Occurrence of *cis*-Isomers of Provitamin A in Brazilian Fruits

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Brazil has a wide variety of tropical, subtropical, and temperate fruits with widely differing carotenoid compositions, providing a good setting for investigating the natural occurrence of *cis*-isomers of provitamins. Seventy-five samples were analyzed. The fruits could be classified into two main groups: (1) those having β-carotene as the principal provitamin and (2) those with β-cryptoxanthin as the major provitamin. Some fruits also had α-carotene, γ-carotene, α-cryptoxanthin, and β-apo-10′-carotenal, usually at low levels. *cis*-Isomers were not found in cajá, papaya (two cultivars), passion fruit, pitanga, and West Indian cherry. Traces of 13-*cis*-β-carotene were found in some samples of loquat, mango (two cultivars), and piqui. Buriti, mamey, nectarine, and peach had 0.1–4.2 μg/g 13-*cis*-β-carotene and 0.1–1.0 μg/g 9-*cis*-β-carotene; the latter two fruits and piqui also had 0.2–0.4 μg/g neo-β-cryptoxanthin. Overestimations of only 3–10% of the retinol equivalents occurred when the isomers were not separated, indicating that this separation is not important in fresh fruits.

INTRODUCTION

Carotenoids are found in nature primarily in the more stable *trans*-form. *cis*-Isomers, however, do occur naturally. The first two carotenoids in the biosynthetic pathway, phytoene and phytofluene, predominantly exist in the 15-*cis*-configuration. The presence of small amounts of the *cis*-isomers of other carotenoids has been increasingly reported.

Since the *cis*-isomers have lower biological potency than the corresponding *trans*-carotenoids, concern has been raised over the necessity of separating and quantitating the isomers of provitamin A individually so as to determine the vitamin A value (activity) of foods more accurately. This operation, however, is not easily accomplished. In gravity-flow column (also called open column) chromatography, it requires rechromatography of the provitamins isolated by a MgO:HyfloSupercel column on a $Ca(OH)_2$ column, and, especially with the second column, separation efficiency and reproducibility depend heavily on the analyst's skill.

With HPLC, isomer separation and quantitation are still not well established. Even when separation of the isomers is achieved, the determination of the absolute concentrations is difficult. Quackenbush and Smallidge (1986) tested more than 20 C_{18} and C_8 commercial columns, and only 3 C_{18} columns showed some separation of the isomers of β-carotene, with Vydac 201 TP being the most effective. They also demonstrated the variation in the purity (0.6–88.7%) of commercial *trans*-β-carotene used as standard. Using methanol/chloroform (94:6) as mobile phase, some food samples were analyzed, and the 13-*cis*- and 9-*cis*-β-carotene levels were given as percentages of the total β-carotene (Quackenbush, 1987). The *cis*-isomers appeared as a shoulder and were quantified with *trans*-β-carotene in Khachik and Beecher (1988), Khachik et al. (1986, 1989, 1992), and Heinonen et al. (1989), using C_{18} columns with gradient elution. Craft et al. (1990) compared five C_{18} columns and eventually chose Vydac 201 TP with methanol/water (97:3) as mobile phase to separate *cis*- and *trans*-isomers of β-carotene in commercial preparations. Quantitative data were calculated as area percentages; these would be rough estimates, considering that the isomers have different absorption coefficients and absorb maximally at different wavelengths. To assess the nutritive value of foods, the absolute concentrations are necessary. Craft et al. (1990) also reported that impurities accounted for 16–75% of the absorbance of commercial β-carotene preparations at 450 nm.

The *cis*-isomers of β-carotene were separated on a laboratory packed $Ca(OH)_2$ column developed with 0.1 or 0.5% acetone in hexane (Tsukida et al., 1982) or with acetone/hexane (3:997) (Chandler and Schwartz, 1987). The latter system was applied to some food samples, and, on the basis of the absorbance at 436 nm, the relative percentages were calculated. Pettersson and Jonsson (1990) used the same column with 0.5–1.0% acetone in hexane as mobile phase to separate the isomers of α- and β-carotene. Although the isomers could be detected, the quantitative data (for heat-treated carrot juice) were given in terms of α- and β-carotene, without distinguishing the isomers. These workers emphasized the problems of obtaining and maintaining reliable carotenoid standards for quantitation and the sensitivity of $Ca(OH)_2$ to water and other polar solvents. In addition, the late-eluting *cis*-isomers of α-carotene overlapped with the early-eluting β-carotene isomers.

O'Neil et al. (1991) evaluated three C_{18} and $Ca(OH)_2$ columns and various solvent systems, finding Vydac 201 TP and $Ca(OH)_2$ most effective. The $Ca(OH)_2$ column was more efficient, being able to separate 15-*cis*- from 13-*cis*- and 9-*cis*-β-carotene with fewer interferences. Greater sensitivity was obtained with the C_{18} column, however. Absorbance at 410 nm, close to an isosbestic point, was employed so that the *trans*-β-carotene calibration curve could be used for the quantification of the *cis*-isomers. Other investigators reported the separation of the isomers of β-carotene (Bushway, 1985; Lesellier et al., 1989; Schmitz et al., 1989; Saleh and Tan, 1991), but no quantitation was carried out. Saleh and Tan (1991) noted the difficulty in separating the *cis*-isomers of α-carotene in the C_{18} column. Separation of the isomers of β-cryptoxanthin by HPLC has not been reported. In none of the HPLC studies were the provitamin A data converted to retinol equivalents.

An aggravating problem is the possibility of *trans*–*cis*-isomerization occurring during analysis. Catalyzed by light, heat, acids, and actives surfaces, this isomerization is the most common artifact problem in carotenoid analysis (Liaaen-Jensen, 1989). Formation of *cis*-isomers of β-car-

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otene, for example, was observed on hot saponification (Kimura et al., 1990) and on standing in chlorinated solvents, such as chloroform and methylene chloride (Pesek et al., 1990), in the dark. Thus, any report of the presence of provitamin *cis*-isomers should guarantee that they are natural constituents and not artifacts formed during analysis.

In addition, Khachik et al. (1988) found that interaction among carotenoid molecules, injection solvent, and mobile phase could produce HPLC peaks that could be misidentified as impurities or *cis*-carotenoids. HPLC peak splitting occurred when *trans*-carotenoids were injected in methylene chloride but not in acetone, with a mixture of methanol, acetonitrile, methylene chloride, and hexane as mobile phase. Other injection solvents that did not result in HPLC artifacts were acetonitrile, methanol, and hexane; aside from methylene chloride, chloroform, tetrahydrofuran, benzene, and toluene produced HPLC artifacts. Peak distortion and multiple peak formation might be avoided by injecting concentrated samples in small volumes.

Before a difficult and error-prone step is required in official methods for provitamin A determination of foods, the extent of the natural occurrence of *cis*-isomeric provitamins needs to be appraised. With its vast expanse of land, subject to different climatic conditions, Brazil has a wide variety of tropical, subtropical, and temperate fruits with various carotenoid compositions. This provides a good setting to investigate the *cis*-isomer distribution in fruits.

MATERIALS AND METHODS

Sample Collection and Preparation. Most of the fruit samples were purchased from farmer's markets, supermarkets, and groceries in Campinas, São Paulo. Mango cv. Haden, papaya cv. Solo, nectarines, passion fruit, peach cv. Diamante, and loquat were produced in the state of São Paulo. Mango cv. Tommy Atkins and papaya cv. Tailândia came from Mato Grosso and Bahia, respectively. One type of peach came from Chile. West Indian cherry and pitanga were collected from home gardens in Campinas. Mamey and caja were bought in Maranhão and Rio Grande do Norte, respectively, and piqui and buriti in Piaui. The latter samples were transported by air to Campinas on the same day they were purchased. All samples were analyzed on arrival in our laboratory.

For the larger fruits (papaya, mango, and mamey), three to five fruits from each lot were quartered, opposite sections were taken, seeds and peel were removed, and the combined pulp was homogenized in a Waring blender. For medium-sized fruits (peach, nectarines, loquat, piqui, and buriti), 5–10 fruits from each lot were homogenized after removal of inedible portions (seeds and peel). About 30 fruits were taken from each lot of the small fruits (West Indian cherry, cajá and pitanga), which were deseeded and homogenized. For passion fruit, the pulp with the seeds was blended, facilitating the removal of the seeds. Depending on the carotenoid content, 5–100 g of the homogenized samples was submitted to analyses.

All of the necessary precautions were taken to avoid *cis*-isomerization during analysis, such as immediate and rapid analysis and protection from light and high temperature.

Determination of Absorption Coefficients. Freshly purified carotenoids were weighed on a Perkin-Elmer Model AD-6 microbalance (precision ±0.0001 mg) and dissolved in reagent grade solvents with ultrasonic agitation. The absorption spectra were recorded on a Perkin-Elmer Model Lambda-6 spectrophotometer.

trans-β-Carotene and *trans*-α-carotene crystals were gifts from Hoffmann-La Roche (Basle, Switzerland) and were used immediately after the ampule was opened. *trans*-β-Cryptoxanthin and *trans*-lycopene were isolated from papaya. 13-*cis*-β-Carotene and 9-*cis*-β-carotene were isolated from kale and watercress, and neocryptoxanthin was isolated from peach and nectarine. The new carotenoid β-apo-8′-carotenol was isolated from mamey. Structure elucidation of this pigment has been reported (Godoy et al., 1992).

The carotenoids from natural sources were extracted and separated on a MgO:HyfloSupercel column as described in the succeeding section; prior to concentration and chromatographic separation, sterols were precipitated by leaving the extracts in petroleum ether in a freezer overnight at −10 °C. The β-carotene fraction was rechromatographed on a $Ca(OH)_2$ column. As first mentioned by Bickoff et al. (1949), not all brands of $Ca(OH)_2$ are capable of separating the isomers. 13-*cis*-Carotene and *trans*-β-carotene were eluted with petroleum ether, and 9-*cis*-β-carotene was eluted with 4% ethyl ether in petroleum ether. Each isomer was rechromatographed on a $Ca(OH)_2$ column two more times, only the major portion of the band being collected each time. The β-cryptoxanthin fraction was also rechromatographed on a $Ca(OH)_2$ column. Neocryptoxanthin and *trans*-β-cryptoxanthin were eluted with 10% and 20% acetone in petroleum ether, respectively. *trans*-Lycopene and *trans*-β-apo-8′-carotenol were purified by rechromatography on a neutral alumina (activity II–III) column. An aliquot of collected *trans*-lycopene was also passed through a $Ca(OH)_2$ column to confirm the absence of the *cis*-isomers. Solvents were removed with N_2. *trans*-Lycopene was crystallized, but we had difficulty crystallizing β-cryptoxanthin. The amount of *cis*-isomers that could be obtained was too small to allow crystallization. In any case, reversion to the *all-trans*-configuration could occur on crystallization (Davies, 1976). The purity of the carotenoids was monitored on a silica gel thin layer developed with 3% methanol in benzene and, principally, by HPLC using a Vydac 201 TP54 C_{18} column with methanol/water (98:2) as mobile phase. Absorption was scanned not only in the visible but also in the UV region to detect possible colorless contaminants such as sterols.

The absorption coefficients ($A^{1\%}_{1cm}$) were determined immediately after purification. The absorption coefficients were calculated according to Beer's law (Davies, 1976). Twenty determinations were carried out for each pigment in each solvent. A few outliers were rejected using the Q test (Dean and Dixon, 1951).

Open Column Carotenoid Determination. The steps from extraction to separation on a MgO:HyfloSupercel column (1:2) were carried out according to the method of Rodriguez et al. (1976). Briefly, this involved extraction with cold acetone in a Waring blender, filtration in a Büchner funnel (extraction and filtration being repeated until the residue became colorless), transfer to petroleum ether in a separatory funnel with the addition of water, washing, drying over anhydrous sodium sulfate, concentration in a rotary evaporator ($T < 35$ °C), and separation on a MgO:HyfloSupercel (1:2) column. Elution was accomplished with increasing concentrations of ethyl ether and acetone in petroleum ether. Each provitamin A fraction obtained was rechromatographed once on a $Ca(OH)_2$ column for the separation of the isomers, elution being carried out as described in the previous section.

Confirmation of the identity of the carotenoids was done as described in detail previously (Rodriguez et al., 1976). This involved the visible absorption spectra, chromatographic behavior on column and TLC, chemical reactions, such as acetylation and methylation for hydroxycarotenoids, $NaBH_4$ reduction of apocarotenals, and iodine-catalyzed isomerization to distinguish *cis*- and *trans*-isomers. The major *cis*-isomers of β-carotene, eluting before and after *trans*-β-carotene in the $Ca(OH)_2$ column, were shown to be 13-*cis*-β-carotene and 9-*cis*-β-carotene, respectively, on the bases of their 200-MHz ^1H NMR and 50.3-MHz ^{13}C NMR spectra (Tsukida et al., 1981). The structure of the *cis*-isomer of β-cryptoxanthin has not been elucidated.

Quantitation of the isolated provitamins was carried out spectrophotometrically as described by Davies (1976), using the absorption coefficients determined in this study.

Confirmation of Presence of *cis*-Isomers by HPLC. It had been repeatedly shown in our laboratory that, under the conditions used, *trans*–*cis*-isomerization did not occur on the MgO:HyfloSupercel and $Ca(OH)_2$ columns and good separation of the *cis*- and *trans*-isomers of provitamins could be achieved in the latter column (Rodriguez-Amaya and Tavares, 1992). Nevertheless, verification of the incidence of the *cis*-isomers was

Table 1. Absorption Coefficients of Common *trans*-Carotenoids

carotenoid	$A^{1\%}_{1cm}$	λ (nm)	solvent	reference
α-carotene	2800	444	petroleum ether	Schwieter et al. (1965)
	2800	445	hexane	Goodwin (1955)
	2730	446	hexane	Zscheile et al. (1942)
	2770 ± 60[a]	444	petroleum ether	our results
β-carotene	2592	453	hexane	Isler et al. (1956)
	2620	453	ethanol	Isler et al. (1956)
	2592	453	petroleum ether	Schwieter et al. (1965)
	2580	450	hexane	Zscheile et al. (1942)
	2505	451	hexane	Goodwin (1955)
	2540	450	methanol	Craft and Soares (1992)
	2580 ± 40[a]	449	petroleum ether	our results
β-cryptoxanthin	2386	452	petroleum ether	Isler et al. (1957)
	2470	452	hexane	Zscheile et al. (1942)
	2330 ± 90[a]	447	petroleum ether	our results
lycopene	3450	472	petroleum ether	Schwieter et al. (1965)
	3460	472	hexane	Zechmeister et al. (1943)
	3370	487	benzene	Surmatis and Ofner (1963)
	3420 ± 60[a]	470	petroleum ether	our results

[a] Means and standard deviations of 20 determinations.

Table 2. Absorption Coefficients[a] of *cis*-Isomers of β-Carotene, β-Cryptoxanthin, and β-Apo-8′-carotenol

carotenoid	$A^{1\%}_{1cm}$	λ (nm)	solvent
13-*cis*-β-carotene	1740 ± 30[a]	443	petroleum ether
	1780 ± 40	444	hexane
	1690 ± 30	445	methanol
	1930[b]	444	1.5% *p*-methylanisole in petroleum ether
9-*cis*-β-carotene	2380 ± 40	445	petroleum ether
	2400 ± 30	445	hexane
	2240 ± 30	446	methanol
	2360[b]	449	0.5% *p*-methylanisole in petroleum ether
neocryptoxanthin	2180 ± 70	445	petroleum ether
	2200 ± 30	444	hexane
	2090 ± 30	445	methanol
β-apo-8′-carotenol	2050 ± 20	398	petroleum ether
	1970 ± 30	398	hexane
	2030 ± 10	396	methanol

[a] Means and standard deviations of 20 determinations. [b] Taken from Sweeney and Marsh (1970) for comparison.

also done by HPLC. A Varian liquid chromatograph equipped with a Varian UV–visible detector Model 5100 was used. The instrument had a ternary solvent delivery system Model 5010, a Rheodyne manual injection switching valve (10-μL sample loop), and an integrator–recorder Model 4400. Separation was carried out with a 250 × 4.6 i.d. mm Vydac 201 TP54 C_{18} (5-μm particle size) column (Vydac Separation Group, Hesperia, CA), protected by a 30 × 4.6 i.d. mm C_{18} (10 μm) Varian Micropore MCH-120 guard column. Methanol/water (98:2) was used as mobile phase, and detection was set at 450 nm. The *cis*- and *trans*-provitamins were identified by cochromatography with isomers isolated as described above for the determination of absorption coefficients and those produced by iodine-catalyzed isomerization and by the spectra obtained with a Waters diode array detector Model 994.

Calculation of the Vitamin A Value. The NAS-NRC (1989) conversion ratio of 6 μg of β-carotene to 1 retinol equivalent (RE) was used in the calculation of the vitamin A values, also taking into consideration currently accepted bioactivities [13% for 13-*cis*-α-carotene, 50% for *trans*-α-carotene, 53% for 13-*cis*-β-carotene, 100% for *trans*-β-carotene, 38% for 9-*cis*-β-carotene, 30% for *trans*-α-cryptoxanthin, 42% for cis-β-cryptoxanthin, 57% for *trans*-β-cryptoxanthin, 42% for *trans*-γ-carotene, and 100% for *trans*-β-apo-10′-carotenal (Deuel et al.,1944, 1945a,b; Bauernfeind, 1972; Zechemeister, 1962)]. For β-apo-8′-carotenol, the biopotency (72%) of β-apo-8′-carotenal (Bauernfeind, 1972) was used.

RESULTS AND DISCUSSION

Whether the separation of the carotenoids is done by open column chromatography or by HPLC, the quantitation is ultimately done spectrophotometrically. Thus, the accuracy of the results depends on the absorption coefficients used in the calculation of the concentrations. Most authors adopt published values, although discrepancies in reported coefficients, in the same solvent, can be seen in Table 1. In addition, the coefficients of *cis*-isomers of provitamin are not known, except for those of 13-*cis*- and 9-*cis*-β-carotene in petroleum ether with 1.5% and 0.5% *p*-methylanisole, respectively (Sweeney and Marsh, 1970). This determination is not easy, and standard deviations observed in our laboratory, using a microbalance, are shown in Tables 1 and 2. Our results, nonetheless, are coherent with most reported data.

Considering the data presented in Table 3, the different fruits can be divided into two main groups: (a) those having β-carotene as the principal provitamin and (2) those with β-cryptoxanthin as the major provitamin. Buriti, loquat, mamey, mango, passion fruit, and West Indian cherry belong to the first group; the mean *trans*-β-carotene content varied from 3.4 to 359.8 μg/g. Buriti is an exceptionally rich provitamin A source, with its β-carotene content being much higher than that of the other fruits. The second group consisted of cajá, nectarine, papaya, peach, piqui, and pitanga, the *trans*-β-cryptoxanthin range being 3.9–16.9 μg/g. β-Carotene was found in all samples. Although β-carotene predominated in West Indian cherry and loquat, β-cryptoxanthin was also found. Small amounts of α-cryptoxanthin appeared in the mango cultivars, while γ-carotene was encountered at high concentration in buriti and at low levels in papaya cv. Solo and pitanga. Mamey had appreciable amounts of β-apo-10′-carotenal and the hitherto unreported β-apo-8′-carotenol. From structural considerations, this new apocarotenoid should have vitamin A activity; thus, it was included in Table 3.

cis-Isomers of provitamin A were not found in cajá, papaya cv. Solo and Tailândia, passion fruit, pitanga, and West Indian cherry. Traces of 13-*cis*-β-carotene were observed in some samples of loquat, mango cv. Haden and Tommy Atkins, and piqui. The mean vitamin A values of the mentioned fruits varied from 64 to 259 RE/100 g (Table 4).

cis-Isomers were found at low levels in mamey (0.5 μg/g 13-*cis*-β-carotene and 0.3 μg/g 9-*cis*-β-carotene), nectarine

Table 3. *Cis*- and *Trans*-Isomer Concentrations of Provitamins in Brazilian Fruits

common name, English or Portugese	scientific name	no. of samples analyzed	pH range	provitamin A	concentration (μg/g)
buriti	*Mauritia vinifera*	5	nd[a]	13-*cis*-α-carotene	1.5 ± 1.4
				trans-α-carotene	80.1 ± 9.0
				13-*cis*-β-carotene	4.2 ± 2.4
				trans-β-carotene	359.8 ± 32.5
				9-*cis*-β-carotene	1.0 ± 0.5
				trans-β-zeacarotene	5.4 ± 1.4
				trans-γ-carotene	36.8 ± 4.5
caja	*Spondias lutea*	5	3.9–4.4	*trans*-α-carotene	0.3 ± 0.1
				trans-β-carotene	1.4 ± 0.3
				neocryptoxanthin	tr[b] in 2 samples
				trans-β-cryptoxanthin	16.9 ± 2.2
loquat	*Eriobotrya japonica*	5	4.1–4.3	13-*cis*-β-carotene	tr in 3 samples
				trans-β-carotene	8.0 ± 0.6
				trans-β-cryptoxanthin	4.8 ± 0.5
mamey	*Mammea americana*	5	4.3–4.6	13-*cis*-β-carotene	0.5 ± 0.1
				trans-β-carotene	14.1 ± 4.1
				9-*cis*-β-carotene	0.3 ± 0.2
				trans-β-zeacarotene	0.8 ± 0.1
				trans-β-apo-10′-carotenal	5.0 ± 1.4
				trans-β-apo-8′-carotenol	11.1 ± 3.7
mango	*Mangifera indica*				
cv. Haden		5	4.1–4.4	13-*cis*-β-carotene	tr in 3 samples
				trans-β-carotene	12.5 ± 4.4
				trans-α-cryptoxanthin	0.3 ± 0.1
cv. Tommy Atkins		5	4.3–4.7	13-*cis*-β-carotene	tr in 2 samples
				trans-β-carotene	15.5 ± 0.9
				trans-α-cryptoxanthin	0.4 ± 0.2
nectarine	*Prunus persica*	5	4.4–4.7	13-*cis*-β-carotene	0.1 ± 0.1
				trans-β-carotene	1.1 ± 0.2
				9-*cis*-β-carotene	0.1 ± 0.1
				neocryptoxanthin	0.3 ± 0.2
				trans-β-cryptoxanthin	3.9 ± 0.7
papaya	*Carica papaya*				
cv. Solo		5	4.0–4.4	*trans*-β-carotene	3.0 ± 0.4
				trans-β-cryptoxanthin	7.6 ± 0.8
				trans-γ-carotene	tr in 3 samples
cv. Tailândia		5	4.0–4.2	*trans*-β-carotene	2.6 ± 0.6
				trans-β-cryptoxanthin	10.2 ± 2.5
passion fruit cv. Mamão	*Passiflora edulis*	5	4.0–4.2	*trans*-β-carotene	4.7 ± 1.0
peach	*Prunus persica*				
Chilean		5	4.2–4.4	13-*cis*-β-carotene	0.2 ± 0.1
				trans-β-carotene	1.2 ± 0.2
				9-*cis*-β-carotene	0.1 ± 0.1
				neocryptoxanthin	0.3 ± 0.1
				trans-β-cryptoxanthin	5.1 ± 0.5
cv. Diamante		5	4.1–4.3	13-*cis*-β-carotene	0.2 ± 0.1
				trans-β-carotene	0.6 ± 0.2
				9-*cis*-β-carotene	0.1 ± 0.1
				neocryptoxanthin	0.2 ± 0.1
				trans-β-cryptoxanthin	4.1 ± 0.3
piqui	*Cariocar villosium*	5	nd	*trans*-α-carotene	0.1 ± 0.1
				13-*cis*-β-carotene	tr in 2 samples
				trans-β-carotene	1.2 ± 0.5
				neocryptoxanthin	0.4 ± 0.2
				trans-β-cryptoxanthin	4.4 ± 0.9
pitanga	*Eugenia uniflora*	5	4.0–4.1	*trans*-β-carotene	3.7 ± 0.6
				trans-β-cryptoxanthin	12.3 ± 1.1
				trans-γ-carotene	0.4 ± 0.1
West Indian cherry	*Malpighia glabra*	5	4.1–4.4	*trans*-α-carotene	tr in 2 samples
				trans-β-carotene	3.4 ± 0.2
				trans-β-cryptoxanthin	0.4 ± 0.1

[a] nd, not determined (the nature of this fruit did not permit determination of the pH, as done with the other fruits). [b] tr, trace.

Table 4. Vitamin A Values Calculated with and without Isomer Separation

fruit/cultivar	vitamin A value[a] (RE/100 g) without isomer separation	with isomer separation	% overestimation
buriti	6992 ± 462	6489 ± 341	8
caja	191 ± 24		
loquat	179 ± 15		
mamey	328 ± 36	293 ± 47	10
mango			
cv. Haden	209 ± 10		
cv. Tommy Atkins	259 ± 17		
nectarine	47 ± 4	45 ± 4	4
papaya			
cv. Solo	122 ± 13		
cv. Tailândia	140 ± 26		
passion fruit	78 ± 17		
peach			
Chilean	75 ± 6	73 ± 6	3
cv. Diamante	58 ± 8	55 ± 8	5
piqui	57 ± 10	54 ± 10	6
pitanga	178 ± 13		
West Indian cherry	64 ± 14		

[a] Each value is the mean and standard deviation of five sample lots analyzed individually.

(0.1 μg/g 13-*cis*-β-carotene, 0.1 μg/g 9-*cis*-β-carotene, and 0.3 μg/g *cis*-β-cryptoxanthin), peach (0.2 μg/g 13-*cis*-β-carotene, 0.1 μg/g 9-*cis*-β-carotene and 0.3 μg/g *cis*-β-cryptoxanthin for the Chilean peach and 0.2 μg/g 13-*cis*-β-carotene, 0.1 μg/g 9-*cis*-β-carotene, and 0.2 μg/g *cis*-β-cryptoxanthin for the peach cv. Diamante), and piqui (0.4 μg/g *cis*-β-cryptoxanthin).

Because of the difficulty in obtaining baseline separation of the isomers and, more importantly, in acquiring *cis*-isomer standards, quantitation was not done by HPLC. Nonetheless, the HPLC chromatograms, some of which are shown in Figures 1 and 2, confirmed the results of the open column determinations. In Figure 1, the first two chromatograms (A and B) show the absence of *cis*-isomers of β-carotene and β-cryptoxanthin in papaya and of β-carotene in passion fruit. The other two chromatograms demonstrate the existence of *cis*-isomers of β-carotene and β-cryptoxanthin in nectarine (C) and peach (D). The chromatograms of the whole extracts were corroborated by those of provitamin fractions collected from the MgO:HyfloSupercel column. The β-carotene fraction of peach (Figure 2A) has *cis*-isomers, while that of papaya does not have these isomers (B). The β-cryptoxanthin fraction of papaya is devoid of *cis*-isomers (C), while that of peach has a *cis*-isomer (D). Our HPLC chromatograms are comparable with those obtained by other authors (Chandler and Schwartz, 1987; Quackenbush, 1987; Pettersson and Jonsson, 1990; O'Neil et al., 1991; Saleh and Tan, 1991) in terms of isomer separation.

The presence of *cis*-isomers of β-carotene had been reported in peach, apricot, and plum by Chandler and

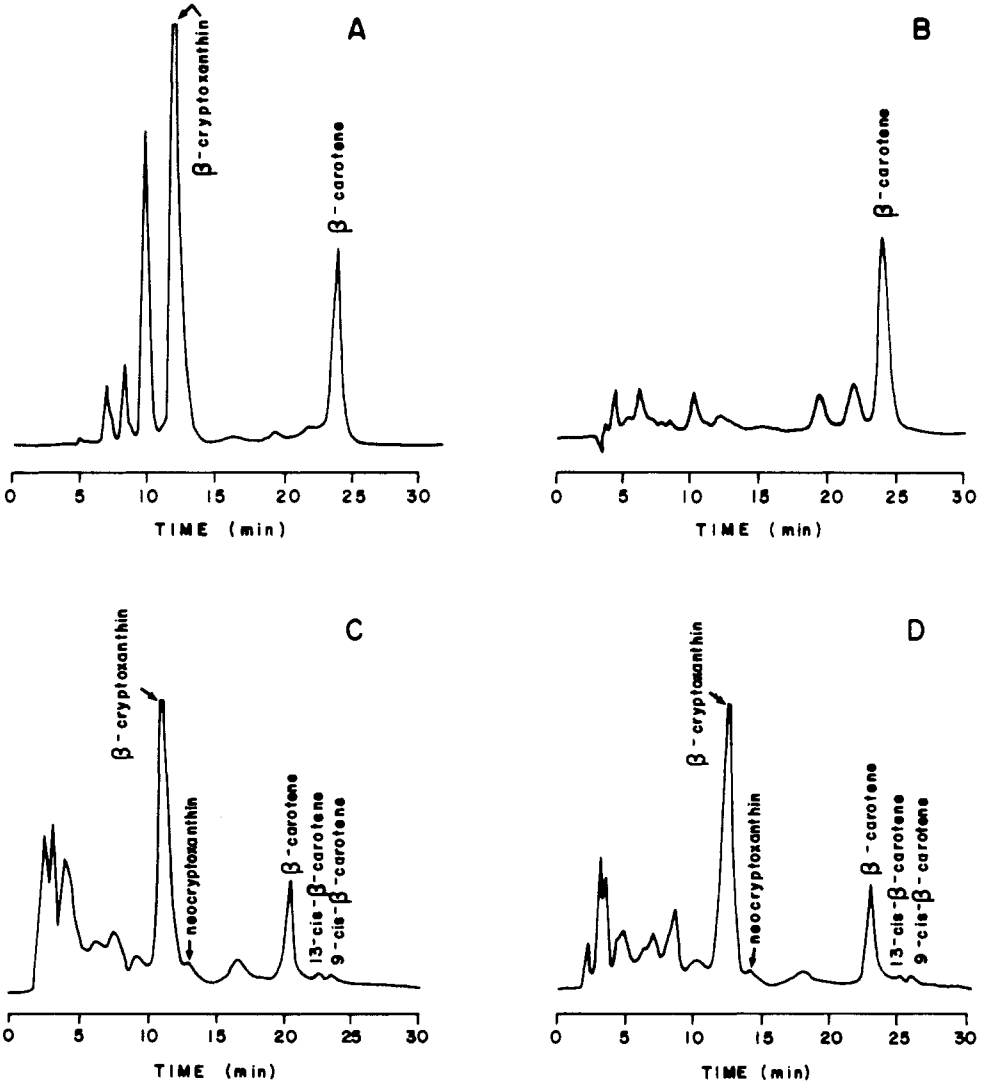

Figure 1. HPLC chromatograms of the total extracts of (A) papaya, (B) passion fruit, (C) nectarine, (D) peach.

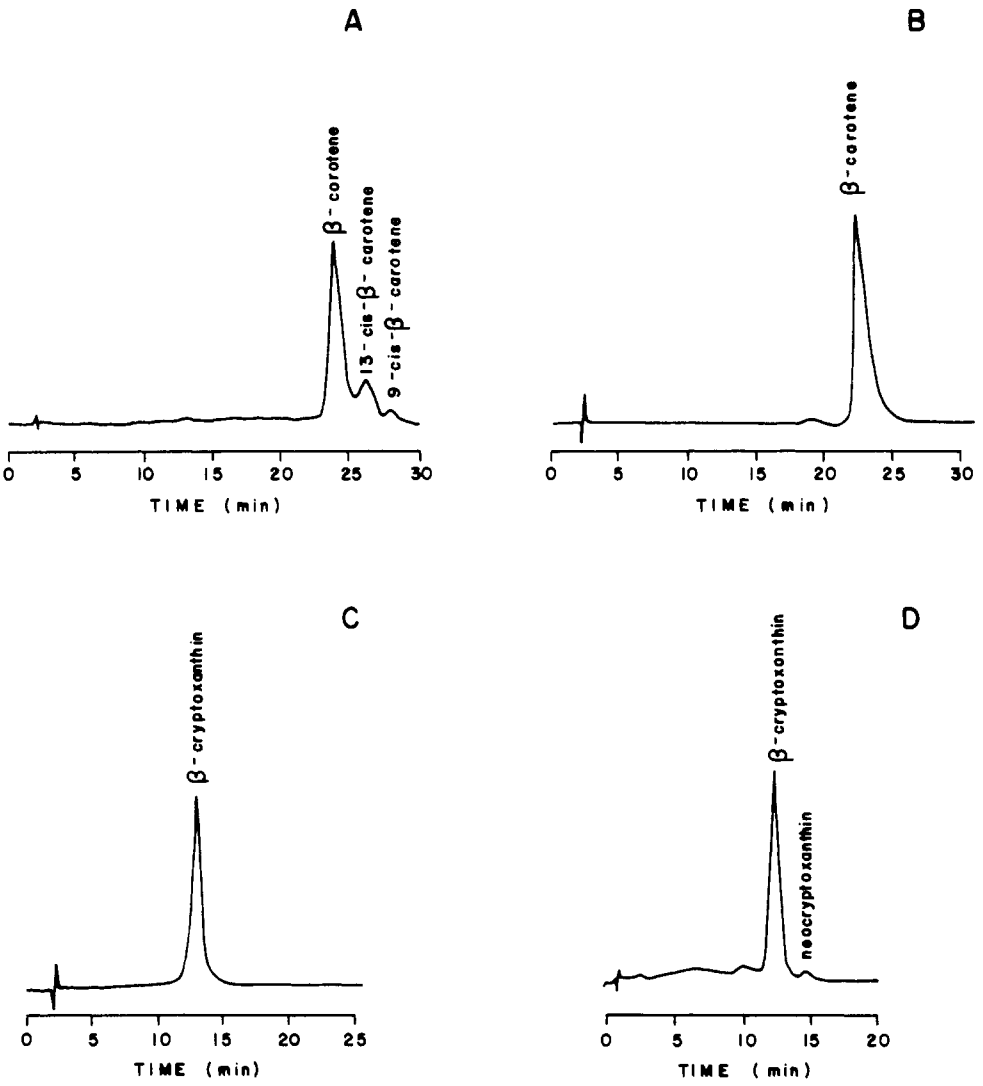

Figure 2. HPLC chromatograms of provitamin fractions obtained from the MgO:HyfloSupercel column: (A) β-carotene fraction of peach; (B) β-carotene fraction of papaya; (C) β-cryptoxanthin fraction of papaya; (D) β-cryptoxanthin fraction of peach.

Schwartz (1987) and in peach and apricot by Quackenbush (1987), using HPLC methods. These authors did not verify the occurrence of β-cryptoxanthin in either isomeric configuration.

The vitamin A values of the fruits containing *cis*-isomers were calculated with (mean RE/100 g, 45–6489) and without isomer separation (mean RE/100 g, 47–6992), showing overestimations of 3–10% when the isomers were not separated (Table 4). Losses on the $Ca(OH)_2$ column were found to be about 4% for *trans*-β-carotene and 6% for *trans*-β-cryptoxanthin in our laboratory. If these losses were considered, the estimated overestimations would either be nonexistent or much lower. In view of these results, and considering the extent of natural variations between samples of the same foods (shown by the standard deviations in Table 3) and the fact that the conversion factors currently used to calculate vitamin A activities are rough estimates, isomer separation does not appear to be necessary in the provitamin A determination of fresh fruits.

Especially with HPLC papers, there is much more concern in the literature over the separation of the *cis*-isomers of β-carotene and sometimes α-carotene than the quantitation of β-cryptoxanthin even in its *trans*-configuration. The results of this study show that, at least for fruits, β-cryptoxanthin is much more important.

In a previous paper, *cis*-isomers of β-carotene were not detected in 10 sample lots of fresh tomatoes. However, in 52 samples of processed tomato products, varying amounts of 13-*cis*- and 9-*cis*-β-carotene were found, reaching high levels in some samples. Formation of *cis*-isomers as a consequence of heat treatment has been reported by several authors (Gortner and Singleton, 1961; Panalaks and Murray, 1970; Sweeney and Marsh, 1971; Lee and Ammerman, 1974; Ogunlesi and Lee, 1979; Khachik et al., 1986; Quackenbush, 1987; Chandler and Schwartz, 1987, 1988; Lawrence et al., 1988). Thus, contrary to fresh fruits, significant amounts of *cis*-provitamins may be present in processed fruits, making their separation and quantitation in provitamin A determination important.

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