Determination of β-Carotene and Other Hydrocarbon Carotenoids in Red Grapefruit Cultivars†

Russell L. Rouseff,* George D. Sadler, Tammy J. Putnam, and John E. Davis

Citrus Research and Education Center, University of Florida, Lake Alfred, Florida 33850

Liquid–liquid extraction sample preparation was employed with nonaqueous reverse-phase HPLC and photodiode array detection to separate and quantify lycopene, β-carotene, ζ-carotene, phytoene, and phytofluene in newer red grapefruit cultivars. These cultivars contained appreciably higher concentrations of both visible and colorless carotenoids when compared to the Ruby Red cultivar. Lycopene and β-carotene were found in highest concentrations in the Star Ruby cultivar. Mean lycopene (all trans) levels in descending order were Star Ruby (33 μg/g), Ray (21 μg/g), and Flame (7.9 μg/g). Lycopene levels in the comparison Ruby Red grapefruit were 2.9 (interior) and 1.6 μg/g (Indian River). Mean β-carotene levels in decreasing order were Star Ruby (9.6 μg/g), Flame (8.6 μg/g), and Ray (7.0 μg/g). The comparison Ruby Red fruits were both 4.2 μg/g. These cultivars could serve as important dietary sources of β-carotene.

INTRODUCTION

Pink and Ruby Red grapefruit cultivars differ from the more common Marsh and Duncan grapefruit cultivars in that they contain significant amounts of lycopene. The first pink grapefruit was discovered among a planting of Marsh (white) grapefruit in 1913 and was called Thompson Pink or Pink Marsh. Its flesh was lightly pink which quickly faded with increasing maturity. It was replaced in the 1930s and 1940s by the more darkly colored Ruby Red. The Ruby Red originated from a limbsport of a Thompson Pink and has been widely grown in both Texas and Florida. However, its color also fades with increasing maturity. The desire to obtain fruit of a more consistent red color led to the development of three intensely colored red grapefruit cultivars. The Star Ruby originated from an irradiated seed of the Hudson grapefruit, a minor seedy red grapefruit. Flame grapefruit was obtained from the seed of a red grapefruit called Henderson. The Ray Ruby was selected as an outstanding tree from a grove of Ruby Red grapefruit in Texas.

Kahn and Mackinney (1953) reported that the major carotenoids in pink grapefruit were lycopene, β-carotene, and ζ-carotene. Huffman et al. (1953) found lycopene and β-carotene in Ruby Red grapefruit juices. Curl and Bailey (1957) examined the pigments in California Ruby Red grapefruit and found the principal pigments to be lycopene and β-carotene with smaller amounts of phytofluene and ζ-carotene. Ting and Deszyck (1958) reported that in Florida the lycopene concentration and visual color of Ruby Red grapefruit decreased rapidly with maturity but β-carotene concentration increased with maturity. Cruse et al. (1979) reported on the seasonal changes of lycopene and β-carotene in Ruby Red and Star Ruby grapefruit juices from Texas. Gross (1987) examined the carotenoids in Ruby Red grapefruit grown in Israel. In all of these studies lycopene was the predominant carotenoid. β-Carotene was always found in much smaller amounts. Reports on the relative amounts of these two carotenoids vary considerably. Curl and Bailey (1957) reported that the ratio of lycopene to β-carotene was approximately 1.5:1, whereas Gross (1987) reported values that would correspond to 6:1.

Recent studies (Colditz et al., 1985; Bendich, 1989; Zeigler, 1989) have shown that there is a relationship between diets rich in β-carotene and reduced incidence of certain cancers. Because of this apparent beneficial relationship between β-carotene and cancer, the hydrocarbon carotenoid content of several newer, red grapefruit cultivars was examined to determine if they contained elevated levels of β-carotene. Since these cultivars were visibly more intensely colored than Ruby Red cultivars grown under similar conditions, it was expected that they should contain elevated levels of lycopene because Ting and Deszyck (1958) had demonstrated that the red color in red grapefruit juice is primarily associated with lycopene. However, since it is not a major color contributor in grapefruit, the β-carotene content was for the most part unknown.

One purpose of this study was to examine the hydrocarbon carotenoid content of these cultivars to determine if they contained elevated levels of β-carotene. Since early workers used lengthy sample preparation and analysis techniques, there was some concern about possible carotenoid degradation and/or artifact formation. Therefore, another goal of this study was to use a more rapid sample preparation and analysis procedure. HPLC is generally accepted as the modern method of choice to separate, identify, and quantify carotenoids so as to minimize if not eliminate carotenoid degradation or artifact formation.

MATERIALS AND METHODS

Reagents and Standards. All solvents were of chromatographic or spectral grade from Fisher Scientific (Fair Lawn, NJ). Lycopene and β-carotene standards were obtained from Sigma Chemical Co. (St. Louis, MO) and were used without further purification.

Equipment. A Perkin-Elmer (Norwalk, CT) Model 410 quaternary liquid chromatographic pump was used with a Waters Associates (Milford, MA) Model 990+ photodiode array detector. An Analytichem C_{18} column (25 cm × 4.6 mm i.d.) was used with a Supelco C_{18} (Bellefonte, PA) precolumn.

Chromatographic Conditions. The isocratic mobile phase consisted of acetonitrile, methylene chloride, and methanol (65:25:10 v/v/v). Flow rate was 1.0 mL/min. Injection volume for samples and standards was 20 μL. The separation was carried out at ambient temperature.

Carotenoid Identification and Quantification. Chromatographic peaks were identified by comparing both the

† Florida Agricultural Experiment Station Journal Series No. R-01312.

0021-8561/92/1440-0047$03.00/0 © 1992 American Chemical Society

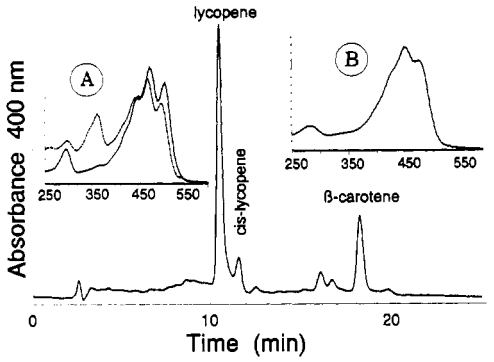

Figure 1. Chromatogram of a Ray Ruby extract at 400 nm. (Inserts A and B) Spectra obtained from the photodiode array from the lycopene, *cis*-lycopene (norlycopene), and β-carotene peaks. Note the characteristic cis peak from *cis*-lycopene (broken line) at 362 nm shown in insert A.

retention time and the absorbance spectra of standards where standards were available. Since standards for phytoene and phytofluene were not available, they were identified by comparison of the spectra obtained at each peak maximum with those found in the literature. A calibration curve of lycopene and β-carotene was obtained to determine the relationship between peak area and concentration. Concentrations of phytoene and phytofluene were determined from published 1% absorptivity coefficients (Davies, 1976). The 1% absorptivity coefficients used for β-carotene, phytoene, phytofluene, and ζ-carotene in hexane were 2592 (453 nm), 915 (286 nm), 1577 (347 nm), and 2555 (400 nm), respectively. The analytical procedures used are similar to those discussed by De Ritter and Purcell (1981). The procedure used to calculate concentrations for materials for which there are no commercial standards is based on the Beer's law, $A = \epsilon bc$, where ϵ is absorptivity, b is a cell constant, and c is the concentration (w/v). A is the absorbance, which is typically a unitless number. Peak area, which is a function of both absorbance and time, was substituted in place of absorbance since the time component is a function of flow rate and should be constant. Using a commercial standard of β-carotene of known concentration and published values of the 1% extinction coefficients, the b value for the detector cell was determined by rearranging the above equation and solving for b. Using this value for b, the concentration (w/v) for other identified components was determined using the above ϵ values and chromatographic peak area.

Sample Preparation. Commercial fresh fruit harvested in January and February 1987 from interior central Florida (sometimes called the Ridge) and the Indian River (east coast) growing regions were used in this study. Authenticated fruit was supplied by Dr. Mohammed Ismail of the Florida Department of Citrus. The flesh (intact juice vesicles and segment membranes) of fresh grapefruit was removed with a scalpel and macerated with a Waring blender. Duplicate samples from three fruit (i.e., six samples) of each cultivar were prepared. Approximately 12 g of the macerated flesh was precisely weighed and extracted in the manner used by Sadler et al. (1990). This liquid–liquid extraction procedure was modified only in that the final carotenoid containing portion was reduced to dryness with a rotary evaporator and redissolved with 1–2 drops of THF and diluted to 1.8 mL with chromatographic mobile phase. Samples were prepared in low light, blanketed with nitrogen, and analyzed immediately after extraction. Each sample was analyzed in duplicate to determine average values (n = 12). Samples were filtered and placed in amber vials prior to HPLC analysis.

RESULTS AND DISCUSSION

Chromatographic Separation. The solvent composition and column material were the same ones used by Fisher and Rouseff (1986) for orange juice carotenoids. Shown in Figure 1 is the chromatogram obtained at 400 nm, from an extract of Ray grapefruit. Most of the peaks appear to be well resolved. The predominant peak at approximately 11 min is most likely lycopene as it had the same retention time as standard lycopene. The other major peak at approximately 18.5 min had the same retention time as standard β-carotene. However, the matching of retention times is no longer considered sufficient information for identification purposes. Independent information such as absorbance spectra or mass spectra would be minimum additional information required. Identification of these and other chromatographic peaks based on spectral and chromatographic information is presented in the following section.

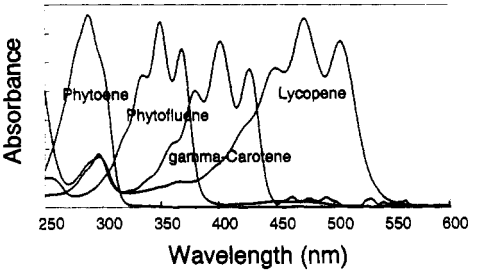

Figure 2. Photodiode array spectra of chromatographic peaks at 23.7 (phytoene), 19.6 (phytofluene), 16.9 (ζ-carotene), and 10.9 min (lycopene) from Figures 1 and 3.

Table I. Spectral Information from Chromatographic Peaks Shown in Figure 3 and the Literature

RT, min	wavelength exp, nm	wavelength lit., nm	compd	ref[a] and solv[b]
10.90	449, 474, 506	447, 472, 504	lycopene	1, a
11.84	445, 470, 500	443, 469, 500	neolycopene	2, b
12.84	412, 430, 457		unidentified	
16.26	381, 402, 426	380, 400, 424	ζ-carotene	1, a
16.92	383, 406, 430		unidentified	
18.44	430, 454, 482	425, 450, 478	β-carotene	1, a
19.54	334, 350, 368	331, 348, 367	phytofluene	1, a
19.84	423, 446, 471		unidentified	1, a
23.68	286	276, 286, 297	phytoene	3, c

[a] (1) Davies, 1976; (2) Kjøsen et al., 1969; (3) Curl, 1962. [b] (a) Petroleum ether; (b) acetone; (c) hexane.

Carotenoid Spectral Identification. The spectral identification of many compounds is often difficult because the spectra usually consist of one or two broad absorbance bands in the UV region. Fortunately, hydrocarbon carotenoids have structural characteristics that produce highly unique absorbance spectra, which greatly adds to identification confidence. Their absorbance maxima occur over a wide range spanning both visible and UV wavelengths. Spectra obtained from four chromatographic peaks are shown in Figure 2. The spectra have been normalized for comparison purposes. It can be clearly seen that all of these peaks exhibit the usual "three-fingered" spectra which are characteristic of carotenoids. However, as the number of conjugated double bonds decreases, going from lycopene (11) to phytoene (3), the distance between the three absorbance bands decreases. In the case of phytoene the two outer bands are barely discernible. As seen from Table I, the absorbance maxima compare favorably with that reported in the literature. With the exception of phytoene, all of the absorbance maxima are 2–4 nm higher than literature values, which were recorded in different solvents. This consistent bias suggests the differences are due to solvent effects and not random error. The overall absorbance band shape also matches that which is described in the literature for these compounds.

The peak immediately following lycopene (at 11.84 min in Figure 1) appears to be a cis isomer of lycopene because its spectral maxima are shifted to slightly shorter wavelengths (see Table I) and it has pronounced absorbance

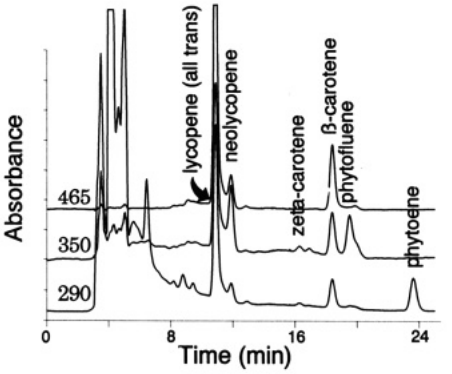

Figure 3. Chromatograms from a Ray Ruby extract monitored at 290, 350, and 465 nm. The chromatograms at 350 and 290 nm are shown at lower attenuation (greater signal amplification) for display purposes.

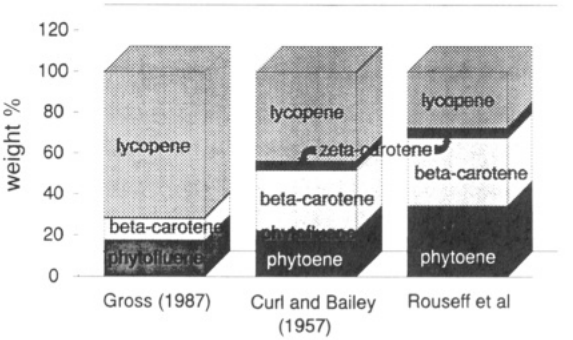

Figure 4. Comparison of relative carotene distribution in Ruby Red grapefruit.

Table II. Comparison of β-Carotene Values (μg/g) for Ruby Red Grapefruit

Ting and Deszyck	Philip and Chen	Rouseff et al.	Khachik et al.
5.0–14	2.6–3.6	4.2	23

at approximately 360 nm, which is typical of carotenoid cis isomers. Since the position of the cis double bond is not known, it is usually reported as neolycopene (Khachik et al., 1990).

The small peak following the *cis*-lycopene peak in Figure 1 (12.84 min) has not been identified. Its absorbance spectra have the typical carotenoid shape, and its absorbance maxima are listed in Table I. γ-Carotene has been reported by Gross (1987) in Ruby Red grapefruit. However, the absorbance maxima reported in the literature [414, 438, and 468 nm (petroleum ether) (Andrews and Liaaen-Jensen, 1973)] are close but the fit is not exceptionally good. The central absorbance maximum is 8 nm higher than the photodiode array value, but the photodiode array maxima were equal to or greater than literature values for all of the other carotenoids. Therefore, it is unlikely that this peak is γ-carotene. The peak at 16.26 min appears to be ζ-carotene because of its excellent spectral match and because the elution order fits with that reported by previous workers. The peak after ζ-carotene has not been identified. It displayed the typical carotenoid shape and its absorbance maxima were at slightly higher wavelengths than those of ζ-carotene (see Table I).

The location of the absorbance maximum for β-carotene was 2–4 nm higher than the closest literature values. This discrepancy is entirely due to solvent differences as the observed spectral characteristics were an exact match with that obtained from standard β-carotene.

Types of Carotenoids Quantified. Phytoene, phytofluene, ζ-carotene, β-carotene, and lycopene were quantified at the wavelength closest to their absorbance maximum. They are all C_{40} carotenoids but differ greatly in their number of conjugated double bonds. Therefore, their absorbance maxima range from the ultraviolet to the visible. Although this may help characterize these compounds, it makes it very difficult to quantify them using a single-wavelength detector. Therefore, a multiwavelength or photodiode array detector would be the detector of choice.

Chromatograms at 290, 350, and 465 nm are shown in Figure 3. As expected, the phytoene peak can only be seen from the 290-nm chromatogram. Phytofluene (19.54 min) is not chromatographically resolved from the unidentified peak, which has a spectrum very similar to that of α-carotene. However, the absorbance maxima of these two compounds are separated by almost 100 nm (see Table I). Since one of the absorbance maxima of phytofluene occurs at 334 nm and the unidentified peak has essentially no absorbance at this wavelength, phytofluene can be accurately quantified with little interference from this peak. However, it should be noted in Figure 3 that even at 350 nm the unidentified peak can be seen as a slight shoulder on the phytofluene peak. ζ-Carotene was chromatographically resolved at all wavelengths but quantified at 400 nm for maximum sensitivity (see Figure 1).

Comparison of β-Carotene Results in Ruby Red Grapefruit. To test the validity of our analytical procedure, the β-carotene results obtained from two different growing regions in Florida were compared with other literature results in Table II. There was generally good agreement between our average β-carotene value and most other literature values. Ting and Deszyck (1958) reported a range of values just slightly greater, and Philip and Chen (1988) reported a range of values slightly below that which was found in this study. The value reported by Khachik et al. (1989) was appreciably greater than the other values. Gross (1987) reports a value that would be equivalent to 2.6 μg/g. Ting and Deszyck (1958) demonstrated that concentrations of β-carotene and lycopene vary considerably with fruit maturity, and this may be the major cause for the wide range of published results.

Since the β-carotene values generated from this study are within the range of published results for Ruby Red grapefruit, the values are reasonable and thus acceptable.

Comparison of Relative Carotene Composition for Ruby Red Grapefruit. A comparison of the reported values for other carotenes in Ruby Red grapefruit is shown in Figure 4. While there are many reports of lycopene and β-carotene concentrations in red grapefruit, there are only a few reports of more extensive carotenoid analyses. In this study only hydrocarbon carotenoids (carotenes) were determined, but Gross (1987) and Curl and Bailey (1957) reported a total of 1.4 and 8.3% of oxygenated carotenoids (xanthophylls) in Ruby Red grapefruit, respectively. To compare their values equitably, total carotene values were determined (by summation) and the corresponding percent total carotene values calculated and used in Figure 4. There are considerable differences among studies as to the kinds and relative amounts of carotenes found in this cultivar. Gross (1987) does not report finding any phytoene, whereas we and Curl and Bailey (1957) report 19.3 and 17.4% of this colorless carotene. On the other hand, Curl and Bailey and Gross report finding 0.4 and 0.9% γ-carotene, respectively. We could not unambiguously identify any of the minor peaks as this compound. Gross did not report any ζ-carotene, but we and Curl and Bailey found 5.1 and 3.8%, respectively.

Table III. Distribution of Hydrocarbon Carotenoids in Pigmented Grapefruit from Florida (Concentrations in μg/g ± SD)

cultivar	lycopene	β-carotene	phytoene	phytofluene	ζ-carotene	total carotene
Ruby Red (Int)	2.9 ± 0.9	4.2 ± 1.4	2.5 ± 0.5	1.9 ± 0.5	0.6 ± 0.15	12.1
Ruby Red (IR)	1.6 ± 0.3	4.2 ± 0.4	2.5 ± 0.2	1.7 ± 0.2	0.5 ± 0.08	10.5
Flame	7.9 ± 2.0	8.6 ± 1.6	11.0 ± 1.2	6.0 ± 0.6	0.9 ± 0.09	34.4
Ray Ruby	20.6 ± 9.2	7.0 ± 1.7	5.0 ± 0.4	2.5 ± 0.1	0.7 ± 0.04	35.8
Star Ruby	33.3 ± 3.1	9.6 ± 1.6	51.3 ± 4.5	16.9 ± 4.4	0.6 ± 0.03	111.7

Quantitative differences could be due in part to the different geographic and climatic environments of the fruits used in the various studies. Fruit from the Curl and Bailey study was from Texas, fruit from the Gross study was from Israel, and fruit used in this study was from Florida. Maturity differences are another source of variation. As discussed previously, fruit maturity can have a profound effect on carotene concentration. Lycopene values ranged from our 27.4 to 71.4% (Gross). Curl and Bailey's lycopene was 43.6% of the total hydrocarbon carotenoids. There was reasonable agreement among phytofluene values in all three studies.

Carotene Composition. As shown in Table III, Star Ruby contained the greatest total concentration of carotenes. The flesh of this cultivar was also more intensely red than any of the other cultivars. This was due to the very high levels of lycopene (a red pigment) and to a lesser extent β-carotene, because β-carotene is orange. (ζ-Carotene is yellow, and phytoene and phytofluene are essentially colorless.) The Star Ruby contained more than 10 times more lycopene than any of the Ruby Red grapefruit. It is interesting to note the carotene in highest concentration was phytoene.

For the most part the carotene content of these new cultivars has not been established. However, Cruse et al. (1979), using the spectrophotometric technique of Lime et al. (1957), reported lycopene and β-carotene values for Star Ruby grapefruit juice. Their lycopene values ranged from 5.7 to 7.2 μg/g, and β-carotene values ranged between 3.4 and 5.6 μg/g throughout the harvesting season. As shown in Table III, values for these carotenes were considerably greater in this study. Red grapefruit develops its most intense color under hot, humid growing conditions (Reuther, 1988). Some of the observed differences can be attributed to different growing climates (Texas vs Florida) and different seasons studied. Another source of difference would be due to juice vs fruit flesh. Lower carotenoid content would be expected in juice, and juice carotenoid content will be dependent upon the pulp content of the juice.

Ray Ruby grapefruit was also intensely colored and was second only to Star Ruby in β-carotene content. However, it contained only 1/10th the colorless phytoene of Star Ruby. Otherwise, the carotenoid patterns were very similar. Lycopene was the major carotenoid, followed by phytofluene and β-carotene. The concentration of ζ-carotene found was relatively consistent in all cultivars.

The Flame grapefruit was qualitatively and quantitatively different from either the Ray, the Star Ruby group, or the Ruby Red group. It contained more lycopene than either Ruby Red cultivar but contained considerably less than Ray or Star Ruby. Surprisingly, it contained more β-carotene than the Ray Ruby and almost as much as the Star Ruby. Even though the absolute amounts differed, the relative concentrations of phytoene, phytofluene, and ζ-carotene were the same as those of the Ruby Red fruit.

Geographical growing area seemed to have little effect on the relative distribution or absolute concentrations in the Ruby Red carotenoids. The interior (Ridge) and Indian River growing areas are separated by over 100 mi and differ primarily in soil composition. The major difference observed was the Ruby Red cultivar was slightly redder than the comparable Indian River fruit. Not surprisingly, the interior fruit contained more lycopene. The distributions of the other carotenoids were essentially identical.

CONCLUSION

The Star Ruby, Ray Ruby, and Flame red grapefruit are highly pigmented red grapefruit whose total carotene contents are 3–5 times greater than that of the standard Ruby Red cultivar. These newer cultivars also contain approximately twice the β-carotene of Ruby Red grapefruit. Therefore, these new varieties of red grapefruit have a more pleasing visual appearance and can also serve as significant sources of β-carotene. However, additional testing should be conducted spanning several seasons to determine the natural concentration range of these carotenes.

ACKNOWLEDGMENT

We gratefully acknowledge an equipment grant from the Florida Department of Citrus for the photodiode array detector and the quaternary gradient pump used in this study and for the study of peel carotenoids. We thank Dr. Mohammed Ismail, Assistant Director of Scientific Research of the Florida Department of Citrus, for the gift of the authenticated fruit used in this study.

LITERATURE CITED

Andrews, A. G.; Liaaen-Jensen, S. Animal carotenoids 8. Synthesis of β-γ-carotene and γ,γ-carotene. *Acta Chem. Scand.* **1973**, *27*, 1401–1409.

Bendich, A. Carotenoids and the immune response. *J. Nutr.* **1989**, *119*, 112–115.

Colditz, G. A.; Branch, L. G.; Lipnick, R. J.; Willett, W. C.; Rosner, B.; Posner, B. M.; Hennekens, C. H. Increased green and yellow vegetable intake and lowered cancer deaths in an elderly population. *Am. J. Clin. Nutr.* **1985**, *41*, 32–36.

Cruse, R. R.; Lime, B. J.; Hensz, R. A. Pigmentation and Color Comparison of Ruby Red and Star Ruby Grapefruit Juice. *J. Agric. Food Chem.* **1979**, *27*, 641–642.

Curl, A. L. The carotenoids of red bell pepper. *J. Agric. Food Chem.* **1962**, *10*, 504–509.

Curl, A. L.; Bailey, G. F. The carotenoids of Ruby Red grapefruit. *Food Res.* **1957**, *22*, 63–68.

Davies, B. H. Carotenoids. In *Chemistry and Biochemistry of Plant Pigments*; Goodwin, T. W., Ed.; Academic Press: London, 1976; Vol. 2, pp 38–165.

De Ritter, E.; Purcell, A. E. Carotenoid Analytical Methods. In *Carotenoids as Colorants and Vitamin A Precursors*; Bauernfeind, J. Christopher, Ed.; Academic Press: New York, 1981; pp 815–882.

Fisher, J. F.; Rouseff, R. L. Solid-Phase Extraction and HPLC Determination of β-Cryptoxanthin and α- and β-Carotene in Orange Juice. *J. Agric. Food Chem.* **1986**, *34*, 985–989.

Gross, J. *Pigments in Fruits*; Academic Press: New York, 1987; Appendix A.18, p 218.

Huffman, W. A. H.; Lime, B. J.; Scott, W. C. Processed juices from red and pink grapefruit. *Proc. Rio Grande Valley Hortic. Inst.* **1953**, *7*, 102–105.

Kahn, M.; Mackinney, G. Carotenoids in grapefruit, *Citrus paradisi*. *Plant Physiol.* **1953**, *28*, 550–552.

Khachik, J. K.; Beecher, G. R.; Lusby, W. R. Separation, Identification and Quantification of the major carotenoids in Extracts of Apricots, Peaches Cantaloupes and Pink Grapefruit. *J. Agric. Food Chem.* **1989**, *37*, 1465–1473.

Kjøsen, H.; Liaaen-Jensen, S. Major carotenoids of Shepherdin Canadensis: isolation and synthesis of methylapo-6′-lycopenoate. *Phytochemistry* **1969**, *8*, 483–491.

Lime, B. J.; Griffiths, F. P.; O'Conner, R. T.; Heinzelman, D. C.; McCall, E. R. Spectrophotometric Methods for Determining Pigmentation—β-Carotene and Lycopene—in Ruby Red Grapefruit. *J. Agric. Food Chem.* **1957**, *5*, 941–944.

Philip, T.; Chen, T. S. Development of a method for the Quantitative Estimation of Provitamin A Carotenoids in Some Fruits. *J. Food Sci.* **1988**, *53*, 1703–1706.

Reuther, W. The effect of climate on fruit quality. In *Citrus Short Course Proceedings: Factors Affecting Fruit Quality*; Ferguson, J. J., Wardowski, W. E., Eds.; University of Florida, Institute of Food and Agricultural Sciences: Gainesville, 1988; pp 9–24.

Sadler, G.; Davis, J.; Dezman, D. Rapid Extraction of lycopene and β-carotene from Reconstituted Tomato Paste and Pink Grapefruit Homogenates. *J. Food Sci.* **1990**, *55*, 1460–1461.

Ting, S. V.; Deszyck, E. J. The internal color and carotenoid pigments of Florida red and pink grapefruit. *Proc. Am. Soc. Hortic. Sci.* **1958**, *71*, 271–277.

Zeigler, R. G. A review of the epidemiologic evidence that carotenoids reduce the risk of cancer. *J. Nutr.* **1989**, *119*, 116.

Received for review December 10, 1990. Revised manuscript received October 3, 1991. Accepted October 15, 1991.

Registry No. Lycopene, 502-65-8; β-carotene, 7235-40-7; phytocene, 13920-14-4; phytofluene, 27664-65-9; ζ-carotene, 72746-33-9.