± 6.0
Valencia	10.1 ± 0.6	7.9 ± 0.4	15.4 ± 0.6	62.8 ± 1.0	72.5 ± 3.7	180.8 ± 8.0	147.8 ± 7.4	170.5 ± 7.3
Robinson	97.5 ± 4.5	199.4 ± 10.6	295.8 ± 7.0	281.1 ± 2.1	243.5 ± 6.5			
Dancy	130.5 ± 1.5	187.4 ± 3.0	154.4 ± 0.0	265.4 ± 13.4	350.0 ± 0.0	404.8 ± 5.6	378.6 ± 7.8	
Orlando	76.3 ± 1.3	143.0 ± 3.0	95.5 ± 5.5	152.0 ± 4.0	277.0 ± 0.0	354.2 ± 13.4	334.0 ± 7.2	
Murcott	57.0 ± 3.0	119.0 ± 5.0	274.4 ± 7.6	232.2 ± 6.0	250.0 ± 0.0	235.0 ± 22.0	413.5 ± 19.1	840.0 ± 32.0
				cis-Violaxanthin				
Hamlin	32.9 ± 0.2	21.4 ± 0.4	30.7 ± 1.5	27.6 ± 1.0	48.9 ± 0.2	55.8 ± 1.0	94.7 ± 4.3	110.8 ± 4.0
Pineapple	41.1 ± 0.3	16.8 ± 1.4	22.1 ± 0.1	95.1 ± 0.4	133.0 ± 3.0	177.0 ± 3.6	115.2 ± 4.0	240.0 ± 6.0
Valencia	22.4 ± 0.8	18.1 ± 0.4	36.0 ± 0.0	115.0 ± 0.0	154.0 ± 10.0	317.0 ± 1.0	216.0 ± 4.0	170.5 ± 7.3
Robinson	163.0 ± 3.0	304.0 ± 4.0	436.0 ± 12.0	452.7 ± 2.7	528.0 ± 8.0			
Dancy	41.8 ± 0.9	67.0 ± 2.2	53.2 ± 0.8	87.0 ± 5.0	206.0 ± 0.0	222.6 ± 4.8	191.4 ± 0.0	
Orlando	42.3 ± 1.8	76.3 ± 2.5	112.0 ± 7.0	67.0 ± 0.9	160.0 ± 4.0	184.8 ± 6.0	158.2 ± 2.6	
Murcott	106.0 ± 2.0	223.2 ± 0.0	414.8 ± 25.2	304.2 ± 4.8	445.0 ± 2.0	396.9 ± 17.1	620.4 ± 40.0	840.0 ± 32.0

[a] Standard error of mean of duplicate determinations.

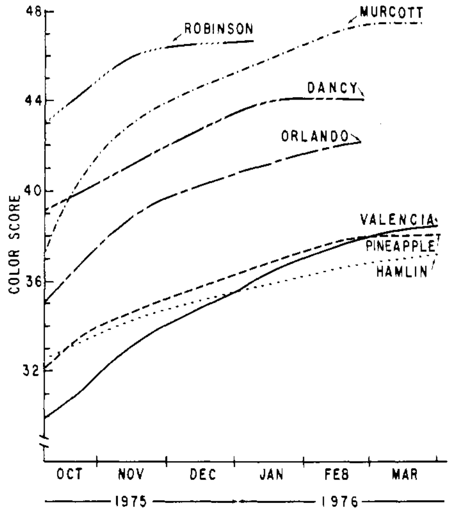

Figure 3. Changes in color score of juice from seven citrus cultivars during maturation of the fruit.

Table II. Provitamin A Content of Citrus Juice[a]

Cultivar	Vitamin A[b] value, international units	Daily recommended percentage[b]
Hamlin	80	1.6
Pineapple	133	2.7
Valencia	83	1.7
Robinson	1142	23
Dancy	965	19
Orlando	236	4.7
Murcott	3195	64

[a] Values obtained from the last date sampled for each cultivar, Table I. Calculations based on: β-carotene equals 1.667 international units vitamin A/μg, equals 100%; α-carotene, 52.7%; β-cryptoxanthin, 57%. Conversion factor from 100 mL of juice to 6 oz, 1.77 (Bauernfeind et al., 1971). [b] Values based on 6 oz of juice and calculated on the dietary daily allowance of 5000 IU.

toxanthin (Table I). But, each does not contribute equally to the color of juice and the contribution of each is not known. Antheraxanthin and violaxanthin are yellow pigments with the main cis forms of the latter in citrus having visible absorption maxima of 466, 437, and 414. Regardless of the amounts, if these two pigments were the only ones in citrus juice the color would be yellow. Cryptoxanthin is an orange carotenoid having visible absorption maxima of 477, 449, and 426. Increasing amounts of this pigment in juice imparts an orange color which is desirable. Cultivars having the highest color scores also had the largest total amount of carotenoids. This also included the relatively colorless compounds, phytofluene and ζ-carotene. The carotenoids continued to increase in the juice of most cultivars during the sampling period.

Provitamin A activity comes from a limited number of carotenoids. β-Carotene and carotenoids which possess one unsubstituted β end group are converted in mammals into vitamin A (Weeden, 1965). The principal provitamin A carotenoids occurring in citrus juice are α-carotene, β-carotene, and β-cryptoxanthin (Tables I, II). Since α-carotene and β-cryptoxanthin each have only one unsubstituted β end group, the provitamin A activity is calculated to be only one-half that of β-carotene (National Academy of Sciences, 1974). Because of the relatively large amounts of β-cryptoxanthin in citrus juice, this carotenoid must be considered as the main source of provitamin A. However, most of the information on provitamin A activity

Table III. Comparison of Duplicate Extractions and Duplicate Chromatograms of the Juice Carotenoid Content (μg/100 mL of Juice) from Three Citrus Cultivars

Samples		Carotenoids: α-Carotene	β-Carotene	ζ-Carotene	α-Cryptoxanthin	β-Cryptoxanthin	Lutein	Antheraxanthin	Violaxanthin	*cis*-Antheraxanthin	*cis*-Violaxanthin
Hamlin	1[a]	0.6	1.6	3.5	1.1	8.5	9.7	11.3	8.7	32.2	25.6
	2	0.7	1.6	3.7	1.1	8.1	9.2	11.5	9.1	30.0	24.7
	3	0.7	1.6	3.9	1.1	8.7	10.6	12.5	11.3	33.3	27.4
	4	0.7	1.6	3.6	1.1	8.6	10.5	12.8	11.1	35.0	26.3
	Av	0.7	1.6	3.7	1.1	8.5	10.0	12.0	10.0	33.0	26.0
		±0.0[b]	±0.0	±0.1	±0.0	±0.1	±0.3	±0.4	±0.7	±1.0	±0.6
Dancy	1	8.8	32.8	110.4	16.8	168	123.2	78.4	23.6	171.0	74.0
	2	8.8	30.8	95.6	12.8	158	114.0	82.4	29.2	206.8	87.6
	3	10.4	24.4	91.0	14.0	151	143.6	89.4	36.8	244.0	92.8
	4	10.4	26.8	81.0	13.2	141	126.4	82.4	21.2	194.8	70.0
	Av	9.6	28.7	94.5	14.2	155.0	127.0	83.0	28.0	205.0	81.0
		±0.5	±1.9	±6.1	±0.9	±5.7	±6.2	±2.3	±3.5	±15.0	±5.4
Orlando	1	4.2	5.8	15.9	5.7	8.4	27.8	45.8	27.2	69.0	40.6
	2	4.6	6.2	15.9	5.4	9.9	33.4	44.2	26.4	82.4	43.8
	3	4.6	5.9	14.7	5.2	9.3	32.2	45.2	22.0	71.2	41.6
	4	4.3	6.1	16.2	5.3	9.2	33.4	46.8	23.4	74.2	43.2
	Av	4.4	6.0	15.7	5.4	9.2	32.0	46.0	25.0	74.0	42.0
		±0.1	±0.1	±0.3	±0.1	±0.3	±1.3	±0.5	±1.2	±2.9	±0.7

[a] Samples 1 and 2 are duplicate chromatograms of one extraction. Samples 3 and 4 are duplicate chromatograms of a separate extraction. [b] Standard error of the mean.

is based on the amount of β-carotene alone (National Academy of Sciences, 1974). The reason β-cryptoxanthin has not been included has been due to the lack of suitable analytical techniques. Recently, Reeder and Park (1975) published on the provitamin A values of three samples of California Valencia orange juice in which cryptoxanthin was included. However, in their method no distinction was made between α-cryptoxanthin from β-cryptoxanthin. We have reported (Stewart, 1977) that α-cryptoxanthin isolated from citrus juice contained the OH group on the β ring and, therefore had no provitamin A activity. The provitamin A values varied greatly in citrus juice depending on the cultivar (Table II). Orange juice was found to have the least amount of any of the cultivars while Murcott had the highest. The latter cultivar was found to contain one of the highest amounts of provitamin A of any common fruits listed by Adams (1975). The provitamin A content of juice from all cultivars increased with maturity of the fruit. Although these calculations have not been included, it can be readily seen from an inspection of the amounts of provitamin A carotenoids in Table I.

The carotenoid content of citrus juices is usually reported as a percentage of the total, where the total is based on the extinction at the maximal absorption wavelength for β-carotene. Using HPLC, this technique of standardization has not been satisfactory. In this laboratory, two procedures have been used. The first consisted of isolating and crystallizing the major pigments and using these samples to run concentration curves. The advantage of this procedure is that the pigments can be positively identified. However, the procedure for isolation and purification is laborious and the samples once isolated are not stable even when stored under N at −25 °C. The other method and the one used for the data reported here was based on collecting the compounds as they were eluted from the column, and then determining an extinction for each pigment at three or more concentrations. The amount of each pigment was plotted against the area of the peaks as each carotenoid was eluted (Stewart, 1977).

The techniques used in this study are new; therefore, some indication of the validity of the methods would be useful (Table III). Samples of fruit were divided and separate extractions made on each. Each extract was chromatographed twice. Both the extraction and the chromatographic procedures gave reasonably good reproducible values. However, the main source of error in this study was probably sampling as the amounts of carotenoids fluctuated considerably from date to date. Citrus trees bloom and set fruit over a period of 1 month or longer. At the time of sampling, it was not possible to distinguish fruit set at one bloom date from another. Studies similar to the one reported in this paper were carried out for 3 years. Similar sampling dates and the same trees were used. However, each year analytical techniques were improved to increase the precision of the data. Nevertheless, the amounts of carotenoids and color scores were similar for these years. Also, in the interest of brevity, only the analysis of a limited number of samples are given in Table I.

Carotenoids are difficult to separate due to the similarity of structures; for example, lutein and zeaxanthin. These pigments can be separated on a $ZnCO_3$–$MgCO_3$ column, but the separation requires several hours (Stewart and Wheaton, 1971). The retention times of lutein and zeaxanthin may be reversed depending on the quantity of oils in the sample and other unknown factors. There is a mixture of zeaxanthin and lutein in citrus juice; however, numerous samples taken of the mixed band from the HPLC would indicate that lutein was the main pigment as determined by visible absorption spectra. It is for this reason that the mixture is reported as lutein in Table I.

The peaks labeled *cis*-antheraxanthin and *cis*-violaxanthin (Figure 2) are the main forms of these compounds to occur in citrus. The exact cis configuration has not been determined. The chromatographic bands are relatively pure as determined by numerous samples taken from single bands. These two carotenoid isomers can also be crystallized which is an indication that they are not isomer mixtures. The peaks labeled antheraxanthin and violaxanthin are both mixtures of isomers. The trans and the 5,8 epoxide forms are found in these bands. We have been unable to crystallize them presumably because they are a mixture of isomers. The amounts of these isomers usually increase with aging of the sample or passing them through certain silica or alumina columns. Although considerable effort has been made to determine if this group of isomers occurs in nature, it still remains to be determined if they are artifacts formed during extraction.

Data in this paper were obtained by techniques that represent substantial improvements over conventional procedures. The HPLC methods increased sensitivity and resolution and eliminated the need for packing a new column for each sample. Equally important was the use of a flow cell and detector in place of collecting each band from the column, evaporating the solvent, making to a standard volume, and determining the extinction. Finally, the method decreases the time the sample is on the column, thereby reducing the amount of decomposition and formation of artifacts.

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