Table II. Recoveries of Metalaxyl Added to Chopped Lettuce (mg/kg) Estimated with Method II

metalaxyl added	BP 10 column			SIL 5 column		
	control	spiked	% rec[a]	control	spiked	% rec
0.050	–[b]	0.044	88	–	0.043	86
0.050	–	0.043	86	–	0.048	96
0.050	–	0.038	76	–	0.044	88
0.050	–	0.037	74	–	0.043	86
av ± SD			81 ± 7.0			89 ± 4.8
0.50	–	0.44	88	–	0.41	82
0.50	–	0.49	98	–	0.45	90
0.50	–	0.44	88	–	0.47	94
0.50	–	0.42	84	–	0.43	86
av ± SD			89.5 ± 6.0			88 ± 5.2

[a] Difference between the amounts found in spiked and control samples, expressed as a percentage of the amount of metalaxyl added. [b] Below detection limit (0.01 mg/kg).

gives overlapping matrix peaks on the SIL 5 column (Figure 1B,D).

Method II has been used in our laboratory in the period 1985–1987 for determining residues of metalaxyl in about 10.000 lettuce samples. In 1987 method I was developed. To verify the reliability of method I, 200 field-treated samples were analyzed with both methods. When positive residues of metalaxyl were found, they were identified by both methods I and II. Metalaxyl concentrations of 23 samples were above 0.04 mg/kg. For these 23 observations the correlation coefficient between both methods is 0.9996.

CONCLUSION

Metalaxyl peaks can be identified on two columns in one run. Cleanup by solid-phase extraction saves work and chemicals. Comparing both methods, we may conclude that method II is more specific and more sensitive than method I. However, method I takes less time. Depending on the number of samples to be analyzed and the sensitivity required, a suitable method can be chosen.

Registry No. Metalaxyl, 57837-19-1.

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Detection of Adulteration of California Orange Juice Concentrates with Externally Added Carotenoids by Liquid Chromatography

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A liquid chromatographic procedure to separate and quantitate externally added common color adulterants to orange juice concentrates is described. The procedure involved treatment of extracted carotenoids with methanolic HCl to convert carotenoids with 5,6-epoxide end groups to 5,8-epoxides and measurement of HPLC peaks at 465 nm. This procedure allowed the quantitation of fatty acid esters of cryptoxanthin, citraurin, and lutein and free cryptoxanthin and β-carotene, without interference from the 5,6- and 5,8-epoxides, which constituted two-thirds of the total carotenoids in commercially processed orange juice concentrate. Although the total carotenoids in California navel and Valencia orange juice concentrates varied with variety, season, and location, the percentage composition of individual carotenoids remained within a narrow range. The mean concentrations of cryptoxanthin esters in California Valencia and navel orange concentrates were 15.5 and 23.5%, respectively, of the total carotenoids measured at 465 nm. The cryptoxanthin ester concentration in tangerine juice concentrates exceeded 40% of the total. Cryptoxanthin palmitate predominated in navel orange concentrates whereas, in tangerine concentrates, myristate and laurate esters predominated. The HPLC procedure permitted detection of commonly used adulterants in orange juice concentrates.

Color is an important quality aspect in commercially processed orange juice concentrates. The color of citrus fruits is due to carotenoids and detection of added colorants to orange juice is of concern to consumers, regulatory agencies, and citrus processing industries. The most common adulterants are synthetic β-carotene and β-apo-8′-carotenal, marigold flower (*Taqetes erecta*) and citrus peel extracts, and tangerine and mandarin juices. Valencia orange is the major source of concentrates produced in the United States. Navel oranges and other citrus fruits are also processed for juice.

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The current procedure for carotenoid analysis adapted by the European Federation of Fruit Juice Producers (Koch and Sajak, 1965) involves open-column separation of extracted carotenoids on deactivated alumina by gradient elution with petroleum ether and benzene. Different fractions are collected and quantitated by spectrophotometry as β-carotene equivalents. Column chromatography is tedious, does not quantitate individual carotenoids, and has a number of sources of errors. The major carotenoids in fresh citrus fruits are 5,6-epoxides (violaxanthin and antheraxanthin), which are converted to their mono-5,8-epoxides (luteoxanthin and mutatoxanthin) during heat concentration (Philip et al., 1988). Luteoxanthin and mutatoxanthin, which account for nearly two-thirds of the total carotenoids in heat-processed citrus concentrates, have light absorption around 425 nm in petroleum ether. Therefore, the column procedure that measures absorbance at 450 nm does not measure luteoxanthin and mutatoxanthin concentrations accurately. Benzene, which is used as the polar eluant, shifts the absorption maximum of carotenoids toward higher wavelengths with decreased extinction coefficients. This would result in an error in the calculation of different column fractions as β-carotene based on absorbance at 450 nm in petroleum ether.

Liquid chromatographic procedures for the separation of saponified citrus carotenoids have been reported (Fisher and Rouseff, 1986; Noga and Lenz, 1983; Stewart, 1977). The authors have recently developed liquid chromatographic procedures for the separation of a number of carotenoid fatty acid esters isolated from fruits (Philip and Chen, 1988). Attempts to separate citrus juice concentrate carotenoids by HPLC, however, have failed because of the presence of numerous isomers of violaxanthin with very close retention times (Stewart, 1980). A new liquid chromatographic procedure for the qualitative and quantitative analyses of major carotenoid esters in processed citrus juice concentrates was developed recently (Philip et al., 1988). The procedure involved treatment of extracted carotenoids with HCl to convert the 5,6-epoxides to 5,8-epoxides and quantitating the 5,8-epoxides at 400 nm. The other carotenoids, including cryptoxanthin, lutein, β-carotene, and β-citraurin, were quantitated separately at 465 nm without interference from the 5,8-epoxides. This procedure reduced the number of peaks attributed to violaxanthin and its isomers with close retention times and permitted accurate quantitation of minor, but important, carotenoids in the juice concentrates. The major carotenoid esters in the concentrates were separated into eight fractions on the basis of their functional group by silica gel thin-layer chromatography, and each fraction was further separated into individual esters by reversed-phase liquid chromatography and characterized by spectrophotometry. This adsorption–partition chromatography gave chromatographically pure carotenoid esters for characterization. It was also found that cryptoxanthin, lutein, and citraurin could be quantitated by HPLC in the presence of auroxanthin esters at a wavelength above 450 nm where auroxanthin esters had no absorbance and peak purity was not an essential prerequisite for peak quantitation.

A profile of the carotenoid ester composition of commercially processed California Valencia and navel orange concentrates was recently completed using the dual-wavelength analyses (Philip et al., 1988); such profiles are useful in the characterization of citrus cultivars (Gross, 1977). This study was undertaken to develop the HPLC methodology for the detection of common adulterants in commercially processed California orange juice concentrates as an alternate method to column chromatography.

MATERIALS AND METHODS

Orange Juice Concentrates. The Valencia and navel citrus juice concentrates used in this study were processed by Sunkist Growers, Inc., Ontario, CA, during the 1986–1987 season. Four concentrates each of early-season, mid-season, and late-season were analyzed in duplicate. The tangerine, temple orange, tangelo, and tangor var. Murcott concentrates were obtained by Sunkist Growers, Inc.

Materials and Reagents. The chemicals and solvents used were ACS grades, and the solvents used for liquid chromatography were HPLC grade. Alumina (Brockmann grade) and β-carotene with defined purity were purchased from Sigma Chemical Co., St. Louis, MO. The concentration of β-carotene was determined on the basis of absorbance values provided by the manufacturer. Synthetic β-apo-8′-carotenal was obtained through the courtesy of Hoffman La Roche, Inc., Nutley, NJ. Sudan 1 [1-(phenylazo)-2-naphthol], purchased from Aldrich Chemical Co., Milwaukee, WI, was purified as follows: Commercial-grade Sudan 1 was dissolved in hot ethanol, filtered hot, and cooled slowly. The crystals formed were collected by filtering, washed with cold ethanol, and dried at 50–60 °C under vacuum to constant weight. The crystals (0.1241 g) were dissolved in 50:50 acetone–2-propanol and diluted to 100 mL in a volumetric flask (5 mM stock solution). The stock solution was diluted 1 to 10 to make 100 mL of 0.5 mM solution, which was diluted 1 to 10 again to make 100 mL of 0.05 mM working standard. The stock solution (5 mM) was stable in refrigerator for 3 months. The 0.5 mM solutions can be stored in a refrigerator for 2–4 weeks. The 0.05 mM working standard solution was prepared fresh daily for use as internal standard.

Marigold flower and tangelo peel extracts were prepared by extracting the plant tissues with a mixture of acetone and petroleum ether (80:20). The extracts were washed with distilled water, dried with anhydrous sodium sulfate, and evaporated to dryness. The concentrations of the extracts were calculated and expressed as β-carotene equivalents.

Apparatus. Liquid chromatographic analyses were done on a Perkin-Elmer Series 4 HPLC with microprocessor-controlled solvent delivery system, variable-wavelength detector with 1.4-μL cell, and integrator. The electronic spectra were obtained with a Gilford Response spectrophotometer using 1-cm cells or 0.5-mL flow-through cell.

Chromatographic Procedure. *Column Chromatography.* The column chromatographic procedure, using deactivated alumina (Brockmann grade alumina with 8% water) and petroleum ether–benzene as eluant, was the same as that described by Koch and Sajak (1965).

Thin-Layer Chromatography. Silica gel G thin layers (0.250 mm) and 20% acetone in petroleum ether were used for the detection of citraurin esters (Philip et al., 1988).

Reversed-Phase HPLC. Octadecylsilane column (Waters Resolve C_{18}, 3.9 × 150 mm stainless steel) was used for HPLC separations. A guard column (2.5 cm) with the same reversed-phase packing was used. The mobile system consisted of methanol with a convex gradient of 0.2 over 15 min to 50:50 mixture of methanol and ethyl acetate followed by a linear gradient (1.0) to 100% ethyl acetate over 5 min. The gradient was nearly linear between 5 and 15 min. Other conditions were as follows: flow rate, 1.0 mL/min; chart speed, 10 mm/min; detection, 465 nm with an AUFS of 0.16 (limit of detection 0.1 μg/g of carotenoid).

Extraction. The juice concentrate (10.0 g) was homo-

genized with methanol (50 mL) in a Brinkman Polytron Homogenizer for 1 min at high speed. The homogenate was filtered through a medium-porosity Buchner funnel (100-mL capacity) under vacuum. The residue was extracted with 50 mL of acetone by mixing with a glass rod under low vacuum in the Buchner funnel, and the cake was further washed with 50 mL of acetone. A 10% methanolic HCl solution (10 mL) was added to the combined filtrate (150 mL) in the Buchner flask. After the mixture was shaken thoroughly, the filtrate was immediately transferred to a separatory funnel (500-mL capacity) containing 50 mL of petroleum ether. Any insolubles left in the Buchner flask were dissolved in 250 mL of distilled water and added to the separatory funnel. The aqueous phase was drained off immediately. A volume of 5 mL of 0.05 mM (0.062 mg) Sudan-1 solution and 100 mg of $MgCO_3$ were added to the petroleum ether layer. After mixing, the petroleum ether layer was washed with 100 mL of distilled water. The aqueous phase was drained and the petroleum ether layer washed again with 100 mL of distilled water. The petroleum ether layer then was dried with 10 g of anhydrous sodium sulfate and filtered. The filtrate was evaporated at low temperature in a rotary evaporator. The resulting residue was dissolved in 1 mL of acetone and filtered through a 0.45-μm filter. Filtrate portions of 5–10 μL were used for HPLC analyses.

The extraction procedure for the column chromatographic analysis was the same except that the extracts were not treated with HCl and carotenoids were quantitated as β-carotene at 450 nm in petroleum ether with $E_{1\%}$ value of 2650.

Partition. The extracted carotenoids were partitioned between equal volumes (10 mL/extract from 10 g of concentrate) of 96% aqueous methanol and petroleum ether. The methanol phase containing citraurin esters was diluted with water to less than 30% methanol concentration and extracted with petroleum ether. The petroleum ether layer was evaporated to dryness and used for the detection of citraurin by TLC or HPLC. The quantity of citraurin esters in mandarins was small, and the amount of concentrate to be used for extraction depended on the concentration of citraurin esters. A 10-g quantity was sufficient for tangerine concentrates, and orange concentrates containing 10% added tangerine concentrate required a sample size of 50–100 g.

Quantitative Analyses. Quantitative analyses by reversed-phase HPLC were done in duplicate immediately after extraction to minimize isomerizations. Peak area measurements at 465 nm were used for quantitation. HPLC peaks at 465 nm were calculated as β-carotene. The response factor for β-carotene at 465 nm was determined by injecting mixtures of known concentrations of β-carotene and internal standard and measuring the area responses. The response factor, f, was determined at 465 nm in duplicate for β-carotene by

$$\frac{\text{concn int std}}{\text{concn } \beta\text{-carotene}} = f\frac{\text{area int std}}{\text{area } \beta\text{-carotene}}$$

The response factor thus determined (f = 2.4057) was used for calculating the concentrations in micrograms per gram. The percentage concentrations were reported as percentage areas of peaks.

The *trans*-dilaurate and laurate–myristate esters of lutein did not separate from the myristate and palmitate esters of cryptoxanthin, respectively. This error was corrected by reducing the peak area of *cis*-lutein myristate–palmitate from each peak. This correction was based on the relative HPLC peak areas of lutein diesters isolated from thin layers and was approximate. This correction was not necessary for routine quality control for the detection and quantitation of common adulterants.

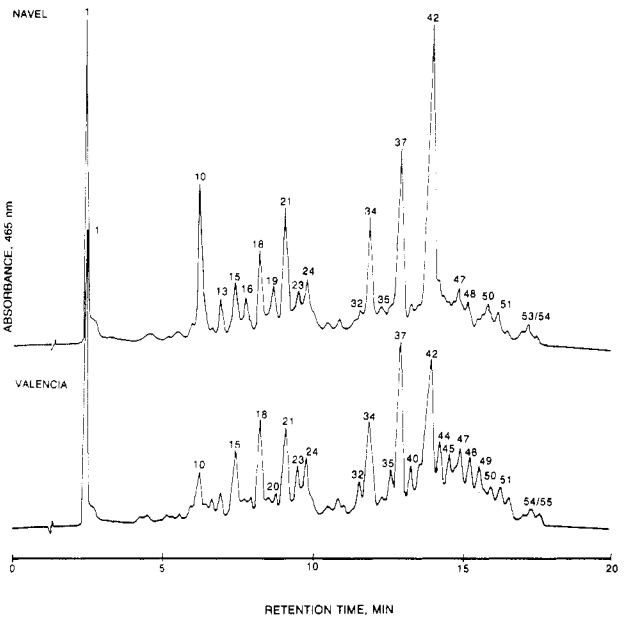

Figure 1. HPLC chromatograms of carotenoids of commercially processed Valencia and navel concentrates detected at 465 nm.

RESULTS

A recent study of the carotenoid composition of the 1986–1987 early-season, mid-season, and late-season commercially processed California Valencia and navel orange concentrates found that the total carotenoid concentration varied with season, variety, and location (Philip et al., 1988). The procedure reported involved treating the extracted carotenoids with HCl to convert the 5,6-epoxides to 5,8-epoxides and quantitating the carotenoids by liquid chromatography at 400 and 465 nm. Auroxanthin esters, which accounted for nearly two-thirds of the carotenoids in orange juice concentrates, were quantitated at 400 nm. The minor carotenoids cryptoxanthin, lutein, citraurin, and carotenes were quantitated at 465 nm without interference from auroxanthin. Mutatoxanthin esters appeared in both chromatograms. Figure 1 shows the HPLC chromatograms of carotenoids in Valencia and navel orange juice concentrates detected at 465 nm. The peak identification based on previous work (Philip et al., 1988) is given in Table I.

Although the total carotenoids varied from 20 to 100 μg/g in navel concentrates and 70 to 160 μg/g in Valencia concentrates, it was found that the percentage of individual carotenoids remained within a narrow range in a given variety (Philip et al., 1988). Table II shows the mean, coefficient of variation (CV), and 95% confidence limits ($t_{0.05}$) of percentage composition of selected carotenoids of navel and Valencia orange concentrates. The percentage composition was based on carotenoids detected at 465 nm and did not include 5,8-epoxides (auroxanthin and mutatoxanthin esters), which were not detected at 465 nm.

Comparison of Column and HPLC Procedures. Carotenoids from each individual fraction of column chromatography on alumina were separated by HPLC into individual esters and characterized. Figure 2 shows the HPLC chromatograms at 465 nm of Valencia orange concentrate and column fractions 1–3. Fraction 1 contained colorless carotenoids and β-carotene (peaks 23 and 24). Fraction 2 contained cryptoxanthin esters (peaks 34, 37, and 42). The carotenoids found in fraction 3 were cis and trans isomers of lutein diesters (peaks 38, 39, 43, 44, 47, 48, 50, 51, 53, 54). Table III shows comparison of HPLC

Table I. HPLC Peak Identification and Retention Data of Carotenoid Esters of Citrus Fruits and Common Adulterants

peak no.[a]	source	peak identity	ret time, min
1	synthetic	Sudan-1	2.47
5	synthetic	β-apo-8′-carotenal	4.74
10	tangerine	free cryptoxanthin	6.23
13	tangerine	citraurin laurate	6.92
16	tangerine	citraurin myristate	7.76
19	tangerine	citraurin palmitate	8.67
23	Valencia	carotenes (isomers)	9.54
24	navel	β-carotene	9.82
30	tangor, Murcott	cryptoxanthin caprate	10.91
34	navel	cryptoxanthin laurate	11.91
37	navel	cryptoxanthin myristate	12.95
38	Valencia	lutein dilaurate (trans)	12.96
39	Valencia	lutein dilaurate (cis)	13.25
42	navel	cryptxanthin palmitate	13.98
43	Valencia	lutein laurate–myristate (trans)	13.90
44	Valencia	lutein laurate–myristate (cis)	14.28
47	marigold	lutein dimyristate (trans)	14.96
48	Valencia	lutein dimyristate (cis)	15.28
50	marigold	lutein myristate–palmitate (trans)	15.99
51	Valencia	lutein myristate–palmitate (cis)	16.32
53	marigold	lutein dipalmitate (trans)	17.10
54	Valencia	lutein dipalmitate (cis)	17.36
55	marigold	lutein palmitate–stearate (trans)	18.21

[a] Peak numbers refer to all figures.

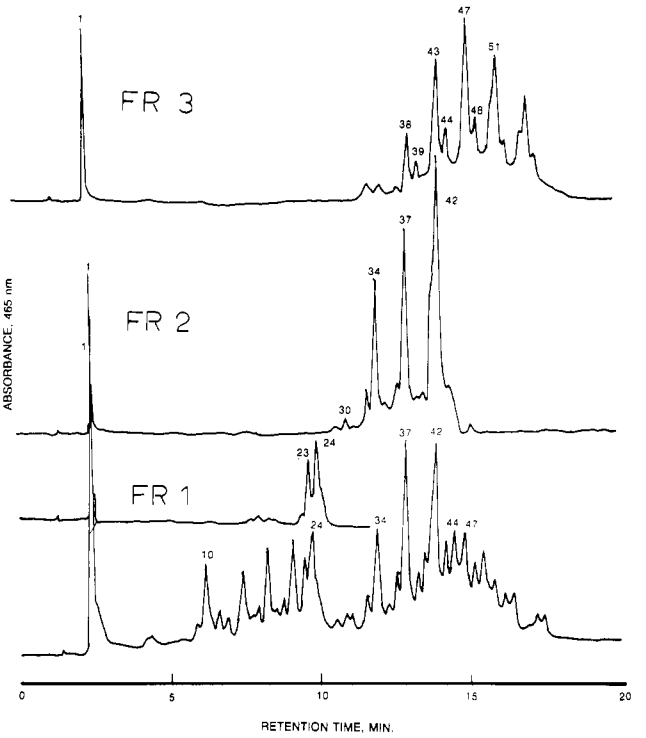

Figure 2. HPLC chromatograms of carotenoids in Valencia concentrate (bottom) and column fractions 1–3 of the same concentrate at 465 nm.

and column data on the same juice concentrates. The HPLC data on individual carotenoid fractions were slightly higher than those by column, but were comparable. The column procedure used absorbance at 450 nm for detection of extracted carotenoids, and the 5,6- and 5-8-epoxides with absorption maxima in the region 400–435 nm were not measured accurately. The epoxides accounted for nearly two-thirds of the total carotenoids in orange concentrates.

Figure 3 shows the liquid chromatographic separation of carotenoids from tangerine and tangor var. Murcott concentrates at 465 nm, and Table IV shows the percentage composition of individual carotenoids in tangerine and some tangerine hybrids. These results and the carotenoid composition in the peel and pulp of several orange and tangerine hybrids (Philip and Chen, 1988) indicate that the concentrations of cryptoxanthin esters were low in oranges (*Citrus sinensis*) and relatively high in tangerines (*Citrus reticulata*). In tangerine hybrids, tangelo or mineola (*C. reticulata* × *Citrus paradisi*) and tangor (*C. sinensis* × *C. reticulata*), the cryptoxanthin concentration is dependent on the inherited trait. Thus, in temple orange fruit (tangor), the cryptoxanthin concentration was similar to oranges than tangerine, whereas in tangor var. Murcott the cryptoxanthin concentration was slightly higher than those of tangerines (Figure 3; Table IV). Cryptoxanthin was found to be acylated with capric, lauric, myristic, and palmitic acids. Cryptoxanthin palmitate (peak 42) was the major ester in navel oranges whereas in Valencia oranges (Figure 1) the distribution of laurate, myristate, and palmitate esters was more or less equal. In tangerine and tangerine hybrids, laurate (peak 34) and myristate (peak 37) esters predominated (Figure 3). The peel and fruit

Table II. Mean Percent Composition of Some Carotenoids in Commercially Processed California Navel and Valencia Orange Juice Concentrates[a]

	navel			Valencia		
carotenoid	mean	$t_{0.05}$	CV	mean	$t_{0.05}$	CV
free cryptoxanthin	7.23	1.21	23.40	3.65	0.33	14.37
citraurin esters, total	6.22	0.64	13.43			
β-carotene	4.43	0.40	12.40	5.85	0.66	17.63
cryptoxanthin laurate	5.69	0.55	13.42	5.62	0.67	18.76
cryptoxanthin myristate	6.54	1.16	35.50	3.99	0.86	34.00
cryptoxanthin palmitate	11.22	1.79	22.27	5.85	0.88	23.76
cryptoxanthin esters, total	23.45	3.39	20.20	15.46	2.06	20.90
lutein diesters, total	18.30	2.76	21.04	22.99	1.31	8.97

[a] Data based on duplicate analyses of 12 samples each of navel and Valencia orange juice concentrates processed during the 1986–1987 season. CV = coefficient of variation; $t_{0.05}$ = 95% confidence limit.

Table III. Comparison of Column and HPLC Methods of Analyses of Carotenoids of Valencia, Navel, and Tangerine Concentrate (μg/g)

	Valencia		navel		tangerine	
carotenoid	column	HPLC	column	HPLC	column	HPLC
β-carotene	0.9	2.6	0.2	1.3	1.2	1.8
cryptoxanthin esters	4.8	6.5	5.4	7.0	13.4	15.8
lutein diesters	7.3	10.8	2.1	3.9	2.5	4.6
total carotenoids	55.9	46.5	36.0	30.5	38.7	39.3

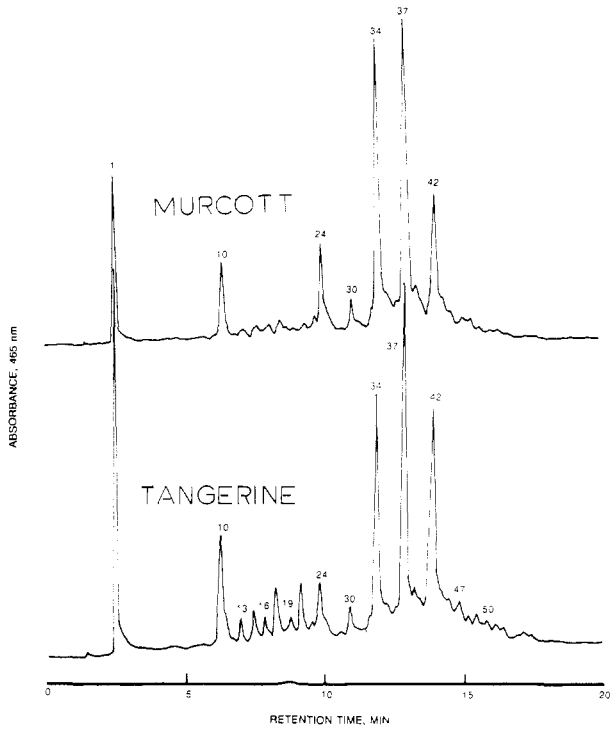

Figure 3. HPLC chromatograms of tangerine and tangor, var. Murcott, carotenoids at 465 nm.

Table IV. Percentage Composition of Carotenoids of Tangerine and Tangerine Hybrids[a]

carotenoid	tangerine	tangor, Murcott	temple	tangelo
free cryptoxanthin	6.1	4.4	7.5	5.8
citraurin esters	5.9	4.4	5.6	3.7
β-carotene	4.5	7.1	5.0	5.5
cryptoxanthin caprate	1.8	2.4	0.0	2.1
cryptoxanthin laurate	14.0	20.1	3.5	7.8
cryptoxanthin myristate	17.0	16.7	2.8	7.2
cryptoxanthin palmitate	10.9	7.8	8.6	6.3
lutein diesters	11.7	14.3	24.7	25.7
cryptoxanthin esters	43.7	47.0	14.9	21.3

[a] The percentage composition is based on HPLC peak areas detected at 465 nm.

carotenoid composition was qualitatively the same in a given variety. However, in some hybrids with intensely red peel such as temple orange, β-citraurin was found in high concentrations in the peel, but in low concentrations in the fruit (Philip and Chen, 1988). Citraurin is a C_{30} carotenoid specific to citrus species and was found in all oranges and mandarins examined except Valencia oranges. Lutein diesters were found in high concentrations in oranges and low concentrations in tangerines. Provitamin A carotenoid, β-carotene, was found in very low concentrations in all the citrus fruits examined. These findings formed the basis for detecting adulteration of orange juice with mandarin juices, with synthetic carotenoids, and with peel and *Taqetes* carotenoids.

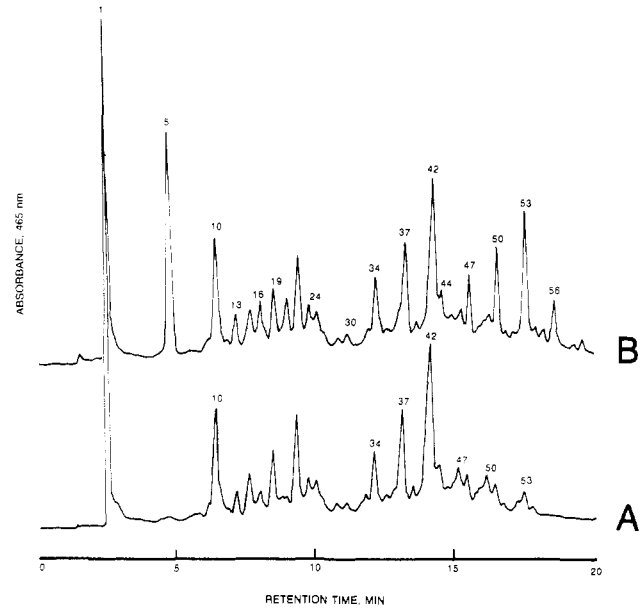

Figure 4. HPLC chromatograms of carotenoids of Valencia concentrate before (A) and after (B) the addition of β-apo-8′-carotenal, tangelo peel carotenoids, and Taqetes carotenoids at 465 nm.

Detection of Adulteration. *Tangerine and Mandarin Juices.* Juices from tangerine (*C. reticulata*) and tangerine hybrids, tangor (*C. sinsensis* ×*C. reticulata*) and tangelos (*C. reticulata* × *C. paradisi*), were characterized by a high concentration of cryptoxanthin compared to Valencia and navel orange juices. Table V summarizes some useful quantitative data for the detection of added tangerine juices to orange juices. The cryptoxanthin laurate concentration (percentage) was found to be fairly constant in both navel and Valencia concentrates and was less than half the amount found in tangerine. The ratio of cryptoxanthin palmitate to cryptoxanthin laurate was around 1 in Valencia, 2 in navel, and less than 1 in tangerines. The ratio of cryptoxanthin esters to lutein diesters was less than 1 in Valencia and slightly higher than 1 in navel but was found to be higher than 3 in tangerine. These ratios could be used to detect addition of tangerine juices to orange juices at levels above 25%. Detection of added tangerine juices below 25% to Valencia juices require quantitation of citraurin esters. Citraurin was not found in Valencia oranges, and adulteration of Valencia juices with small amounts of other citrus juices can be detected by thin-layer chromatography or by HPLC of extracts after partitioning with 96% aqueous methanol and petroleum ether. The methanol phase containing the citraurin esters was used for the detection of citraurin by TLC where they appeared as red spot with R_f value of 0.85 in 20% acetone in petroleum ether. Citraurin was the only red carotenoid in oranges and tangerines and was readily distinguishable on thin layer by its color.

Table V. Range of Values of Esters of Cryptoxanthin and Lutein in Orange Concentrates and Some Useful Ratios for Detection of Adulteration

	navel		Valencia		tangerine:
	mean	$t_{0.05}$	mean	$t_{0.05}$	av
cryptoxanthin laurate,[a] %	5.69	0.55	5.62	0.67	14.04
cryptoxanthin esters,[a] %	23.45	3.39	15.46	3.24	43.70
lutein diesters,[a] %	18.30	2.76	22.99	1.31	11.65
cryptoxanthin myristate/cryptoxanthin laurate	1.13	0.22	0.69	0.11	1.21
cryptoxanthin palmitate/cryptoxanthin laurate	1.97	0.29	1.06	0.17	0.78
cryptoxanthin esters/lutein diesters	1.37	0.35	0.68	0.11	3.74

[a] The percentage composition is based on area of peaks detected at 465 nm.

Citrus Peel and Marigold Flower Carotenoids. Figure 4 shows the HPLC chromatograms of Valencia orange concentrate with added tangelo peel and marigold flower extracts and synthetic β-apo-8′-carotenal. Peel carotenoids from red skinned citrus fruits were characterized by the presence of citraurin esters (peaks 13, 16, and 19). Citraurin was also detected by thin-layer chromatography where the citraurin appeared as a red spot. Myristate and palmitate esters of lutein predominated in marigold flowers (Gregory et al., 1986), whereas in oranges the predominant form was esters of myristate and laurate. Added lutein esters were readily recognizable in the chromatogram (peaks 47, 50, 53, and 56).

Synthetic Carotenoids. The commercially available synthetic carotenoids β-carotene (retention time 9.54 min, peak 24) and β-apo-8′-carotenal (retention time 4.74 min, peak 5) were readily detectable on the chromatogram (Figure 4). Apo-8′-carotenal had a retention time lower than that of free cryptoxanthin and eluted in an area free of interference from other carotenoids (peak 5 in Figure 4).

DISCUSSION

The HPLC procedure using Sudan-1 as internal standard was found suitable for the detection of common adulterants used in citrus juice concentrates. Measurement of HPLC peaks at 465 nm of HCl-treated juice extracts permitted the quantitation of minor carotenoids cryptoxanthin, lutein, carotenes, and citraurin, which constituted less than 35% of the total carotenoids, without interference from the major carotenoids violaxanthin, antheraxanthin, and their 5,8-epoxides. Quantitation by HPLC at 465 nm is not a measure of total carotenoids in citrus concentrates. However, the accurate quantitation of the minor carotenoids is adequate in determining the type and extent of adulteration.

Detection of adulteration of orange juice concentrates with small amounts externally added carotenoids and tangerine concentrates was difficult by this HPLC procedure. However, added carotenoids above 0.5 mg/100 g orange concentrate and addition of tangerine concentrate above 25% level can be detected. Valencia orange concentrate did not contain citraurin esters, and trace amounts of this red carotenoid were readily detectable by thin-layer chromatography.

The rapid HPLC procedure quantitated individual carotenoid esters, and the results were comparable to those of the column procedure. A maximum limit of 15% cryptoxanthin esters in true orange concentrates was established by the column procedure. This limitation was found to be valid for Valencia concentrates. However, the 15% limit was found to be too low for late-season navel concentrates, and an upper limit of 20% by column procedure would be more appropriate. The column procedure was developed in Europe in the mid-1960s and does not measure free cryptoxanthin, which accounted for 20–30% of the total cryptoxanthin in the concentrates. In the past 20 years, the quality of heat-concentrated citrus juice has improved largely due to the development of multistage evaporators. The smaller degree of hydrolysis of cryptoxanthin esters and heat-induced losses in modern evaporators may account for the lower setting of cryptoxanthin (15%) concentration by the column procedure.

Registry No. β-apo-8′-Carotenal, 1107-26-2; cryptoxanthin, 472-70-8; citraurin laurate, 49795-37-1; citraurin myristate, 49795-33-7; citraurin palmitate, 113464-23-6; β-carotene, 7235-40-7; cryptoxanthin caprate, 117191-85-2; cryptoxanthin laurate, 49795-34-8; cryptoxanthin myristate, 113481-37-1; lutein dilaurate (trans), 23852-66-6; lutein dilaurate (cis), 117305-37-0; cryptoxanthin palmitate, 6081-47-6; lutein laurate myristate (trans), 117116-36-6; lutein laurate myristate (cis), 117180-16-2; lutein dimyristate (trans), 86853-02-3; lutein dimyristate (cis), 117305-38-1; lutein myristate palmitate (trans), 104784-49-8; lutein myristate palmitate (cis), 117180-18-4; lutein dipalmitate (trans), 547-17-1; lutein dipalmitate (cis), 117305-39-2; lutein palmitate stearate (trans), 79313-82-9.

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