

Carotenoid Content of 50 Watermelon Cultivars

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The lycopene content of 50 commercial cultivars of seeded and seedless red-fleshed watermelons was determined. Scanning colorimetric and spectrophotometric assays of total lycopene were used to separate watermelon cultivars into low (<50 mg/kg fw), average (50–70 mg/kg fw), high (70–90 mg/kg fw), and very high (>90 mg/kg fw). Cultivars varied greatly in lycopene content, ranging from 33 to 100 mg/kg. Most of the seeded hybrid cultivars had average lycopene contents. Sixteen of the 33 seedless types had lycopene contents in the high and very high ranges. All-*trans*-lycopene was the predominant carotenoid (84–97%) in all watermelon cultivars measured by high-performance liquid chromatography, but the germplasm differed in the relative amounts of *cis*-lycopene, β -carotene, and phytofluene. Red-fleshed watermelon genotypes vary extensively in carotenoid content and offer opportunities for developing watermelons with specifically enhanced carotenoids.

KEYWORDS: Lycopene; free radical scavenger; antioxidant; *Citrullus lanatus*

INTRODUCTION

There have been numerous reports that high dietary lycopene consumption (4 mg or more/day) may be beneficial in reducing the incidence of prostate and oral cancers and in the prevention of oxidative damage to cells (1–5). Epidemiological and intervention studies suggest that increased lycopene intake may reduce the risk of cardiovascular disease (6, 7) and may protect the skin from ultraviolet light damage (8). Tomatoes and tomato products provide most of the dietary intake of lycopene in Western diets. However, red-fleshed watermelon contains more lycopene per unit fresh weight than fresh tomatoes and is equally bioavailable to humans (9, 10).

Lycopene imparts the red color to watermelon, with red-fleshed fruit containing an average of 48.2 mg/kg lycopene (10). There are several hundred watermelon cultivars used commercially, and these are either seeded (diploid, 2*n*) or seedless (triploid, 3*n*). Lycopene is reported to be the prevailing carotenoid in red-fleshed watermelons (70–90% of total carotenoids), while remaining carotenoids include phytofluene, phytoene, β -carotene, lutein, neurosporene, and ζ -carotene (11–13). Plant breeders have introduced new watermelon varieties that have a deep red color, but the lycopene and total carotenoid contents of these varieties are not known. Advertising watermelon as a source of lycopene is enhancing watermelon sales in the United States, and this has stimulated interest among breeders to determine the relative pigment content of their selections (Xingping Zhang, Syngenta Seeds, personal com-

munication). The purpose of this experiment was to identify watermelon cultivars that are high in lycopene content and to determine how carotenoid profiles differed among cultivars.

MATERIALS AND METHODS

Plant Material. A total of 50 watermelon cultivars considered important in U.S. watermelon production were grown in research plots at Lane, Oklahoma, in 2002 and 2003 (Tables 1 and 2). Watermelons were transplanted into a Bernow silty loam soil (fine loamy, siliceous, thermic Glossic Paleudalf, cation exchange capacity of 4 mequiv/100 g) with in-row spacing of 91 cm and between-row spacing of 274 cm. Four blocks per variety were used with 15 plants per block. Recommended cultural practices and fertility rates for Oklahoma watermelons were followed (14). Temperatures during the 2002 and 2003 growing season averaged 30, 32, and 35 °C (max) and 20–21 °C (min) for June, July, and August.

Watermelons were harvested on multiple harvest dates from late July to mid-September, with 3–5 ripe melons harvested at random per block per variety used each year, a total of 12–20 melons sampled for each year for each variety. No reliable index for watermelon ripeness is available, such as heat units, so field ripeness was judged by various methods, including tendril browning, yellowing of the groundspot, loss of surface gloss, and by a slight crunching sound when pressed at the blossom end (on oblong diploid melons).

Compositional Analysis. All fruits were transported carefully to the laboratory to avoid internal bruising. Fruits were cut and sampled under fluorescent lights equipped with polarization filters (Ergomart, Dallas, TX) to prevent carotenoid degradation. Tissue samples were taken from the center of the melon in the heart and locular areas from the center of the melon to best replicate sampling from different sizes and types of melons (4–20 kg, round to oblong). Only melons with $\geq 9\%$ soluble solids content were sampled for lycopene to ensure that all fruits were fully ripe. About 200 g of flesh without seeds was

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Table 1. Total Lycopene Concentration and Soluble Solids Content of Ripe Seeded (2*n*) Watermelon Cultivars from Oklahoma, 2002 and 2003^a

variety	total no. of melons sampled	type	total lycopene (mg/kg)	soluble solids content (%)
		low		
Black Diamond	20	OP	33.8 ± 4.5	9.7 ± 0.9
Allsweet	16	OP	47.4 ± 7.9	10.7 ± 0.6
total	36	mean	40.60 ± 6.20	10.20 ± 0.75
		range	30.4–51.0	9.0–11.8
		average		
RojoGrande	12	F1	52.0 ± 11.5	10.2 ± 0.6
Sangria	40	F1	52.3 ± 4.9	10.9 ± 0.9
HSR2965	12	F1	57.5 ± 8.1	11.3 ± 1.4
Jamboree	24	F1	58.3 ± 5.2	10.9 ± 0.8
Dulce	16	F1	59.4 ± 5.8	11.5 ± 0.8
Campeche	12	F1	60.5 ± 9.1	11.0 ± 0.9
WX264	12	F1	63.1 ± 9.8	11.0 ± 0.8
HSR3034	16	F1	63.2 ± 10.3	10.8 ± 0.7
HYR133	12	F1	65.0 ± 9.0	10.5 ± 0.5
Summer Flavor 800	40	F1	66.0 ± 7.8	11.2 ± 0.6
Dixie Lee	20	OP	68.1 ± 9.1	10.3 ± 1.1
Minipool	20	F1	68.7 ± 12.2	11.6 ± 1.1
total	236	mean	61.18 ± 8.57	10.93 ± 0.85
		range	47.6–77.3	9.5–12.3
		high		
Ole'	16	F1	71.7 ± 9.3	11.6 ± 0.5
Summer Flavor 810	12	F1	75.4 ± 7.1	11.8 ± 0.5
Summer Flavor 710	12	F1	82.3 ± 5.9	11.9 ± 0.8
total	40	mean	76.47 ± 7.43	11.77 ± 0.60
		range	64.7–88.3	10.2–12.5

^a The total lycopene was determined by spectrophotometric methods. F1, hybrid; OP, open pollinated. Means ± standard deviation.

collected per fruit and placed into double plastic bags and frozen quickly at -80°C . All tissue was held at -80°C and analyzed within 4 months of harvest. Twelve to 40 melons that were judged to be ripe [soluble solids content (SSC) $\geq 9\%$, full red color] were used for each cultivar. A composite sample of 40–50 g of frozen tissue was ground for 3 min to a fine puree using a homogenizer (Polytron, Brinkman, Westbury, NY). The SSC was determined by placing 0.5 mL of puree onto a digital refractometer (Atago model PR 100, Gardiner, NY). The lycopene content of the puree of individual melons was measured by modified hexane extraction (2:2:1 ethanol:hexane:acetone) using a wavelength of 503 nm and an extinction coefficient of 172000 (15–17). The lycopene spectra and contents were verified using tomato lycopene (Sigma, St. Louis, MO) and synthetic all-*trans*-lycopene standards (Lycovit 10 cwd, BASF, Mt. Olive, NJ).

Determination of Carotenoid Profiles. Following spectrophotometric analysis, the 50 watermelon cultivars were separated into groups depending on lycopene content. Fruits from cultivars with <50 mg/kg fw mean total lycopene were coded “low”; those from 50 to 70 mg/kg fw were coded “average” (comparable to values listed in the U.S. Department of Agriculture database); those from 70 to 90 mg/kg fw were coded “high”, and those >90 mg/kg fw were coded “very high” (Tables 1 and 2). About half of the total cultivars in each lycopene group (low to very high) of seeded or seedless types were selected at random for high-performance liquid chromatography (HPLC) carotenoid profiling, with a total of 27 cultivars used.

Twenty grams of puree from each of four melons per cultivar was combined into composite samples, with 3–5 replicates per cultivar. Subsamples of 0.3–0.6 g of puree from the composite samples were weighed into amber glass bottles, and carotenoids were extracted with spectrophotometric grade solvents in a ratio of 2:2:1 hexane, ethanol, and acetone (10 mL:10 mL:5 mL) (Pharmco, Brookfield, CT). These samples were tightly sealed and placed on orbital shaker (Lab Line, Melrose Park, IL) for 15 min at 200 rpm, and then, 3 mL of ddi water was added, samples were shaken again for 10 min, and samples stood for 15 min to develop solvent phase separation. Duplicate samples were

Table 2. Total Lycopene and Soluble Solids Content of Ripe Seedless Watermelon Cultivars (3*n*) from Oklahoma, 2002 and 2003^a

variety	no. melons sampled	total lycopene (mg/kg)	soluble solids (%)
	average		
Sugar Slice	12	55.3 ± 6.6	10.6 ± 0.7
HSR2877	16	55.7 ± 7.7	11.2 ± 1.1
HSR2965	12	57.5 ± 8.1	11.3 ± 1.4
Sugar Shack	20	58.1 ± 8.5	11.7 ± 0.7
Summer Sweet 5244	20	58.8 ± 5.6	11.0 ± 0.6
Sweet Slice	16	59.2 ± 7.9	10.9 ± 0.7
HSR3005	12	59.6 ± 10.4	11.2 ± 0.9
Afternoon Delight	16	60.1 ± 6.1	10.3 ± 0.4
DRX4040	12	60.7 ± 9.0	11.2 ± 0.7
Tri-x Carousel	16	60.7 ± 5.9	10.8 ± 1.1
Tri-x Palomar	12	62.4 ± 9.5	11.2 ± 0.8
Tri-x 313	40	64.1 ± 7.0	11.2 ± 0.6
Mt. Shavano	12	64.1 ± 5.8	11.1 ± 0.6
HSR2920	12	64.3 ± 11.3	10.8 ± 1.6
Summer Sweet 7167	12	65.0 ± 10.0	11.4 ± 0.5
Sweet Eat'n	12	65.7 ± 6.1	11.1 ± 0.6
Dillion	12	66.5 ± 11.2	11.0 ± 1.2
total/mean	266	61.05 ± 8.04	11.06 ± 0.84
range		45.1–74.8	9.5–12.1
	high		
Imagination	16	70.0 ± 5.3	10.2 ± 0.8
Sweet Delight	12	70.1 ± 6.9	11.3 ± 0.8
Samba	12	74.9 ± 6.7	11.8 ± 0.5
HtRWM8133	12	73.0 ± 10.0	11.6 ± 0.7
Hazera 5116	16	73.8 ± 8.5	10.7 ± 0.7
Summer Sweet 7187	12	74.7 ± 9.8	11.9 ± 0.5
ACX8238	12	75.2 ± 4.3	11.7 ± 0.6
Millenium	16	75.2 ± 5.8	11.8 ± 0.7
HRS2492	12	75.7 ± 7.9	11.7 ± 0.5
Scarlet Trio	20	75.8 ± 8.8	11.2 ± 0.8
Mielhart (Hazera 5133)	12	82.6 ± 7.8	10.5 ± 0.7
Lilliput (HazeraSW1)	12	86.5 ± 11.1	10.8 ± 0.8
Hazera 6009	24	88.6 ± 12.5	11.3 ± 0.6
total/means	188	76.62 ± 8.11	11.27 ± 0.67
range		59.9–93.2	10.1–12.1
	very high		
Extazy (HZ6008)	16	93.2 ± 8.0	10.5 ± 0.7
Hazera 5109	16	95.6 ± 16.3	10.8 ± 0.6
Xite (Hazera 6007)	32	99.8 ± 14.8	10.9 ± 0.8
total/means	64	96.20 ± 13.03	10.73 ± 0.70
range		65.6–119.8	9.5–12.0

^a The total lycopene was determined by spectrophotometric methods. Means ± standard deviation.

filtered using 0.45 μm PTFE syringe filters (Daigger, Vernon Hills, IL) into 2 mL amber crimp-top vials (Daigger) and then loaded onto a HPLC equipped with autosampler, photodiode array detector, and integration software (Hewlett-Packard 1100, Wilmington, DE). A C₃₀ YMC carotenoid column (4.6 × 250 mm²) and YMC carotenoid guard column S-3 (4.0 × 20 mm²) (Waters, Milford, MA) and a gradient method with three solvent mixtures were used to separated carotenoids (6). Solvent mixtures consisted of (A) 90% methanol and 10% ddi water containing 0.5% triethylamine and 150 mM ammonium acetate, (B) 99.5% 2-propanol and 0.5% triethylamine, and (C) 99.95% tetrahydrofuran and 0.05% triethylamine. Gradient conditions were as follows: initial conditions 90% solvent A plus 10% solvent B; 24 min gradient switched to 54% solvent A, 35% solvent B, and 11% solvent C; final gradient conditions were 11 min gradient of 30% solvent A, 35% solvent B, and 35% solvent C and then held for 8 min. The mobile phases were returned to initial conditions over 15 min. Injection volumes of 0.1 mL were used for samples and standards. Standards (β -carotene, lycopene, phytoene, and phytofluene) obtained from Sigma and Carotenature (Geneva, Switzerland) were used to verify peaks and calculate concentrations following the method of Craft (18).

Statistics. The experimental design used was a completely randomized design. Data were subjected to analysis of variance (SAS, v. 8.0, Cary, NC), using PROC GLM, and means were separated by standard

Table 3. Carotenoid Concentration Profiles of Red-Fleshed Seeded Diploid (2n) and Seedless Triploid (3n) Watermelon Cultivars Determined by HPLC^a

Diploid (Seeded)						
selection	mg/kg					
	total lycopene	all-trans-lycopene	cis-lycopene	β -carotene	phytofluene	total carotenoids
Black Diamond	35.2 \pm 2.3	29.3 \pm 2.3	5.8 \pm 0.8	0.9 \pm 0.2	0.2 \pm 0.02	37.1 \pm 2.5
			open pollinated			
Rojo Grande	53.6 \pm 13.6	45.8 \pm 10.2	7.8 \pm 2.8	3.6 \pm 1.6	1.2 \pm 0.3	58.4 \pm 16.7
Summer Flavor 800	60.3 \pm 4.7	56.3 \pm 5.1	4.0 \pm 1.2	1.2 \pm 0.4	0.4 \pm 0.1	61.9 \pm 5.1
Dulce	63.5 \pm 7.2	60.7 \pm 9.9	2.8 \pm 1.5	4.4 \pm 0.8	1.3 \pm 0.1	69.2 \pm 8.6
Jamboree	65.7 \pm 4.8	57.7 \pm 4.3	8.0 \pm 0.9	5.1 \pm 1.0	1.6 \pm 0.2	72.4 \pm 5.1
Minipool	66.5 \pm 5.8	63.8 \pm 8.8	2.7 \pm 1.4	9.1 \pm 2.7	2.3 \pm 0.3	77.9 \pm 8.1
Campeche	69.5 \pm 10.0	60.5 \pm 8.2	9.0 \pm 3.1	4.9 \pm 2.7	1.9 \pm 0.3	76.3 \pm 13.0
Ole'	76.1 \pm 8.1	70.8 \pm 4.2	5.6 \pm 2.3	9.3 \pm 1.7	1.9 \pm 0.1	87.3 \pm 10.8
			hybrid			
Triploid (Seedless)						
selection	mg/kg					
	total lycopene	all-trans-lycopene	cis-lycopene	β -carotene	phytofluene	total carotenoids
Afternoon Delight	60.9 \pm 4.3	53.8 \pm 3.8	7.1 \pm 2.0	5.8 \pm 1.1	1.4 \pm 0.2	68.1 \pm 4.6
Summer Sweet 7167	60.9 \pm 13.9	51.1 \pm 10.1	9.8 \pm 6.1	1.5 \pm 0.4	0.5 \pm 0.1	62.9 \pm 14.4
Sweet Slice	62.4 \pm 4.2	57.6 \pm 4.2	4.8 \pm 1.3	6.7 \pm 0.7	1.8 \pm 0.3	70.9 \pm 3.5
Tri-x 313	63.3 \pm 14.3	59.0 \pm 4.9	4.3 \pm 3.0	2.4 \pm 1.4	1.0 \pm 0.4	66.7 \pm 12.0
Sweet Eat'n	63.6 \pm 6.3	55.9 \pm 5.3	7.8 \pm 2.3	5.1 \pm 1.5	1.5 \pm 0.4	70.2 \pm 7.0
Mt Shavano	65.4 \pm 13.6	61.0 \pm 8.1	4.4 \pm 2.9	4.1 \pm 1.2	1.3 \pm 0.2	70.8 \pm 14.8
Sugar Slice	66.2 \pm 4.7	62.1 \pm 11.9	4.1 \pm 3.0	4.3 \pm 0.9	1.3 \pm 0.3	71.8 \pm 4.8
Tri-x Carousel	69.5 \pm 6.5	68.0 \pm 5.9	1.5 \pm 0.8	3.9 \pm 0.4	1.3 \pm 0.1	74.7 \pm 6.7
Scarlet Trio	71.7 \pm 5.3	64.9 \pm 4.7	6.8 \pm 2.6	2.3 \pm 0.4	0.8 \pm 0.2	74.8 \pm 5.9
Sweet Delight	73.4 \pm 5.5	71.5 \pm 4.9	1.9 \pm 0.3	4.8 \pm 1.2	1.3 \pm 0.2	79.5 \pm 6.3
Samba	75.4 \pm 4.0	67.0 \pm 3.6	8.4 \pm 5.1	3.4 \pm 0.8	1.4 \pm 0.3	80.2 \pm 4.6
Summer Sweet 7187	77.4 \pm 13.2	64.9 \pm 12.8	12.5 \pm 7.8	2.0 \pm 0.3	0.6 \pm 0.1	80.0 \pm 0.3
Imagination	77.7 \pm 2.1	68.8 \pm 1.8	8.9 \pm 5.2	7.4 \pm 2.1	2.5 \pm 0.5	87.6 \pm 4.1
Millenium	80.0 \pm 5.4	60.7 \pm 4.4	19.3 \pm 1.8	2.0 \pm 0.7	0.7 \pm 0.1	82.7 \pm 5.8
ACX 8238	80.2 \pm 3.6	65.8 \pm 3.8	14.4 \pm 4.2	2.7 \pm 0.1	0.6 \pm 0.1	83.5 \pm 3.5
Mielhart (Hazera 5133)	82.5 \pm 3.7	76.9 \pm 2.0	5.6 \pm 1.2	10.2 \pm 0.6	4.3 \pm 0.2	97.0 \pm 3.1
Hazera 6009	87.6 \pm 9.7	81.1 \pm 9.4	6.5 \pm 0.3	4.2 \pm 0.8	1.9 \pm 0.2	93.7 \pm 2.6
Extazy (Hazera 6008)	103.3 \pm 8.4	86.1 \pm 7.3	7.2 \pm 0.7	8.8 \pm 1.4	2.9 \pm 0.3	115.0 \pm 9.6
Xite (Hazera 6007)	112.4 \pm 12.9	101.2 \pm 12.1	11.2 \pm 6.4	6.5 \pm 1.0	3.0 \pm 0.8	121.9 \pm 14.8

^a Means \pm standard deviation.

deviation. Carotenoids were subjected to Pearson's correlation coefficient to determine correlated means.

RESULTS AND DISCUSSION

Germplasm Variation in Total Lycopene and SSC. Although fruits from a few seeded cultivars, such as Summer Flavor 710 and Ole, were high in lycopene, seedless watermelon cultivars were most often high in lycopene (Tables 1 and 2). All melons used in this study were considered fully ripe, with a minimum SSC of 9%. Many of the cultivars used in this study that were high or very high in lycopene are of recent introduction (last 2–10 years; the Hazera selections, Sweet Delight, Ole, Imagination, and Summer Sweet 7187). Extazy, Hazera 5109, and Xite fruits had 93–99 mg/kg lycopene, higher than any values reported for watermelon. The range of lycopene values within a group increased in both minimum and maximum contents as the average lycopene content increased.

SSC was positively correlated with lycopene, but the relationship was poor, with an R^2 of 0.18 using linear or quadratic regression (data not shown). In our study, only fully ripe watermelons were used, with a range of soluble solids values of 9–12.5%, and a wide range of lycopene concentrations (35–100 mg/kg) (Tables 1 and 2). Elmstrom and Davis (19) found that total sugars of various open-pollinated watermelon cultivars increase in a sigmoidal pattern during watermelon fruit growth. They noted that the red color was present in melons as soon as

12–16 days postanthesis and gradually increased. A strong linear or quadratic relationship between SSC and lycopene concentration would most likely be seen if these quality characteristics were followed from anthesis through full ripeness.

HPLC Carotenoid Profiles of Watermelons. Total carotenoid content varied from 37 to 122 mg/kg fw among red-fleshed watermelon cultivars (Table 3). Lycopene provided the largest portion of the total carotenoids (84–97%), as reported by others using open column chromatography and HPLC (11–13, 20). *cis*-Lycopene was 2–18% of the total lycopene, with most cultivars having 4–8% *cis*-lycopene; the remainder was all-*trans*-lycopene (Table 3). Edwards et al. (9) reported that the *cis*-lycopene in watermelon was predominately the 5-*cis* form, with some 13-*cis* isomer. Although the *cis*-lycopene was a small amount of the total lycopene found in the watermelon cultivars, there is some evidence that the *cis* form is more bioavailable to humans (21); β -carotene made up 2–11% of the total carotenoids, and phytofluene was 0.5–7% of the total carotenoids. The carotenoids β -carotene, phytofluene, and *cis*- and all-*trans*-lycopene were identified by comparing retention times and peaks to standards in HPLC analysis of watermelon puree (Figure 1). An unknown carotenoid was found at a retention time of about 32 min (peak 2, Figure 1). It did not match γ - or δ -carotene standards and may be 9-*cis*- β -carotene. Lutein (2–3 mg/kg fw) has been found in watermelon using HPLC and a C₁₈ column (4). We found lutein at 1–2 mg/kg in

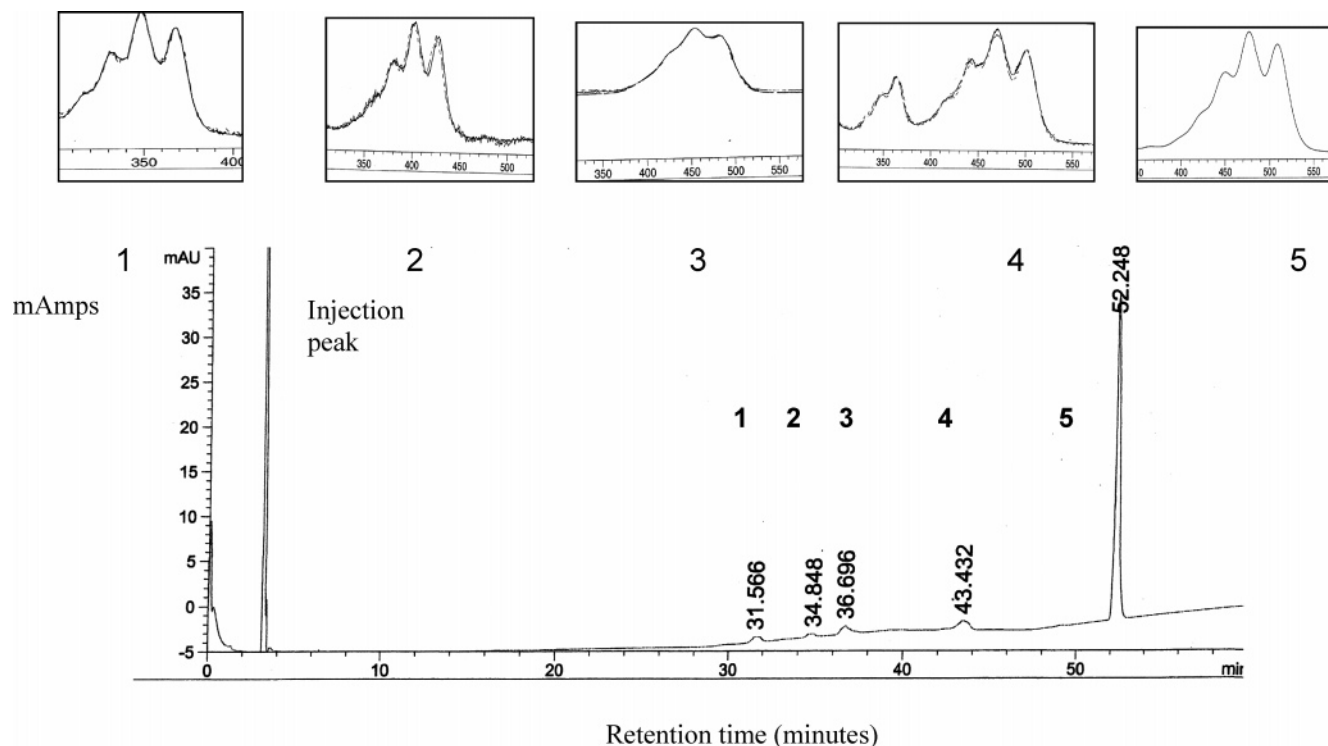


Figure 1. HPLC chromatogram of the watermelon cultivar Xite (Hazera 6007). Peaks 1–5 represent the following: 1, phytofluene; 2, unknown; 3, β -carotene; 4, *cis*-lycopene; and 5, *trans*-lycopene.

Table 4. Correlations among Carotenoids of Red-Fleshed Watermelons^a

variable	total carotenoids	<i>cis</i> -lycopene	<i>all-trans</i> -lycopene	total lycopene	phytofluene
β -carotene	0.48**	0.31**	0.36**	0.46**	0.69**
phytofluene	0.76**	0.51**	0.71**	0.75**	
total lycopene	0.99**	0.65**	0.93**		
<i>all-trans</i> -lycopene	0.93**	0.34**			
<i>cis</i> -lycopene	0.65**				

^a ** indicates significance at $P < 0.01$, Pearson's correlation coefficient.

other watermelon studies using a C₁₈ column but were unable to resolve this peak under our conditions with a C₃₀ column. Neurosporene, a carotenoid intermediate to ζ -carotene and lycopene, has also been reported to be present in watermelon with open column chromatography (11) and with HPLC using a C₁₈ column (12), but we were unable to resolve this peak using our conditions. Phytoene, γ -carotene, and ζ -carotene were present but too low to quantify in our study using this extraction system.

All carotenoids were positively correlated (Table 4). Phytofluene was strongly correlated with total lycopene and β -carotene. *All-trans*-lycopene was less well-correlated with *cis*-lycopene or with β -carotene, as *cis*-lycopene and β -carotene did not always increase proportionally with lycopene. In some cultivars (Jamboree, Minipool, and Afternoon Delight), β -carotene was high (>5 mg/kg fw) although total lycopene was in the 50–70 mg/kg fw range (Table 3). *cis*-Lycopene was 2.8 and 9.0 mg/kg in Dulce and Campeche, respectively, although *all-trans*-lycopene values were similar between the cultivars (60.7–60.9 mg/kg fw). Like Tomes et al. (11) and Tadmor et al. (12), we found that total carotenoid content and phytofluene generally increased in proportion to total lycopene content, as phytofluene is a required precursor for both carotenoids (22).

In this large cultivar screening study, seven watermelon cultivars were found to have more than 80 mg/kg lycopene content, representing 1.5–2 times more than the average value of 48 mg/kg reported in the U.S. Department of Agriculture database. Generally, melons with more total lycopene also contain more β -carotene and phytofluene. *All-trans*-lycopene was the dominant carotenoid in all red-fleshed melons sampled. These results indicate that a wide range in lycopene content exists among watermelon germplasm and that watermelon cultivars with very high lycopene contents are available. On the basis of our results, a serving size (150 g) from any of the watermelon cultivars tested would provide the suggested intake of 4 mg of lycopene per day (2).

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