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Separation, Identification, and Quantification of the Major Carotenoids in Extracts of Apricots, Peaches, Cantaloupe, and Pink Grapefruit by Liquid Chromatography[†]

Frederick Khachik,*[‡] Gary R. Beecher,[‡] and William R. Lusby[§]

Beltsville Human Nutrition Research Center, Nutrient Composition Laboratory, U.S. Department of Agriculture—Agricultural Research Service, Building 161, BARC-East, Beltsville, Maryland 20705, and Insect and Nematode Hormone Laboratory, U.S. Department of Agriculture—Agricultural Research Service, Building 467, BARC-East, Beltsville, Maryland 20705

The predominant carotenoids and carotenol fatty acid esters in extracts from apricots, peaches, cantaloupe, and a variety of pink grapefruit (Ruby seedless) have been separated and quantitated on C_{18} reversed-phase high-performance liquid chromatography (HPLC) columns with low and high carbon loading. Isocratic and gradient HPLC conditions were developed that separated carotenoids from three classes of xanthophylls, hydrocarbon carotenoids, and carotenol fatty acid esters. The xanthophylls were identified as zeaxanthin and β-cryptoxanthin. The hydrocarbon carotenoids were identified as lycopene, γ-carotene, ζ-carotene, β-carotene, phytofluene, and phytoene, which were accompanied by several of their cis stereoisomers. The carotenol fatty acid esters were identified as saturated straight-chain bis(fatty acid esters) of β-cryptoxanthin, lutein, and zeaxanthin, which were shown to be only present in the extracts from peaches.

Recent laboratory and epidemiological studies have correlated the high consumption of certain foods with reduced incidence of several types of cancers in human beings. These studies have associated several micronutrients as possible active ingredients in certain fruits and vegetables with prevention of cancer (Moon and Micozzi, 1988). Since carotenoids are among one of the most abundant micronutrients found in cancer-preventive foods, the determination of accurate qualitative and quantitative data on these classes of compounds in foods, particularly in fruits and vegetables, has recently become increasingly important. As a result, rigorous analytical techniques have been developed that can separate and quantify carotenoids in several green and yellow/orange fruits and vegetables (Beecher and Khachik, 1984, 1988; Khachik et

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[‡] Nutrient Composition Laboratory.

[§] Insect and Nematode Hormone Laboratory.

al., 1986; Khachik and Beecher, 1987; Chandler and Schwartz, 1987). Recently, we reported the separation, identification, and quantification of carotenoids and carotenol fatty acid esters in several squash varieties (Khachik and Beecher, 1988a). As many as 25 components were separated in an extract from one variety of squash; these components were assigned to four classes of compounds: xanthophylls, carotenol mono(fatty acid esters), hydrocarbon carotenoids, carotenol bis(fatty acid esters). The presence of carotenol fatty acid esters in fruits and vegetables prompted the development of HPLC techniques that separated a wide range of carotenoids and their related esters (Khachik and Beecher, 1988b). Several researchers have investigated the carotenoid constituents of apricots (Curl, 1960; Bureau and Bushway, 1986), peaches (Curl, 1959; Panalaks and Murray, 1970; Bureau and Bushway, 1986), cantaloupe, and grapefruit (Bureau and Bushway, 1986). Although, in the literature reports by Curl (1959, 1960), the carotenoid constituents of apricots and cling peaches were separated by countercurrent chromatography and the structures of some of the carotenoids in these fruits were tentatively identified, the other researchers only reported the β-carotene levels in the fruits they studied.

In this report we have extended our carotenoid studies to apricots, peaches, cantaloupe, and pink grapefruit and have extensively investigated the separation, identification, and quantification of all the predominant carotenoids in these fruits by HPLC. We have developed isocratic and gradient HPLC conditions on two different HPLC columns and have evaluated the efficiency of these columns for the separation of the major carotenoid constituents and their cis stereoisomers in these fruits. The effects of drying and processing on the qualitative distribution of carotenoids in the extracts from fresh, canned, and dried apricots and peaches have been studied.

EXPERIMENTAL SECTION

Apparatus. A Beckman Model 114M ternary solvent delivery system equipped with a Beckman Model 421 controller was interfaced into a Hewlett-Packard 1040A rapid-scanning UV/visible photodiode array detector. The data were stored and processed by means of a Hewlett-Packard 9000/Series 300 (ChemStation) computing system, which was operated with a Hewlett-Packard Model 9153B disk drive, color display monitor, Model 35741, and a Model 7470A plotter. The absorption spectra of the carotenoids were recorded between 200 and 600 nm at the rate of 12 spectra/min; spectra for examination and publication were selected from the apex of each peak in an attempt to minimize contamination in those cases where peaks were not completely resolved. Absorption spectra of the carotenoids in various solvents were recorded on a Beckman DU-7 UV/visible spectrophotometer. Mass spectra were obtained from a Finnigan-MAT Model 4510 mass spectrometer (San Jose, CA) equipped with an INCOS data system and a direct-exposure probe, which was heated by the application of current from 0 to 1000 mA at a rate of 50 mA/s. Desorption chemical ionization (DCI) spectra were obtained, employing ammonia and methane as the reagent gas at a source block temperature of 60 °C. Negative-ion electron capture mass spectra were produced by using methane as a buffer gas at an indicated pressure of 0.3 Torr. For negative-ion spectra, the ionizing chamber was maintained at 60 °C and spectra were collected from m/z 45 to 650.

Column. Analytical separations were carried out on two C_{18} reversed-phase HPLC columns each packed with a different adsorbent. The first column with a high carbon loading was a Microsorb (25-cm length × 4.6-mm i.d.) C_{18} (5-μm spherical particles with a small pore size, 100 Å) column (Rainin Instrument Co.), which was protected with a Brownlee guard cartridge (3-cm length × 4.6-mm i.d.) packed with Spheri-5 C_{18} (5-μm particle size). The second column with a low carbon loading was a Vydac 201 TP54 (25-cm length × 4.6-mm i.d.) C_{18} (5-μm particles with a large pore size, 300 Å) column (The Separation Group), which was protected with a Vydac guard cartridge (3-cm length × 4.6-mm i.d.) packed with the same adsorbent as that of the column. For semipreparative separations a Rainin (25-cm length × 10-mm i.d.) Microsorb C_{18} column (5-μm spherical particles) protected with a Brownlee guard cartridge was employed.

Reagents and Materials. The reference samples of lycopene and *all-trans*-β-carotene (Sigma, St. Louis, MO) were further purified by recrystallization from methylene chloride and methanol. Samples of *all-trans*-zeaxanthin, *all-trans*-β-cryptoxanthin, and δ- and γ-carotene were provided by Hoffmann-La Roche, Basel, Switzerland, and were further purified by thin-layer chromatography. Lutein was isolated from squash extracts according to published procedures (Khachik et al., 1988). Straight long-chain fatty acid esters of lutein, zeaxanthin, and β-cryptoxanthin were prepared from lutein, zeaxanthin, and β-cryptoxanthin and the appropriate fatty acid chlorides according to published procedures (Khachik et al., 1988; Khachik and Beecher, 1988b). *cis*-β-Carotene, ζ-carotene, phytofluene, and phytoene were isolated from the extracts of dried apricots and peaches by semipreparative TLC and HPLC (details described later in the text). An authentic sample of ζ-carotene was also synthesized according to a published procedure (Davis et al., 1966). Ethyl β-apo-8′-carotenoate (Fluka Chemical Co.) was employed as an internal standard and was used without further purification. The purities of the internal standard and carotenoid reference samples were checked by HPLC as well as comparison of their absorptivity data in various solvents with those of the published values (Ritter and Purcell, 1981). When necessary, the samples were further purified by semipreparative TLC and HPLC. HPLC-grade solvents, methanol, acetonitrile, methylene chloride, and hexane (Fisher Scientific, Pittsburgh, PA), were used without further purification. Tetrahydrofuran was stabilized with butylated hydroxytoluene (BHT, 0.01%).

Chromatographic Procedures. The analytical separations were carried out employing eluents A–C, while eluents D and E were employed for the semipreparative separations. Chromatographic analyses with all of the eluents were simultaneously monitored at 470-, 460-, 450-, 400-, 350-, and 286-nm wavelengths. The column flow rate with all of the eluents in analytical separations was 0.70 mL/min.

1. Eluent A. This eluent, which consisted of a combination of isocratic and gradient chromatography, was employed for the separation of carotenoids in various fruit extracts on the Rainin Microsorb column. An isocratic mixture of methanol (10%), acetonitrile (85%), methylene chloride (2.5%), and hexane (2.5%), at time 0, was followed by a gradient beginning at time 10 and completed at time 40 (minutes). The final composition of the gradient mixture was: methanol, 10%; acetonitrile, 45%; methylene chloride, 22.5%; hexane, 22.5%. At the end of the gradient the column was reequilibrated under the initial isocratic conditions for 20 min at a flow rate of 1.5 mL/min and finally for 5 min at 0.70 mL/min.

2. Eluent B. This eluent consisted of an isocratic mixture of methanol (10%), acetonitrile (85%), methylene chloride (2.5%), and hexane (2.5%) and was employed for the separation of carotenoids on the Vydac C_{18} column.

3. Eluent C. This eluent consisted of a combination of isocratic and gradient chromatography and was particularly developed for the separation of carotenoids and carotenol fatty acid esters in peach extracts on the Vydac C_{18} column. An isocratic mixture of methanol (10%), acetonitrile (85%), methylene chloride (2.5%), and hexane (2.5%), at time 0, was followed by a gradient beginning at time 15 and completed at time 30 (minutes). The final composition of the gradient mixture was: methanol (10%), acetonitrile (60%), methylene chloride (15%), and hexane (15%).

4. Eluent D. Isolation of ζ-carotene, *cis*-β-carotene, phytofluene, and phytoene by semipreparative HPLC was accomplished with this eluent, which consisted of an isocratic mixture of methanol (25%), acetonitrile (50%), methylene chloride (12.5%), and hexane (12.5%) at a column flow rate of 2.5 mL/min.

5. *Eluent E.* This eluent was employed in isolation of carotenol fatty acid esters by semipreparative HPLC and consisted of methanol (15%), acetonitrile (50%), methylene chloride (17.5%), and hexane (17.5%) at a column flow rate of 2.5 mL/min.

Source of the Fruits. The scientific names for the fruits studies are *Prunus armeniaca* (apricot), *Prunus persica* (peach), *Cucumis melo* (cantaloupe), and *Citrus paradisi* [grapefruit (ruby red variety)]. Popular name brands of dried apricots (Blenum variety) and peaches (Yellow Elberta variety), which are grown, harvested, and manufactured in California, were purchased from local supermarkets. Canned apricots (Blenum variety) and peaches (Elberta variety) of the same varieties as those of their corresponding dried fruits were also purchased from the local supermarkets. For the present study, fresh samples of apricots and peaches before and after drying were donated by one of the leading fruit drying companies located in California. Fresh cantaloupe and a variety of pink grapefruit (Ruby seedless red) were purchased from local supermarkets on the day of analysis.

Preparation of the Fruits for Extraction. An appropriate amount of the dried fruits was weighed and kept in a beaker containing water for 1 h. The water was drained, and the fruits were rinsed with water and transferred onto paper towels to dry their outside surface. This preparation of the dried fruits allowed the removal of some of the free sugars and facilitated the extraction procedure. Canned fruits were removed from heavy syrup, washed with water, and dried on paper towels, and appropriate amounts were weighed for extraction. The water recovered after washing and rinsing of the fruits was colorless, indicating that no major losses of carotenoids had occurred as a result of this preparation. Cantaloupe and grapefruit were prepared for analysis by removing the inedible parts prior to extraction.

Extraction. A stock solution of the internal standard was prepared by dissolving 56.81 mg of ethyl β-apo-8′-carotenoate in 250 mL of hexane. An appropriate aliquot of the internal standard was added to each sample of fruits prior to homogenization. Extractions were carried out at 0 °C by immersing the Waring blender in an ice bath to prevent the degradation and isomerization of carotenoids. The remainder of the extraction procedure (and where appropriate saponifications) was carried out in a manner similar to a published procedure (Khachik et al., 1986).

Partial Synthesis of Carotenol Fatty Acid Esters. General procedures for preparation of lutein and zeaxanthin fatty acid esters have been described elsewhere (Khachik et al., 1988; Khachik and Beecher, 1988b); therefore, only the preparation of β-cryptoxanthin fatty acid esters is described here.

Partial Synthesis of β-Cryptoxanthin Fatty Acid Esters. A solution of an appropriate fatty acid chloride (0.004 mmol) in dry benzene (2 mL) was added to a solution of β-cryptoxanthin (0.0007 mmol) and triethylamine (0.012 mmol) in benzene (5 mL), and the mixture was heated at 40 °C for 10 min. The reaction mixture was sequentially washed with 10% sodium bicarbonate (3 × 10 mL) and water (3 × 10 mL) and was dried over sodium sulfate. The solvent was evaporated under reduced pressure, and the residue was chromatographed on *n*-silica plates (250-μm thickness), employing petroleum ether/acetone (24/1) as eluent. The main yellow zone of β-cryptoxanthin fatty acid esters was separated. β-Cryptoxanthin decanoate, laurate, myristate, palmitate, and stearate, which were prepared according to this procedure were shown by HPLC (eluent A) to contain a cis stereoisomer. The HPLC peak of this stereoisomer of each of the β-cryptoxanthin fatty acid esters elutes after that of the corresponding all-trans compound and has been designated by the prefix of neo A or A′ in the present study.

The UV/visible absorption maxima of β-cryptoxanthin decanoate [λ_{max} 445 nm ($\epsilon^{1\%}$ 1891), 469.5 ($\epsilon^{1\%}$ 1365)], β-cryptoxanthin laurate [λ_{max} 447 nm ($\epsilon^{1\%}$ 1828), 473.5 ($\epsilon^{1\%}$ 1499)], β-cryptoxanthin myristate [λ_{max} 447 nm ($\epsilon^{1\%}$ 1761), 472 ($\epsilon^{1\%}$ 1028)], β-cryptoxanthin palmitate [λ_{max} 447 nm ($\epsilon^{1\%}$ 1699), 472 ($\epsilon^{1\%}$ 1063)], and β-cryptoxanthin stearate [λ_{max} 447 nm ($\epsilon^{1\%}$ 1641), 473 ($\epsilon^{1\%}$ 1325)] prepared by partial synthesis exhibited 4–6-nm hypsochromic shift (hexane) from the UV/visible absorption maxima of *all-trans*-β-cryptoxanthin in hexane [λ_{max} 451, 483 nm (Ritter and Purcell, 1981)].

Isolation and Identification of Carotenoids. (*a*) *Peaches.* An extract of dried peaches (430 g) was chromatographed on C_{18} reversed-phase plates (20 × 20 cm, layer thickness 1000 μm; Whatman Chemical Separation Inc.) employing methanol (10%), acetonitrile (55%), methylene chloride (17.5%), and hexane (17.5%) as eluent. Six bands were separated.

The first band (R_f 0.56) was shown by HPLC (eluent A) to consist of a major and a minor component, which were identified by comparison of their HPLC retention times and absorption spectra with those of authentic samples as *all-trans*-zeaxanthin (major component) and neozeaxanthin A (minor component). The UV/visible absorption spectrum of neozeaxanthin A monitored by the rapid-scanning detector in the HPLC solvents exhibited a 4-nm hypsochromic shift in its absorption maximum (λ_{max} 450 nm) with respect to that of *all*-trans-zeaxanthin (λ_{max} 454 nm). The absorption spectrum of neozeaxanthin A also contained a strong cis peak at 334 nm. An authentic sample of *all-trans*-zeaxanthin in methanol after 20-min reflux was shown by HPLC (eluent A) to have partially isomerized to neozeaxanthin A.

The second band (R_f 0.45) was shown by HPLC (eluent A) to consist of a single component, which was identified as *all-trans*-β-cryptoxanthin. The absorption spectrum of *all-trans*-β-cryptoxanthin in the HPLC solvents (eluent A) had a maximum at 454 nm. The UV/visible absorption maxima of this band in petroleum ether [λ_{max} 451 nm ($\epsilon^{1\%}$ 2431), 478 ($\epsilon^{1\%}$ 2050)] and benzene [λ_{max} 466 nm ($\epsilon^{1\%}$ 2287), 494 ($\epsilon^{1\%}$ 1892)] were consistent with the absorption maxima of *all-trans*-β-cryptoxanthin in these solvents [literature values: petroleum ether, λ_{max} 450–452 nm ($\epsilon^{1\%}$ at 452 nm, 2386) (Ritter and Purcell, 1981)]. The mass spectrum (DCI, ammonia) of *all-trans*-β-cryptoxanthin contained peaks at *m/z* 570 [100%, $(M + NH_4)^+$] and 553 (40%). The desorption chemical ionization mass spectrum of this compound employing methane as reagent gas possessed a protonated molecular ion at *m/z* 553 [30%, $(M + H)^+$] as well as ions at *m/z* 535 [20%, $(M + H - 18)^+$] and 369 (100%).

The third band (R_f 0.29) was shown by HPLC (eluent A) to consist of four major components and a minor component, which were separated by semipreparative HPLC (eluent D) and identified in order of elution on a C_{18} reversed-phase column as *cis*-β-carotene (neo-β-carotene U), ζ-carotene, *all-trans*-β-carotene (coeluting with neo- and B), phytofluene, and phytoene (minor component).

all-trans-β-Carotene, neo-β-carotene U, and neo-β-carotene A and B were identified by comparison of their HPLC retention times (eluent B, Vydac column) and absorption spectra with those of the thermal isomerization products of *all-trans*-β-carotene monitored by the rapid-scanning photodiode array detector (details described later in this text).

all-trans-ζ-Carotene was identified by comparison of its HPLC retention time (eluent A) and absorption spectra with that of synthetic ζ-carotene prepared in our laboratory according to the procedure described by Davis et al. (1966). UV/visible absorption maxima of ζ-carotene in hexane [λ_{max} 377.5 nm, 399 ($\epsilon^{1\%}$ 2560), 424] were consistent with the reported absorption maxima of ζ-carotene in hexane [λ_{max} 377–380 nm, 397–400 ($\epsilon^{1\%}$ at 400 nm, 2555), 423–426 (Ritter and Purcell, 1981)]. The electron ionization mass spectrum of *all-trans*-ζ-carotene contained a weak molecular ion peak at *m/z* 540 ($C_{40}H_{60}$ requires 540) as well as ions at *m/z* 403 (M − 137), 81, and 69. Negative-ion electron capture mass spectral analysis of *all-trans*-ζ-carotene provided a molecular anion at *m/z* 540 (100%).

Isolated phytofluene was shown by HPLC (eluent A) to contain a cis isomer. The absorption spectra of both the *all-trans*- and *cis*-phytofluene in the HPLC solvents (eluent A) had identical absorption maxima (λ_{max} 350 nm) as determined by the rapid-scanning photodiode array detector. UV/visible absorption maxima of the mixture of *all-trans*-phytofluene and its cis isomer in hexane [λ_{max} 331 nm, 348 ($\epsilon^{1\%}$ 1565), 367] were consistent with the reported absorption maxima for phytofluene in hexane [λ_{max} 331–332 nm, 347–348 ($\epsilon^{1\%}$ at 347 nm, 1577), 366 (Ritter and Purcell, 1981)]. The electron ionization mass spectrum of the isolated phytofluene mixture contained a weak

molecular ion peak at m/z 542 ($C_{40}H_{62}$ requires 542) as well as ions at m/z 410 (M − 132), 81, and 69. Negative-ion electron capture mass spectrum of phytofluene contained molecular anion at m/z 542 (100%).

Isolated phytoene was shown by HPLC (eluent A) to contain a cis isomer. The absorption spectra of both *all-trans-* and *cis*-phytoene in the HPLC solvents (eluent A) had identical absorption maxima (λ_{max} 286 nm) as determined by the rapid-scanning photodiode array detector. UV/visible absorption maxima of the mixture of *all-trans*-phytoene and its cis isomer in hexane [λ_{max} 276 nm, 286 ($\epsilon^{1\%}$ 920), 297] were consistent with the reported absorption maxima for phytoene in hexane [λ_{max} 276 nm, 286 ($\epsilon^{1\%}$ 915), 297 (Ritter and Purcell, 1981)]. The electron ionization mass spectrum of the isolated phytoene mixture contained an intense molecular ion peak at m/z 544 (50%) ($C_{40}H_{64}$ requires 544) as well as ions at m/z 410 [80%, (M − 134)], 339 [100%, (M − 205)], 81, and 69.

The fourth band (R_f 0.22) was shown by HPLC (eluent A) to consist of three major components and a minor component, which were identified as β-carotene (minor component), phytofluene, phytoene, and β-cryptoxanthin myristate. This mixture was chromatographed on a semipreparative HPLC column (eluent D) for isolation and further accumulation of phytofluene and phytoene. The isolated β-cryptoxanthin myristate was identified by comparison of its HPLC retention time and absorption spectrum with those of an authentic sample of β-cryptoxanthin myristate prepared by partial synthesis. The HPLC profile (eluents A and B) of the isolated β-cryptoxanthin myristate revealed the presence of a mono-cis isomer of this compound, which appeared as a trailing shoulder on the HPLC peak of *all-trans*-β-cryptoxanthin myristate. The UV/visible absorption spectrum of *cis*-β-cryptoxanthin myristate (λ_{max} 450 nm) monitored by the rapid-scanning detector in the HPLC solvents (eluent A) exhibited a 4-nm hypsochromic shift in its absorption maximum in comparison with that of *all-trans*-β-cryptoxanthin myristate (λ_{max} 454 nm). The absence of a strong cis peak in the absorption spectrum of this cis isomer of β-cryptoxanthin myristate in the near-UV region excluded the possibility of a 15,15′-cis geometry for this compound. Therefore, since the location of the cis double bond in *cis*-β-cryptoxanthin myristate is not known, this compound has been referred to as neo-β-cryptoxanthin myristate A or A′ throughout this text. Upon saponification, the isolated β-cryptoxanthin myristate was shown by HPLC (eluent A) to have been converted to the parent hydroxycarotenoid, β-cryptoxanthin.

The fifth band (R_f 0.16) was shown by HPLC (eluent A) to consist of two minor components and a major component, which were separated by HPLC (eluent D) and identified as phytoene (minor component), β-cryptoxanthin myristate (minor component), and β-cryptoxanthin palmitate (major component). Isolated β-cryptoxanthin palmitate was identified by comparison of its HPLC retention time and absorption spectrum with those of an authentic sample of β-cryptoxanthin palmitate prepared by partial synthesis. In a manner analogous to that used for β-cryptoxanthin myristate, mono-*cis*-β-cryptoxanthin palmitate was demonstrated to accompany its all-trans isomer. This *cis*-β-cryptoxanthin palmitate has been referred to as neo-β-cryptoxanthin palmitate A or A′ throughout this text, since the location of the cis double bond in this compound is not known with certainty. Saponification of the isolated β-cryptoxanthin palmitate resulted in the formation of β-cryptoxanthin.

The sixth band (R_f 0.09) was shown by HPLC (eluent A) to consist of six components, which were separated by semipreparative HPLC (eluent E) and identified by comparison of their HPLC retention times and absorption spectra with those of the authentic samples as esters of lutein and zeaxanthin. These carotenol fatty acid esters in the order of elution on a C_{18} reversed-phase column were *all-trans*-lutein dimyristate, *all-trans*-zeaxanthin dimyristate, *all-trans*-lutein myristate/palmitate diester, *all-trans*-zeaxanthin myristate/palmitate, *all-trans*-lutein dipalmitate, and *all-trans*-zeaxanthin dipalmitate. For a detailed separation of carotenol fatty acid esters by HPLC, see the publication by Khachik and Beecher (1988b). Saponification of this fraction regenerated lutein and zeaxanthin.

(*b*) *Apricots.* An extract of dried apricots (300 g) was chromatographed on the same TLC system as described for peaches, and four bands were separated.

Table I. Weight of the Internal Standard Added to the Apricot, Peach, Cantaloupe, and Grapefruit Samples at the Beginning of the Extraction and the Final Volume of the Extracts

entry	fruit	wt extr, g	ethyl β-apo-8′-carotenoate (int std), mg	final vol extr, mL
1	apricot			
	fresh	162	0.46	25
	canned	150	0.46	25
	dried	70	0.46	25
2	peach			
	fresh	387	0.23	10
	canned	150	0.23	10
	dried	50	0.23	10
3	cantaloupe	300	1.36	50
4	grapefruit	400	1.82	50

The first band (R_f 0.40) was identified by comparison of its HPLC (eluent B, Vydac C_{18} column) retention time and UV/visible absorption maxima in various solvents with those of an authentic sample as *all-trans*-lycopene.

The second band (R_f 0.35) was shown by HPLC (eluent B, Vydac C_{18} column) to consist of two components, which were identified in the order of elution as *all-trans*-γ-carotene and a mono-cis stereoisomer of this compound referred to as neo-γ-carotene A. The UV/visible absorption spectrum of neo-γ-carotene A (λ_{max} 460 nm) monitored by the rapid-scanning detector in the HPLC solvents (eluent B) exhibited a 4-nm hypsochromic shift in its absorption maximum in comparison with that of *all-trans*-γ-carotene (λ_{max} 464 nm). The UV/visible absorption spectrum of neo-γ-carotene A in the HPLC solvents also contained a low-intensity cis peak in the near-UV region at 350 nm. An authentic sample of *all-trans*-γ-carotene in hexane after 30-min reflux was shown by HPLC to have partially isomerized to neo-γ-carotene A. The mass spectrum (DCI, methane) of the isolated mixture of *all-trans*-γ-carotene and its cis isomer showed a protonated molecular ion at m/z 537 [100%, $(M + H)^+$] and an ion peak at m/z 369 (18%).

The third band (R_f 0.29) was shown by HPLC (eluent A) to consist of three major components and a minor component, which were separated by semipreparative HPLC (eluent D) and identified in the order of elution on a C_{18} reversed-phase column as ζ-carotene, *all-trans*-β-carotene (coeluting with neo-β-carotene A and B), phytofluene, and phytoene (minor component). The identification of these compounds by HPLC and spectroscopy was similar to that of the various isolated fractions from peach extracts described earlier.

The fourth band (R_f 0.22) was shown by HPLC (eluent A) to consist of a minor and two major components, which were separated by semipreparative HPLC (eluent D) and identified as β-carotene (minor component), phytofluene, and phytoene.

(*c*) *Cantaloupe.* A concentrated extract of cantaloupe (300 g) was chromatographed on the semipreparative HPLC column employing eluent D. Five fractions were shown to be present, which were identified from their HPLC retention times and UV/visible absorption spectra as neo-β-carotene U, *all-trans*-ζ-carotene, *all-trans*-β-carotene (coeluting with neo-β-carotene A and B), phytofluene, and phytoene.

(*d*) *Grapefruit (Pink).* A concentrated extract of pink grapefruit extract (400 g) was chromatographed on the semipreparative HPLC column employing eluent D. Five components were shown to be present, which were separated and identified by comparison of their HPLC retention times and UV/visible absorption spectra with those of the authentic samples as *all-trans*-lycopene (coeluting with a cis stereoisomer referred to as a neolycopene A in this text), *all-trans*-ζ-carotene, *all-trans*-β-carotene (coeluting with neo-β-carotene A and B), phytofluene, and phytoene.

Quantification of Carotenoids and Related Esters. The carotenoid constituents in the extract of various fruits were quantified from the HPLC response factors of individually isolated compounds at three or four different concentrations, which were obtained under various HPLC conditions employing eluents A–C. The HPLC peak area of cis carotenoids, which were not

Table II. Peak Identification of the Various Carotenoids of Fruit Extracts Separated by HPLC in the Order of Elution on C_{18} Columns

chem class	peak	carotenoid	λ, nm
xanthophyll	1	*all-trans*-lutein	450
	2	*all-trans*-zeaxanthin	450
	3	neozeaxanthin A	450
	4	*all-trans*-β-cryptoxanthin	450
apocarotenoid	5	ethyl β-apo-8′-carotenoate (int std)	450
hydrocarbon carotenoid	6	*all-trans*-lycopene	470
	7	neolycopene A	470
	8	*all-trans*-γ-carotene	460
	9	neo-γ-carotene A	460
	10	neo-β-carotene U	450
	11	*all-trans*-ζ-carotene	400
	12	*all-trans*-β-carotene	450
	13	neo-β-carotene A	450
	14	neo-β-carotene B (15,15′-cis)	450
	15	*all-trans*- or *cis*-phytofluene	350
	16	*cis*- or *all-trans*-phytofluene	350
	17	*all-trans*- or *cis*-phytoene	286
	18	*cis*- or *all-trans*-phytoene	286
carotenol fatty acid ester	19	*all-trans*-β-cryptoxanthin myristate	450
	20	neo-β-cryptoxanthin myristate A or A′	450
	21	*all-trans*-β-cryptoxanthin palmitate	450
	22	neo-β-cryptoxanthin palmitate A or A′	450
	23	*all-trans*-lutein dimyristate	450
	24	*all-trans*-zeaxanthin dimyristate	450
	25	*all-trans*-lutein myristate/palmitate diester	450
	26	*all-trans*-zeaxanthin myristate/palmitate diester	450
	27	*all-trans*-lutein dipalmitate	450
	28	*all-trans*-zeaxanthin dipalmitate	450

resolved and appeared as a trailing shoulder on their all-trans compound, were combined with the HPLC peak area of all-trans carotenoids. Therefore, in quantification of carotenoids and related esters in the present study it has been assumed that the response factors of cis carotenoids under various chromatographic conditions are reasonably close to that of their all-trans isomer. The HPLC peak area of the internal standard (ethyl β-apo-8′-carotenoate) after each extraction was monitored to determine the losses of carotenoids related to sample preparation and workup procedures. The recovery of ethyl β-apo-8′-carotenoate from numerous extractions of various fruits was more than 97% as determined by the HPLC peak area of this internal standard before and after extraction. This internal standard was found to be suitable for extraction and workup procedures that did not involve saponification.

RESULTS AND DISCUSSION

Qualitative Distribution of Carotenoids. The major carotenoid constituents separated from various fruit extracts and their corresponding HPLC peaks in the order of elution on a C_{18} reversed-phase column are shown in Table II. In the present study, the HPLC separations of carotenoids in the extracts of apricots, peaches, cantaloupe, and pink grapefruit have been examined on two C_{18} reversed-phase HPLC columns each packed with different adsorbents. These columns are a Rainin Microsorb C_{18} reversed phase with a high carbon loading (small pore size, 100 Å) and a Vydac C_{18} reversed phase with a low carbon loading (large pore size, 300 Å). Although both columns are packed with C_{18} reversed-phase adsorbents, the chromatographic conditions employed with each of these columns are significantly different. This is owing to the fact that carotenoids from various classes in general have a much higher adsorption affinity (longer HPLC retention times) for the Microsorb column with high carbon loading than for the Vydac column with a low carbon loading (shorter HPLC retention times). In the present study both of these columns have been employed in evaluating the qualitative distribution of carotenoids in the extracts of various fruits under optimum HPLC conditions [eluents A, Microsorb column; B, Vydac column; and C, Vydac column]. From our extensive HPLC studies with both of these columns under various chromatographic conditions it appears that, among the various classes of carotenoids, the separation of the stereoisomers of the hydrocarbon carotenoids can be better accomplished on a Vydac than a Microsorb C_{18} column. On the other hand the separations of the oxygenated carotenoids (xanthophylls and their stereoisomers are best accomplished on the Microsorb C_{18} column (Khachik et al., 1986).

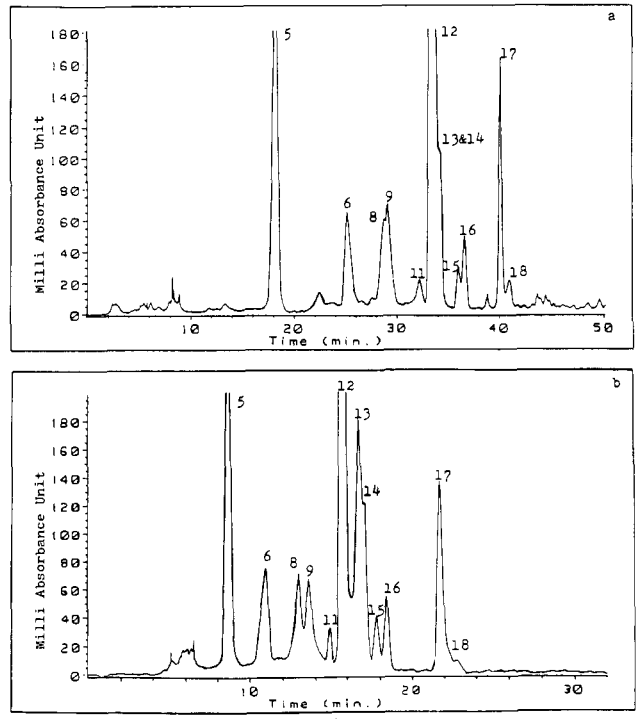

Figure 1. HPLC profiles of dried apricot extracts. Upper trace: extract separated on Microsorb C_{18} column employing eluent A. Lower trace: extract separated on Vydac C_{18} column employing eluent B. Chromatographic conditions and peak identification (Table II) described in the text.

The structural elucidation of carotenoids was based on UV/visible absorption and mass spectra as well as comparison of HPLC retention times of unknowns with those of authentic samples (for details, see the Experimental Section). Several stereoisomers of the hydrocarbon carotenoids were shown to be present in the extracts from fruits studied in this report. Since the locations of the cis double bonds in these isomeric carotenoids are not known with certainty, tentative assignments are based on the UV/visible absorption spectra of these compounds obtained by the photodiode array detector in the HPLC solvents. These tentative assignments and qualitative distribution of cis carotenoids in apricots, peaches, cantaloupe, and pink grapefruit are separately discussed below.

Apricots. The HPLC chromatograms of carotenoids in an extract from dried apricots separated on Rainin Microsorb and Vydac columns are shown in parts a and b of Figure 1, respectively. The HPLC profiles of carotenoids in the extracts from fresh and canned apricots are similar to that of dried apricots. With the exception

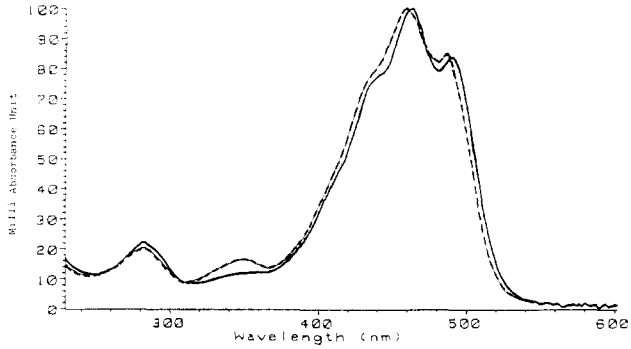

Figure 2. UV/visible absorption spectra of *all-trans*-γ-carotene [(—) λ_{max} 464 nm, 492] and neo-γ-carotene A [(- - -) λ_{max} 460 nm, 486; cis peak at 350 nm] in the HPLC solvents (eluent B) under conditions described in the text.

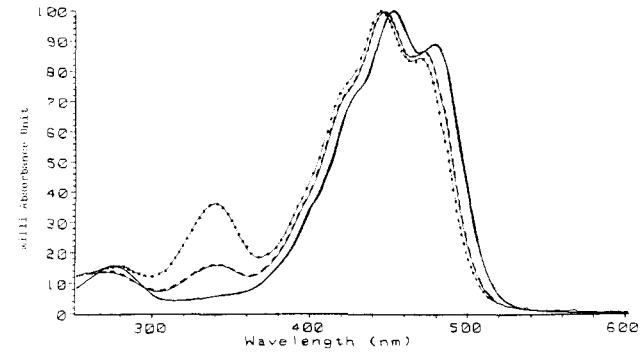

Figure 3. UV/visible absorption spectra of *all-trans*-β-carotene [(—) λ_{max} 454 nm, 480], neo-β-carotene A [(- - -) λ_{max} 448 nm, 474; cis peak at 342 nm], and neo-β-carotene B [(···) λ_{max} 446 nm, 472] in the HPLC solvents (eluent B) under conditions described in the text.

of *all-trans*-lycopene and ζ-carotene, several of the carotenoids in the extracts from apricots were shown to be accompanied by their cis isomers. These were γ-carotene, β-carotene, phytofluene, and phytoene. The qualitative distribution of each of these carotenoids is discussed below.

γ-Carotene. The UV/visible absorption spectra of *all-trans*-γ-carotene (λ_{max} 454 nm) and its cis isomer, neo-γ-carotene A (λ_{max} 460 nm), in the HPLC solvents are shown in Figure 2. These spectra are obtained by monitoring the HPLC peaks of γ-carotene and its cis isomer (peaks 8 and 9, Figure 1b), when the isolated γ-carotene or an apricot extract is chromatographed (eluent B) on a Vydac C_{18} column. The presence of the low-intensity cis peak in the near-UV region in the spectrum of neo-γ-carotene A at 350 nm and small hypsochromic shift (4 nm) in the absorption maximum of this *cis*-γ-carotene with respect to that of its all-trans compound is in agreement with a mono-cis geometry for this carotenoid. At first it may appear that the possible presence of δ-carotene (an structurally related isomer of γ-carotene) in an extract from apricots could result in incorrect assignments of *all-trans*- and *cis*-γ-carotene. However, chromatography of an authentic sample of *all-trans*-δ-carotene spiked with the isolated *all-trans*- and neo-γ-carotene A under various conditions (eluents A and B) results in base-line separation between *all-trans*-δ-carotene (eluting first), *all-trans*-γ-carotene (eluting second), and neo-γ-carotene A (eluting third, partially resolved from its all-trans isomer). Furthermore, the UV/visible absorption maximum of δ-carotene (λ_{max} 458 nm) monitored by the rapid-scanning detector in the HPLC solvents (eluents A and B) is accompanied by a 6-nm hypsochromic shift with respect to that of *all-trans*-γ-carotene (λ_{max} 464 nm), which makes the identification of these carotenes by HPLC–UV/visible spectroscopy possible.

β-Carotene. As it seems apparent from the HPLC profile of dried apricots shown in Figure 1b, the stereoisomers of β-carotene (peaks 13 and 14) are partially separated from *all-trans*-β-carotene (peak 12). Tentative assignments for these carotenoids were based on the UV/visible absorption spectra of these compounds in the HPLC solvents (eluent B), with maxima at λ_{max} 454 and 480 nm for *all-trans*-β-carotene; 448 and 474 nm for neo-β-carotene A; and 446 and 472 nm for neo-β-carotene B (Figure 3). The presence of the low-intensity cis peak in the near-UV region in the absorption spectrum of neo-β-carotene A at 342 nm and small hypsochromic shift (6 nm) in the absorption maximum of this *cis*-β-carotene with respect to that of its all-trans compound is in agreement with a mono-cis geometry for this compound. Neo-

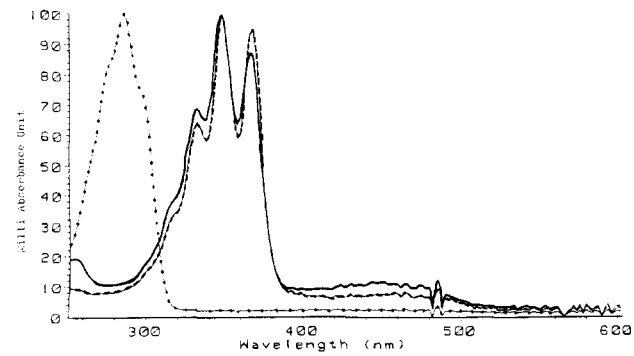

Figure 4. UV/visible absorption spectra of *all-trans*- (—) and *cis*-phytofluene (- - -) [assignments may be reversed; λ_{max} (both isomers) 332 nm, 350, 368] and *all-trans*- and *cis*-phytoene [(···) λ_{max} (both isomers) 286 nm].

β-carotene B contains an intense cis peak in the near-UV region at 340 nm, which is characteristic of the central mono-cis isomer of carotenoids (Vetter et al., 1971). Neo-β-carotene B was therefore identified as 15,15′-*cis*-β-carotene. Thermal isomerization of *all-trans*-β-carotene in refluxing hexane after 30 min resulted, in addition to neo-β-carotene A and B, in the formation of another cis isomer, which was shown by HPLC (eluent B) to elute before *all-trans*-β-carotene on the Vydac C_{18} column. This cis isomer, which has been referred to as neo-β-carotene U throughout this text, possessed UV/visible absorption maxima at 450–452 and 474 nm and had no detectable cis peak in the near-UV region. As it will be shown later, neo-β-carotene U was only present in the extracts from cantaloupe and peaches. Definitive structural elucidation of the cis isomers of β-carotene can only be achieved by evaluation of the NMR spectrum of each individually isolated isomer. This has been elegantly accomplished by Tsukida et al. (1982), who separated the cis isomers of β-carotene on an HPLC Lime column.

Phytofluene and Phytoene. The absorption spectra of *cis*- and *all-trans*-phytofluene and phytoene in the HPLC solvents (eluent B) monitored by the photodiode array detector are shown in Figure 4. Since the absorption maxima of both phytofluene isomers appeared at 332, 350, and 368 nm, it is not possible to determine which one of the two HPLC peaks (peaks 15 and 16) in the chromatogram of apricot extract (Figure 1) is associated with the *all-trans*-phytofluene. Similarly, the UV/visible absorption spectra of both *cis*- and *all-trans*-phytoene in the HPLC solvents (eluent B) were superimposable and had maximum at 286 nm. As a result, the geometrical assignments for the HPLC peaks of phy-

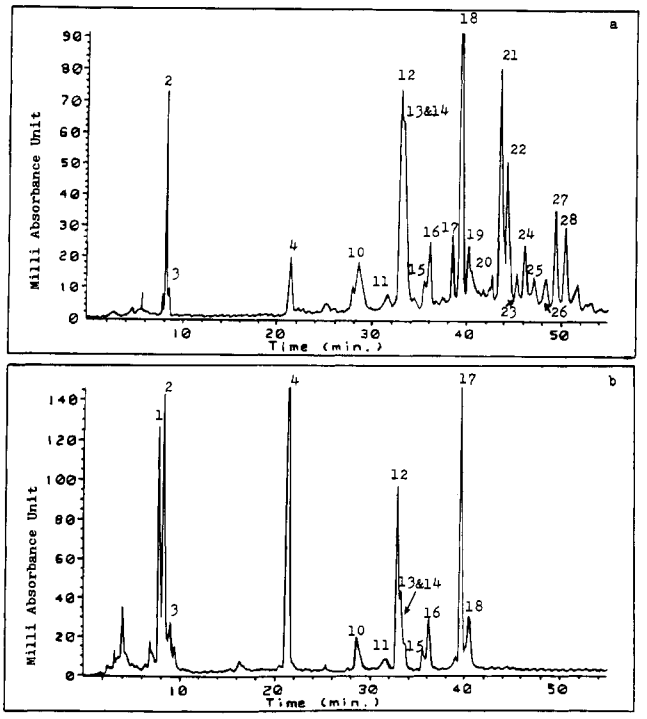

Figure 5. HPLC profiles of dried peaches separated on Microsorb C_{18} column employing eluent A. Upper trace: extract before saponification. Lower trace: extract after saponification. Chromatographic conditions and peak identification (Table II) described in the text.

toene isomers (peaks 17 and 18) in the chromatograms of apricot extracts (Figure 1) are not known with certainty.

Peaches. Carotenoids and carotenol fatty acid esters in an extract from dried peaches before and after saponification are separated on a Microsorb C_{18} column, as shown in the HPLC profiles in parts a and b of Figure 5, respectively. Similarly, the separation of peach carotenoids can also be accomplished on a Vydac C_{18} column employing eluent C. The chromatographic profiles of carotenoids in the extracts from fresh and canned peaches are similar to that of dried peaches. The structural elucidation of carotenoids and carotenol fatty acid esters has been described in the Experimental Section. Of particular interest is the presence of fatty acid esters of lutein, β-cryptoxanthin, and zeaxanthin in peaches as well as ζ-carotene, β-carotene, phytofluene, and phytoene, which are also present in the extracts from apricots, cantaloupe, and grapefruit.

Saponification of peach extracts regenerates the parent hydroxycarotenoids, lutein, zeaxanthin, and β-cryptoxanthin. The separation and structural elucidation of synthetic and naturally occurring carotenol fatty acid esters (isolated from selected squash varieties) by HPLC have been well documented in the literature (Khachik and Beecher, 1988a,b; Khachik et al., 1988).

Cantaloupe. The chromatographic profile of carotenoids in an extract from cantaloupe, separated on a Vydac C_{18} column (eluent B), is shown in Figure 6. The separation of these carotenoids can also be effected on a Microsorb C_{18} column employing eluent A. The major hydrocarbn carotenoids in this fruit are ζ-carotene, β-carotene and its cis isomers, phytofluene, and phytoene. Particularly noticeable is the absence of stereoisomers of phytofluene and phytoene.

Grapefruit (Pink). The HPLC profile of carotenoids in an extract from pink grapefruit (Ruby seedless red), separated on a Vydac C_{18} column, is shown in Figure 7.

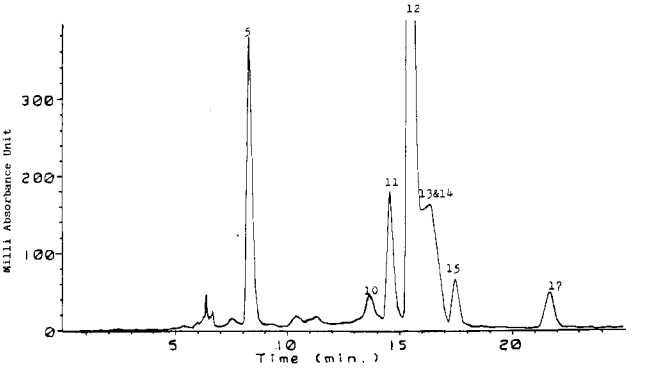

Figure 6. HPLC profile of cantaloupe extract separated on Vydac C_{18} column employing eluent B. Chromatographic conditions and peak identification (Table II) described in the text.

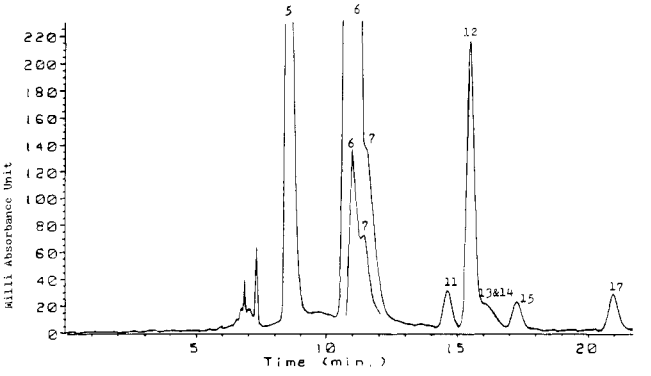

Figure 7. HPLC profile of pink grapefruit (ruby seedless red variety) extract separated on Vydac C_{18} column employing eluent B. Chromatographic conditions and peak identification (Table II) described in the text.

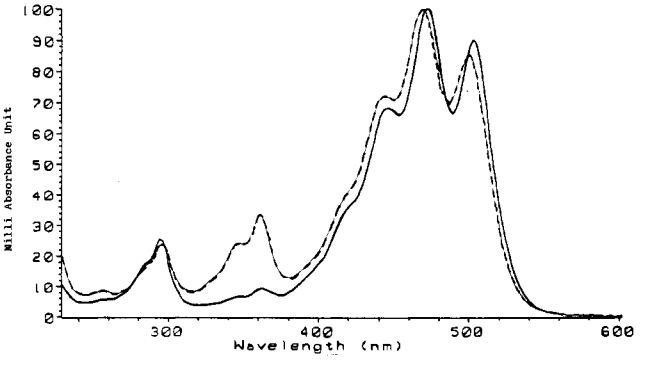

Figure 8. UV/visible absorption spectra of *all-trans*-lycopene [(—) λ_{max} 444 nm, 472, 504] and neolycopene A [(- - -) λ_{max} 442 nm, 468, 498; cis peaks at 346 and 360 nm] in the HPLC solvents (eluent B) under conditions described in the text.

The major carotenoid constituents of pink grapefruit were identified as *all-trans*-lycopene, neolycopene A (a cis isomer of lycopene), ζ-carotene, β-carotene and its cis isomers, phytofluene, and phytoene. The UV/visible absorption spectra of *all-trans*-lycopene (λ_{max} 294 nm, 444, 472, 504) and neolycopene A (λ_{max} 296 nm, 346, 360, 442, 468, 498) in the HPLC solvents (eluent B) are shown in Figure 8. These spectra are obtained by monitoring the partially resolved HPLC peaks of *all-trans*-lycopene (peak 6, Figure 7) and neolycopene A (peak 7, Figure 7) with a photodiode array detector as these isomeric lycopenes are eluted from the HPLC column. The presence of the intense cis peaks in the near-UV region in the absorption spectrum of neolycopene A at 346 and 360 nm as

Table III. Quantitative Distribution of Xanthophylls, Carotenes, and Carotenol Fatty Acid Esters in Apricots, Peaches, Cantaloupe, and Grapefruit

	μg/100 g edible food							
	apricot			peach				grapefruit
	fresh	canned	dried	fresh	canned	dried	cantaloupe	(pink)
			Xanthophylls					
all-trans-zeaxanthin + neozeaxanthin A	–[a]	–	–	4	9	94	–	–
all-trans-β-cryptoxanthin	–	–	–	3	10	72	–	–
total				7	19	166		
			Carotenes					
all-trans-lycopene + neolycopene A	5	65	864	–	–	–	–	3362
all-trans-γ-carotene + neo-γ-carotene A	18	28	290	–	–	–	–	–
all-trans-ζ-carotene	38	21	141	2	4	176	394	138
neo-β-carotene U	–	–	–	30	160	882	3936	–
all-trans-β-carotene + neo-β-carotene A + neo-β-carotene B (15,15′-cis)	6433	19270	34630	82	465	3374	21560	2343
all-trans- and *cis*-phytofluene	25	57	139	2	6	27	44	13
all-trans- and *cis*-phytoene	61	104	235	12	12	11	38	15
total	6580	19545	36299	128	647	4470	25972	5871
			Carotenol Fatty Acid Esters					
all-trans + neo-β-cryptoxanthin myristate A	–	–	–	6	22	81	–	–
all-trans + neo-β-cryptoxanthin palmitate A	–	–	–	6	30	169	–	–
all-trans-lutein dimyristate	–	–	–	1	10	14	–	–
all-trans-zeaxanthin dimyristate	–	–	–	1	10	22	–	–
all-trans-lutein myristate/palmitate diester	–	–	–	2	4	21	–	–
all-trans-zeaxanthin myristate/palmitate diester	–	–	–	1	3	13	–	–
all-trans-lutein dipalmitate	–	–	–	3	10	59	–	–
all-trans-zeaxanthin dipalmitate	–	–	–	2	5	40	–	–
total				22	94	419		

[a] Insufficient level in aliquot of extract applied to HPLC to permit quantification. HPLC limits of detection for carotenoids are about 1 μg/100 g of food extracted.

well as small hypsochromic shift (4 nm) in the absorption maximum of this *cis*-lycopene with respect to that of its all-trans compound is in agreement with a central mono-cis geometry for neolycopene A. It is interesting to note that when an extract from a variety of white fleshed grapefruit (i.e., Duncan) was examined by HPLC (eluents A and B), no significant level of any carotenoid could be detected in this variety of grapefruit.

Quantitative Distribution of Carotenoids. The quantitative distribution of xanthophylls, carotenes, and carotenol fatty acid esters in apricots, peaches, cantaloupe, and pink grapefruit are shown in Table III. The quantitative data shown in Table III for each fruit were obtained from two consecutive extractions from one batch of sample; therefore, these data are not necessarily representative of the levels of these compounds in the products consumed nationwide. The carotenoid levels for dried apricots and peaches are significantly higher than those of fresh and canned for these fruits. This is primarily due to the fact that the carotenoid data presented in Table III for fresh and canned apricots are based on wet weight of these fruits, whereas the carotenoid levels for dried apricots and peaches are based on their dried weights. The moisture contents for fresh and canned apricots, as determined by a microwave moisture analyzer, were 81% and 86%, respectively. Similarly, the moisture contents for fresh and canned peaches were 93% and 82%, respectively.

Effect of Drying and Processing on Distribution of Carotenoids in Apricots and Peaches. A comparison between the HPLC profiles of the carotenoids in the extracts from fresh apricots and peaches before and after drying indicates that no significant changes in the qualitative distribution of carotenoids resulted from the drying process. Similarly, the qualitative distributions of carotenoids in the extracts from canned apricots and peaches were found to resemble those of their fresh and dried fruits. It is difficult to determine the quantitative losses of carotenoids as result of drying and processing of apricots and peaches, since the samples of these fruits before processing were not available. Furthermore, since the moisture content of fresh and canned fruits would be expected to contribute to errors in weight measurement of the actual sample extracted, a comparison between the carotenoid levels in fresh, canned, and dried apricots and peaches (Table III) may not reflect accurate losses of carotenoids as a result of processing. The predominant carotenoids in apricots and peaches belong to the classes of hydrocarbon carotenoids and carotenol fatty acid esters, which have been found to be more heat resistant than other classes of carotenoids such as xanthophylls (Khachik et al., 1986; Khachik and Beecher, 1987, 1988a).

Nomenclature. For convenience, the trivial names of several naturally occurring carotenoids have been used throughout this report. Trivial and systematic names as well as chemical structures of these carotenoids with ε-, β-, and ψ-type end groups have been tabulated by Straub (1987). In cases where definite geometrical configurations of the cis carotenoids are not known, prefixes such as neo U, neo A, and neo B have been used to distinguish these compounds. In the old nomenclature (Zechmeister, 1962) cis carotenoids that chromatographed and eluted before their all-trans compound on certain adsorbents [i.e. calcium hydroxide (+Celite), calcium carbonate, alumina] were designated prefixes such neo A, B, C, etc., and the cis carotenoids that chromatographed after their all-trans compound were designated prefixes such as neo U, V, W, etc. The order of elution for cis carotenoids on C_{18} reversed-phase adsorbent with respect to their all-trans compound is not expected to be the same as that of cis carotenoids on adsorbents such as calcium hydroxide and alumina. Therefore, in the present study we have arbitrarily designated the prefixes such as neo U and neo V cis carotenoids that elute before their all-trans compound and neo A, and neo B to cis carotenoids eluting after their all-trans compound on a C_{18} reversed-phase column.

ABBREVIATIONS

HPLC, high-performance liquid chromatography; TLC, thin-layer chromatography; EI, electron impact; DCI, desorption chemical ionization.

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