

## Carotenoid Changes of Intact Watermelons after Storage

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Watermelon contains lycopene, a red carotenoid pigment that has strong antioxidant properties. The lycopene content of watermelon is substantial, contributing 8–20 mg per 180 g serving. There are no reports on carotenoid changes in whole watermelon during storage. Three types of watermelon, open-pollinated seeded, hybrid seeded, and seedless types, were stored at 5, 13, and 21 °C for 14 days and flesh color, composition, and carotenoid content were compared to those of fruit not stored. Watermelons stored at 21 °C had increased pH, chroma, and carotenoid content compared to fresh fruit. Compared to fresh fruit, watermelons stored at 21 °C gained 11–40% in lycopene and 50–139% in  $\beta$ -carotene, whereas fruit held at 13 °C changed little in carotenoid content. These results indicate that carotenoid biosynthesis in watermelons can be affected by temperature and storage.

**KEYWORDS:** Lycopene;  $\beta$ -carotene; chilling injury; triploid watermelon; *Citrullus lanatus* (Thunb Matsum & Nakai)

### INTRODUCTION

Red-fleshed watermelon contains significant amounts of lycopene, a carotenoid pigment that is a highly efficient free radical scavenger. Intake of tomato products high in lycopene has been associated with a reduced incidence of cardiovascular disease and some types of cancer (1–3). Lycopene is also associated with enhanced skin protection from UV light damage, improved bone mineral density, and improved sperm motility (4–7). Lycopene bioavailability to humans from unheated (unpasteurized) watermelon juice is similar to that of heat-processed tomatoes (8). The carotenoid profile of watermelons is similar to that of tomato, with phytofluene, lutein,  $\beta$ -carotene, and lycopene reported to be in red-fleshed melons (9, 10).

Several factors have been shown to affect the lycopene content of watermelon. Lycopene content varies widely in watermelon germplasm, ranging from 36 to 120  $\mu\text{g/g}$  of fresh weight (11, 12). Environmental conditions during production, such as light intensity, temperature, and irrigation, can alter lycopene content by 10–20% (11, 13). Fresh-cut watermelon held for >7 days at 2 °C had slightly reduced lycopene content (14).

The usual shelf life for watermelons is 14–21 days at 13 °C after harvest (15). Watermelons are chilling sensitive, with symptoms expressed as rind pitting, rind and stem decay, and water-soaked lesions when stored at 5 °C for >7 days (16). Showalter (17) reported that red color faded in uncut seeded watermelon stored below 10 °C for >7 days, and the pigment was reduced when measured as percent transmittance of a benzene extraction. In contrast, watermelons were found to be redder when held at ambient temperatures (22–33 °C) for 7–10

days (17, 18). Watermelons held at 22–33 °C for >10 days developed a visible orange color in the flesh, but carotenoid contents were not measured (18).

Several types of watermelons are grown for U.S. markets. The older, open-pollinated, seeded watermelons are used primarily for local markets, where variety name recognition is important. In the United States, hybrid seeded and seedless (triploid) watermelons dominate commercial markets, with a split of 20 and 80% (National Watermelon Promotion Board, personal communication, 2005). In the first seedless watermelons released, variability in chilling sensitivity was found among cultivars, and also in small (<5 kg) seeded watermelons (16, 19). The purpose of this experiment was to determine the effects of temperature on the quality and carotenoid profiles of three types of watermelon.

### MATERIALS AND METHODS

**Plant Material.** Ripe watermelons of ‘Black Diamond’ (heirloom, light red), ‘Summer Flavor 800’ (hybrid seeded, crimson), and ‘Sugar Shack’ (seedless, crimson) were obtained from local Oklahoma watermelon producers. All melons were held overnight in a cooler at 20 °C and 75% relative humidity. Twenty fruits per cultivar were immediately cut and sampled to provide initial (not stored) measurements. Remaining fruits were randomly assigned to three coolers at three temperatures of 5, 13, and 21 °C for 14 days, with 20 melons per cultivar used per storage temperature.

**Sample Collection and Analysis.** Fruits were weighed before and after storage to determine mass loss and rated for symptoms of chilling injury (pitting, water-soaked lesions, mold) before cutting. Watermelons were cut transversely (midway between blossom and stem ends). Rind thickness (from peel to start of pink color) was measured to 0.1 mm using calipers at the ground spot and directly above the ground spot. Colorimeter measurements were made at days 0 and 14 on two heart and two locule (seed area) locations per fruit using a colorimeter

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**Table 1.** Comparisons of Characteristics of Fruit from Three Watermelon Cultivars (Heirloom Seeded, Seedless, and Hybrid Seeded) before and after Storage for 14 Days at 5, 13, or 21 °C<sup>a</sup>

storage temp (°C)	rind thickness (mm)	pH	SSC (%)	L*	a*	b*	hue (deg)	chroma
Black Diamond								
fresh	18.2ab	5.42a	10.8a	45.0a	21.4a	10.5a	26.2b	23.8ab
5	18.8a	5.07b	10.2ab	41.3b	18.6c	9.7b	27.5ab	21.0c
13	16.8bc	5.16b	10.1b	41.0b	20.2b	10.7a	27.9a	22.9b
21	16.4c	5.49a	10.1b	42.3b	22.1a	11.2a	26.9ab	24.8a
mean	17.5A	5.28B	10.3C	42.4A	20.6B	10.5C	27.1B	23.2B
Summer Flavor 800								
fresh	18.7a	5.34b	12.0a	40.3a	26.6b	13.5a	26.9a	29.8b
5	17.1ab	5.13c	12.1a	38.7a	25.6b	12.9a	26.9a	28.7b
13	16.2b	5.39b	11.9a	38.4a	25.9b	14.1a	27.7a	30.4ab
21	15.4b	5.60a	11.8a	35.7b	28.9a	13.9a	25.7a	32.1a
mean	17.1A	5.34AB	12.0A	38.6B	26.8A	13.6B	26.9B	30.0A
Sugar Shack								
fresh	17.2a	5.43ab	11.9a	42.7a	26.4bc	14.1b	28.1b	29.9c
5	17.3a	5.16c	11.5a	42.4a	25.6c	13.7b	28.1b	29.1c
13	16.6a	5.32b	12.0a	42.9a	27.4b	16.1a	30.4a	31.8b
21	16.1a	5.57a	11.5a	39.4b	29.4a	16.0a	28.5b	33.5a
mean	16.8A	5.37A	11.7B	41.8A	27.2A	14.9A	28.7A	31.0A

<sup>a</sup> Lower case letters indicate mean separation within column and cultivar by Ryan–Einot–Gabriel–Welsch (REGWQ),  $P < 0.05$ . Capital letters indicate mean separation among cultivar means within a column by REGWQ,  $P < 0.05$ .

(Minolta CR 200, Ramsey, NJ) with an aperture of 8 mm diameter, D65 illuminant, and CIE  $L^*a^*b^*$  color scale. A white color tile ( $L = 97.70$ ,  $a = -0.48$ ,  $b = 2.23$ ) was used to calibrate the colorimeter, and hue and chroma were calculated using the formulas  $[\arctan(b/a)] \times 57.3$  and  $(a^2 + b^2)^{1/2}$ , respectively (20).

Soluble solids content (SSC) was determined by sampling a core of heart tissue and expressing  $\approx 0.5$  mL of juice onto a digital refractometer (Atago model PR-100). About 300 g of tissue from the heart and locule was collected, held at  $-80$  °C, and analyzed for carotenoids, pH, and SSC within 4 months of collection.

**Compositional Analysis.** Frozen samples (50 g) were ground in mortar and pestle and again with a homogenizer equipped with a shear-type blade (Polytron; Brinkman, Westbury, NY) to ensure particle sizes of  $< 1$  mm diameter. The pH was determined on 50 g puree aliquots using a pH-meter (Orion, model 1100, Boston, MA) and Ross electrode 8455. A 30 mL aliquot of puree per fruit was subjected to scanning colorimetry using a Hunter XE Colorscan 200 (Hunter, NJ), and lycopene was determined as micrograms per gram using the formula  $[\text{absorbance}_{560\text{nm}} - \text{absorbance}_{700\text{nm}}] \times 37.8$  (21).

**HPLC Analysis.** Composite samples of watermelon were prepared for high-performance liquid chromatography (HPLC) analysis by combining 5 g of puree from each of five watermelons, for a total of four composite samples per cultivar and temperature treatment. Aliquots of the composite samples were then extracted with hexane and analyzed for carotenoid composition by HPLC using a modified method of Fish et al. (22). In brief, two replicates of 0.3–0.6 g of tissue were weighed into amber glass bottles and extracted with HPLC grade solvents of 10 mL of hexane, 10 mL of ethanol, and 5 mL of acetone (Pharmco, Brookfield, CT). Samples were tightly sealed and placed on an orbital shaker (Lab Line, Melrose Park, IL) for 15 min at 200 rpm, and then 3 mL of deionized distilled (ddi) water was added; samples were shaken again for 10 min, and then samples were put in a rack to allow solvent phase separation. The upper hexane layer was measured on a UV spectrophotometer (Shimadzu UV 160, Columbia, MD) at 450, 471, and 503 nm. Samples were filtered using 0.45 mm PTFE syringe filters (Daigger, Vernon Hills, IL) into 2 mL amber crimp-top vials (Daigger) and then loaded into the HPLC with an autosampler, a photodiode array detector, and integration software (Hewlett-Packard 1100, Wilmington, DE). Sample carotenoids were separated using a C<sub>30</sub> YMC carotenoid column (4.6  $\times$  250 mm) equipped with a YMC carotenoid guard column S-3 (4.0  $\times$  20 mm) (Waters, Milford, MA). Methods for HPLC analysis were followed as previously described (23). In brief, a gradient method was used with three solvent mixtures: A, 90% methanol, 10% ddi water containing 0.5% triethylamine and 150 mM ammonium acetate; B, 99.5% 2-propanol and 0.5% triethylamine; C, 99.95%

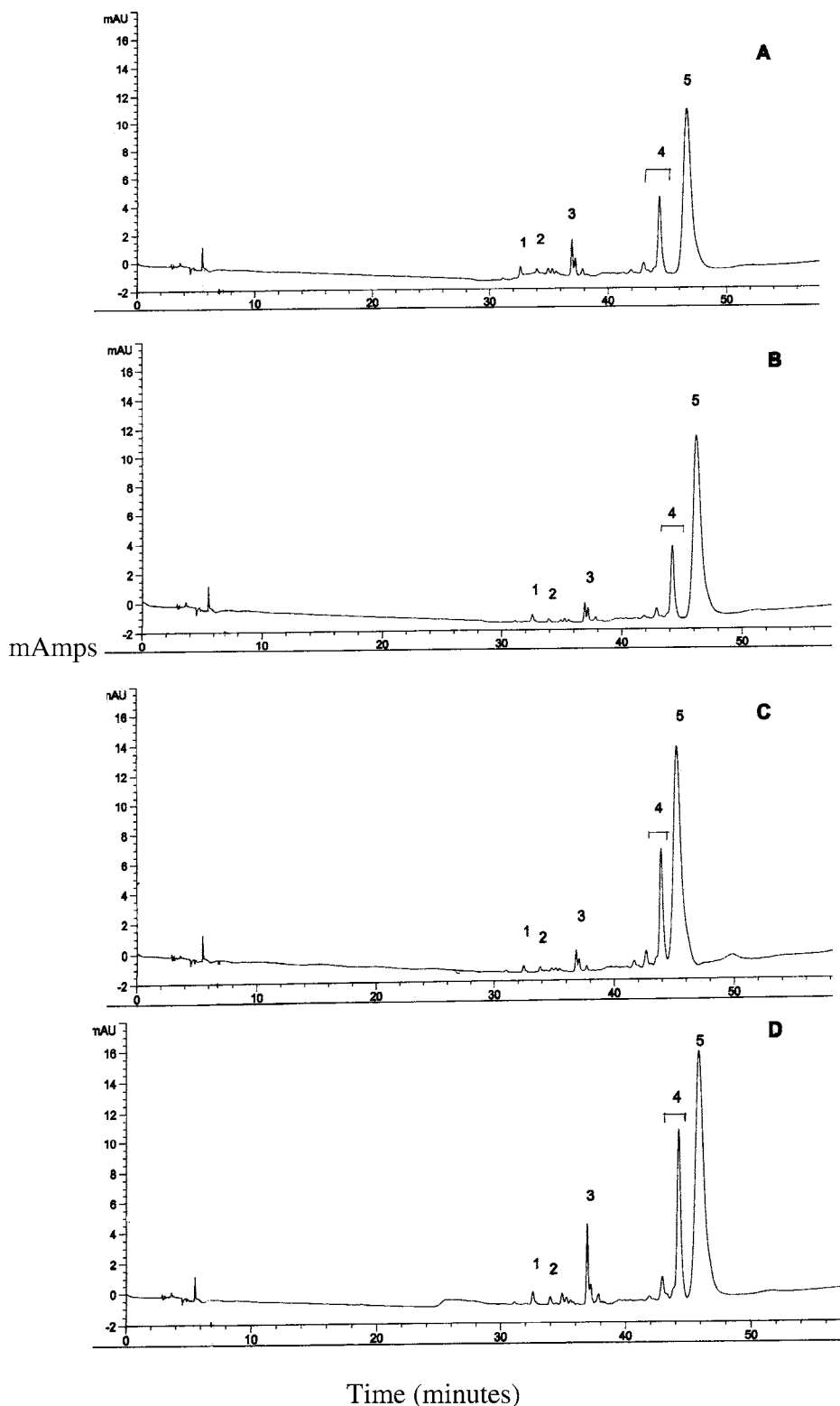
tetrahydrofuran and 0.05% triethylamine. Gradient conditions were as follows: initial conditions, 90% solvent A plus 10% solvent B; 24 min gradient switched to 54% solvent A, 35% solvent B, and 11% solvent C; final gradient conditions were 11 min gradient of 30% solvent A, 35% solvent B, 35% solvent C, and then held for 8 min. The mobile phases were returned to initial conditions over 15 min. Injection volumes of 50 and 100  $\mu$ L were used for samples and standards. External standards ( $\beta$ -carotene, lycopene, phytoene, phytofluene) obtained from Sigma (St. Louis, MO) and Carotenature (Geneva, Switzerland) were used to verify peaks and calculate concentrations following the method of Craft (23).

**Statistics.** The experiment was designed as a split plot, with cultivar as main effect and storage temperature as the split plot. Twenty melons per storage temperature and cultivar were used. Data were subjected to analysis of variance using a General Linear Means model, and mean separation was done using the multiple-range test Ryan–Einot–Gabriel–Welsch (REGWQ) at the 5% level (SAS, v. 8.2, Cary, NC). Correlations were done among dependent variables using Pearson's correlation coefficient,  $P < 0.05$ , and regression was done at linear and quadratic levels with SAS.

## RESULTS

Few symptoms of chilling injury, such as mold and pitting, were seen in fruit held at 5 °C. Watermelon weight loss was  $< 1\%$  at all temperatures after 14 days of storage. Rind thickness decreased in watermelons held at 21 °C, compared to fresh fruit (Table 1). The pH of fruit flesh was lower at 5 °C relative to fresh watermelons (Table 1). SSC changed little with storage temperature and was within 0.5% of that of fresh watermelons. The heirloom seeded cultivar Black Diamond had a lower SSC than the other cultivars (Table 1).

Color changes in watermelon flesh after storage were dependent on storage temperature and cultivar (Table 1). Watermelons from all cultivars were darker (lower  $L^*$  values) after storage at 21 °C than fresh melons. The  $a^*$  values (e.g., redness) were highest in Sugar Shack and Summer Flavor 800 watermelons stored at 21 °C compared to those held at 5 °C. Black Diamond watermelons were less red in color (lower  $a^*$  value) after storage at 5 or 13 °C than fresh watermelons. Overall, Black Diamond fruit, which is visibly light red in color, had lower  $a^*$ ,  $b^*$ , and chroma values than Sugar Shack or Summer Flavor 800. Chroma, or intensity of color, was higher in Summer

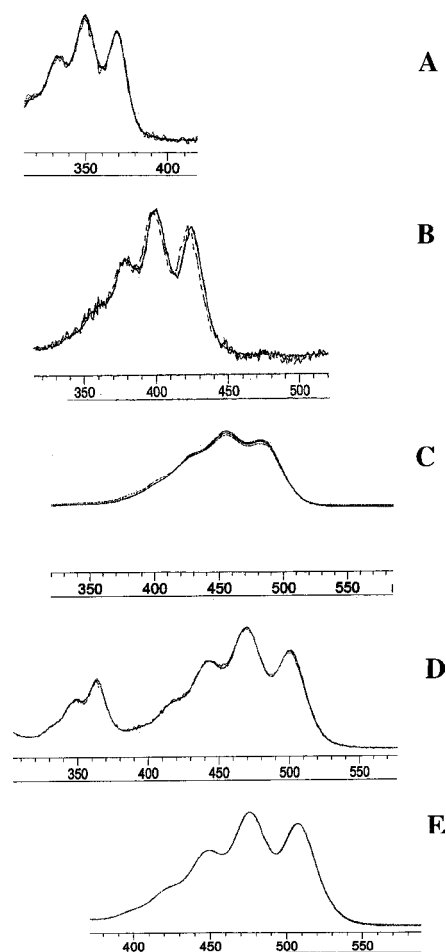


**Figure 1.** HPLC chromatograms from Summer Flavor 800 watermelons not stored (**A**) or held at 5 °C (**B**), 13 °C (**C**), or 21 °C (**D**). Peaks represent phytofluene (1), unknown (2),  $\beta$ -carotene (3), *cis*-lycopene (4), and *trans*-lycopene (5).

Flavor 800 and Black Diamond watermelons held at 21 °C compared to melons held at 5 °C. Hue was higher in Black Diamond and Sugar Shack fruit held at 13 °C compared to fresh fruit (**Table 1**).

Lycopene [*cis* [(*Z*)-lycopene] and all-*trans* [(*all-E*)-lycopene] forms] was the predominant carotenoid and accounted for 94–97% of the total carotenoids (**Figures 1** and **2**; **Table 2**). Other carotenoids found included phytofluene,  $\beta$ -carotene, and an

unknown, possibly  $\zeta$ -carotene (**Figure 2**). Lutein was not found in this study, although it is present in watermelon in small amounts (9). Lutein may have been oxidized by exposure to hexane during extraction. Lycopene content and carotenoid profile were dependent on both cultivar and storage temperature (**Table 2**). Although total lycopene increased in all watermelons held at 21 °C, it was significantly higher only in Summer Flavor 800 fruit. *cis*-Lycopene [(*Z*)-lycopene] increased in Sugar Shack

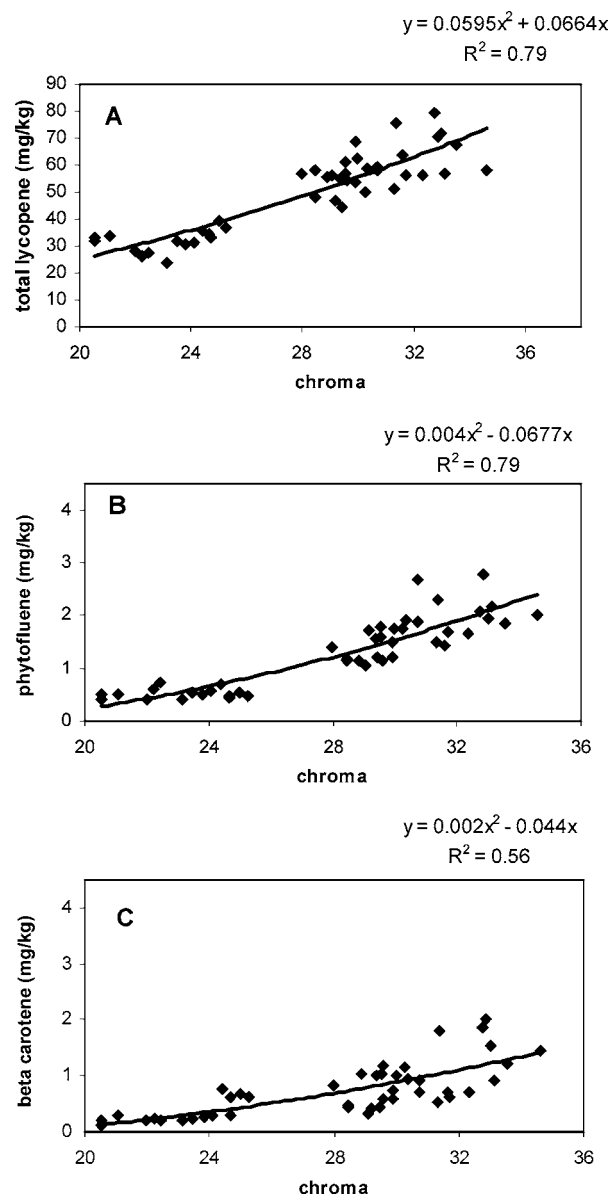


**Figure 2.** Peaks from HPLC chromatograms in **Figure 1**, showing phytofluene (A), unknown (B),  $\beta$ -carotene (C), *cis*-lycopene (D), and *trans*-lycopene (E).

and Summer Flavor 800 watermelons held at 21 °C compared to fresh fruit or those held at 5 or 13 °C (**Table 2**). In contrast, *cis*-lycopene decreased in Black Diamond watermelons. *all-trans*-Lycopene [(*all-E*)-lycopene] increased in all fruit held at 21 °C compared to fresh watermelons or those held at 5 °C. Phytofluene, a precursor of lycopene, was slightly higher in Sugar Shack and Summer Flavor 800 watermelons held at 21 °C.  $\beta$ -Carotene content was higher and increased significantly as a percentage of total carotenoids in all watermelons held at 21 °C compared to fresh fruit. Compared to fresh fruit, total carotenoids were elevated by 11–30% in all watermelons held at 21 °C.

When averaged across storage temperatures, total lycopene and *cis*- and *trans*-lycopene,  $\beta$ -carotene, and total carotenoids were lowest in Black Diamond and similar in Summer Flavor and Sugar Shack watermelons (**Table 2**). Black Diamond watermelons had only 36.2  $\mu\text{g/g}$  lycopene compared to 61.3 and 58.8  $\mu\text{g/g}$  lycopene in Summer Flavor 800 and Sugar Shack, respectively. Black Diamond had the highest amount of *cis*-lycopene as a percent of the total carotenoids and the least  $\beta$ -carotene and phytofluene compared to the other cultivars. Sugar Shack fruit had a greater percent of total carotenoids as phytofluene and slightly less as total lycopene than the other cultivars.

Correlations among variables measured were positive and significant among carotenoids and the color variables  $L^*$ ,  $a^*$ ,  $b^*$ , and chroma (**Table 3**). Hue, which indicates color and incorporates  $a^*$  and  $b^*$  values, was not significantly correlated



**Figure 3.** Relationship of watermelon flesh chroma of stored and unstored fruit to total lycopene content (A), phytofluene (B), and  $\beta$ -carotene (C). Points in each figure represent four composite samples per storage temperature (fresh and 5, 13, and 21 °C) ( $n = 48$ ).

to carotenoids or flesh composition. Rind thickness was negatively correlated with pH, indicating that a loss of thickness corresponded to a rise in puree pH. SSSC and pH were significantly correlated with all of the carotenoids and chroma,  $a^*$ , and  $b^*$  values. Rind thickness was weakly correlated with lycopene,  $\beta$ -carotene, and color variables. When subjected to regression, the relationship of total lycopene to SSC was cubic in nature (data not shown).  $\beta$ -Carotene and pH were quadratically related ( $R^2 = 0.55$ ), due in part to the lower pH values of Black Diamond, which also had very little  $\beta$ -carotene (data not shown).

Chroma, a function of  $a^*$  and  $b^*$  values, was a much better indicator of lycopene content than hue, which had overlapping values for Black Diamond (light red) and Summer Flavor 800 (crimson) watermelons (**Table 1**). Lycopene and phytofluene contents were quadratically related to chroma ( $R^2 = 0.79$ ) (**Figure 3**). Chroma was less effective in predicting  $\beta$ -carotene content ( $R^2 = 0.56$ ) (**Figure 3C**). Among carotenoids, phytofluene was linearly related to total lycopene and  $\beta$ -carotene ( $R^2$

**Table 2.** HPLC Analysis of Carotenoids of Fruit from Three Watermelon Types (Heirloom Seeded, Seedless, and Hybrid Seeded) before and after Storage for 14 Days at 5, 13, or 21 °C<sup>a</sup>

storage temp (°C)	$\mu\text{g/g}$					as a % of total carotenoids					
	total lycopene	<i>all-trans</i> -lycopene	<i>cis</i> -lycopene	$\beta$ -carotene	phytofluene	total carotenoids	total lycopene	<i>trans</i> -lycopene	<i>cis</i> -lycopene	$\beta$ -carotene	phytofluene
<b>Black Diamond</b>											
fresh	32.6a	26.9b	5.75a	0.28b	0.53a	33.4ab	97.6ab	80.6b	17.2a	0.8b	1.6ab
5	31.8a	27.1b	4.71ab	0.21b	0.47a	32.5bc	97.9a	83.3b	14.6ab	0.6b	1.4b
13	27.0b	23.1b	3.89ab	0.24b	0.56a	27.8c	97.1bc	83.1b	14.0ab	0.8b	2.0a
21	36.2a	33.9a	2.27b	0.67a	0.54a	37.4a	96.8c	90.7a	6.1b	1.8a	1.4b
mean	36.2B	27.8B	4.16B	0.35B	0.52C	32.8B	97.4A	84.4A	13.0A	1.0B	1.6C
<b>Summer Flavor 800</b>											
fresh	55.7b	51.3b	4.40c	0.80b	1.35b	57.8b	96.3ab	88.7a	7.6b	1.4b	2.3a
5	51.6b	46.8b	5.32bc	0.42b	1.14b	53.2b	97.0a	86.9ab	10.1a	0.8b	2.2a
13	60.3b	53.8b	6.53b	0.77b	1.53b	62.6b	96.3ab	85.8ab	10.5a	1.2b	2.5a
21	77.6a	68.4a	9.20a	1.84a	2.18a	81.6a	95.1b	83.8b	11.3a	2.2a	2.7a
mean	61.3A	55.1A	6.36A	1.06A	1.55B	63.8A	96.2AB	86.3A	9.9B	1.4A	2.4B
<b>Sugar Shack</b>											
fresh	55.1b	49.9b	5.20b	1.06b	1.94a	58.1b	94.8a	85.9a	8.9ab	1.8b	3.3a
5	54.3b	49.1b	5.18b	0.66c	1.67a	56.6b	95.9a	86.8a	9.1ab	1.1c	3.0a
13	59.0ab	54.4ab	4.59b	0.80bc	1.81a	61.6ab	95.8a	88.3a	7.5b	1.3c	2.9a
21	66.8a	58.6a	8.22a	1.56a	2.15a	70.6a	94.7a	83.1b	11.6a	2.2a	3.1a
mean	58.8A	53.0A	5.80A	1.02A	1.89A	61.7A	95.3B	86.0A	9.3B	1.6A	3.1A

<sup>a</sup> Lower case letters indicate mean separation within column and cultivar by Ryan–Einot–Gabriel–Welsch (REGWQ),  $P < 0.05$ . Capital letters indicate mean separation among cultivar means within a column by REGWQ,  $P < 0.05$ .

**Table 3.** Correlation Coefficients among Variables Measured of Fruit from Three Watermelon Cultivars (Heirloom Seeded, Seedless, and Hybrid Seeded) before and after Storage for 14 Days at 5, 13, or 21 °C<sup>a</sup>

variable	pH	SSC	rind thickness	$L^*$	$a^*$	$b^*$	hue	chroma	total lycopene	<i>all-trans</i> -lycopene	$\beta$ -carotene	phytofluene	<i>cis</i> -lycopene
total carotenoids	0.49**	0.70**	-0.35*	-0.44**	0.90**	0.83**	0.16	0.90**	1.00**	0.99**	0.82**	0.87**	0.58**
<i>cis</i> -lycopene	0.31*	0.31*	-0.25	-0.38*	0.48**	0.40**	0.10	0.46**	0.58**	0.48**	0.52**	0.51**	
phytofluene	0.43**	0.68**	-0.15	-0.32*	0.88**	0.84**	0.21	0.88*	0.85**	0.84**	0.79**		
$\beta$ -carotene	0.73**	0.43**	-0.41**	-0.41**	0.74**	0.65**	0.05	0.74**	0.80**	0.79**			
<i>all-trans</i> -lycopene	0.47**	0.71**	-0.34*	-0.42**	0.89**	0.84**	0.18	0.89**	0.99**				
total lycopene	0.48**	0.69**	-0.36*	-0.44**	0.90**	0.83**	0.16	0.89**					
chroma	0.48**	0.62**	-0.37*	-0.33*	0.99**	0.96**	0.24						
hue	-0.08	0.07	-0.25	0.40**	0.15	0.51**							
$b^*$	0.39**	0.54**	-0.40**	-0.17	0.92**								
$a^*$	0.50**	0.63**	-0.36*	-0.38*									
$L^*$	-0.20	-0.22	0.33*										
rind thickness	-0.40**	0.07											
SSC	0.11												

<sup>a</sup> \*\*, significant differences at  $P < 0.05$  and 0.01 levels, respectively, using Pearson's correlation coefficient.

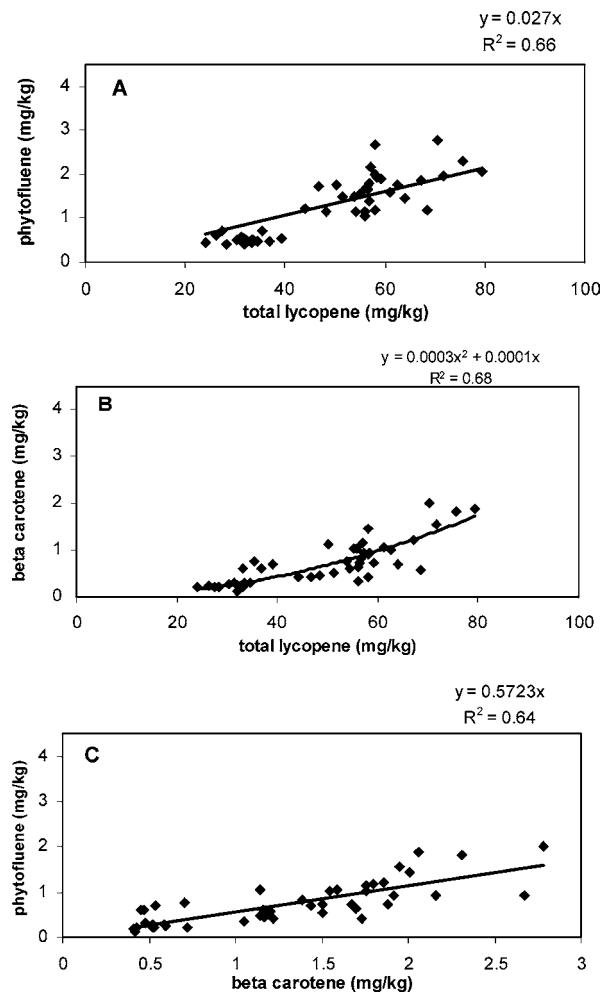
= 0.66 and 0.64, respectively) (**Figure 4A,C**), and  $\beta$ -carotene content was quadratically related to total lycopene ( $R^2 = 0.68$ ) (**Figure 4B**).

## DISCUSSION

In watermelons, thinning of the rind, increased pH and SSC, and increased flesh redness are indicators of ripeness, whereas a slight loss of SSC and a shift in color from red to red orange are indicators of overripeness (24). In our study, fruit held at 21 °C had slightly thinner rinds (1–2 mm loss), elevated pH, lower SSC, higher  $a^*$  and chroma values, and higher lycopene contents than unstored (fresh) fruit (**Table 1**). A similar loss of  $\approx 1.6$  mm in rind thickness was reported for 'Charleston Gray' and 'Congo' seeded melons (open-pollinated types) held at 20 °C for 7–10 days, relative to fresh watermelons (25). Fruit held at 13 °C had little change in rind thickness, lycopene, SSC, color, or pH compared to fresh melons. Watermelon quality was maintained during storage at 13 °C, whereas ripening was advanced in fruit held at 21 °C. In contrast, watermelons held at 5 °C had lower pH, slightly lower  $a^*$  and chroma values, and rind thickness similar to that of fresh fruit.

In a previous study, color measured by reflectance with a colorimeter (Minolta CR 200) was not a good predictor of lycopene content in freshly harvested, red-fleshed watermelon cultivars (11). In the present study, lycopene and  $\beta$ -carotene contents of stored fruit from three watermelon cultivars were highly correlated with Hunter  $a^*$ ,  $b^*$ , and chroma values (**Table 3; Figure 1**). Chroma, a measure of color intensity, increased in watermelons stored at 13 or 21 °C. The increase in total lycopene, the dominant pigment in watermelon, most likely contributed to the increased chroma. Although Showalter (17) found that watermelons held for 10 days at 22–30 °C developed an orange cast, we did not see an orange color in fruit held for 14 days at 21 °C. Although  $\beta$ -carotene increased as a percentage of total carotenoids in watermelons stored at 21 °C, it was below the 3% level estimated by Tomes (10) as necessary to visibly shift color from red to orange red in tomatoes.

All watermelons used in our study had been selected by commercial growers as fully ripe when harvested. Fruits of the three cultivars had a lower pH after storage at 5 °C than fresh fruit, indicating a physiological change. Normal symptoms of chilling injury reported to occur in watermelons held at 5 °C,



**Figure 4.** Relationships among carotenoids in watermelon flesh of stored and unstored fruit: phytofluene and total lycopene (A);  $\beta$ -carotene and total lycopene (B); phytofluene and  $\beta$ -carotene (C). Points in each figure represent four composite samples per storage temperature (fresh and 5, 13, and 21 °C) ( $n = 48$ ).

such as rind pitting, mold, and water soaking of flesh (19), were not apparent in melons held for 14 days at 5 °C in our study. However, at 5 °C, there was a slight loss of phytofluene and  $\beta$ -carotene in all cultivars and a slight loss of lycopene in two cultivars. Tomatoes, which have a carotenoid pathway similar to that of watermelon (9), had a slight loss in total carotenoids in red fruit held at 5 °C (26).

Electrolyte leakage, an indicator of membrane damage, was increased in ethylene-treated watermelon (27) before a loss of cell wall polyuronides was seen. Water soaking of the flesh in ethylene-treated watermelons was thought to occur from lipid catabolism, as increased transcripts and activities for lipoxygenase and phospholipases C and D were found (28, 29). Mature green tomatoes often fail to develop full red color if held at 5 °C before ripening at 20 °C (30), which is attributed to irreparable chloroplast membrane damage (31). In watermelon, loss of carotenoids may be an early symptom of chilling injury, with initial loss of membrane integrity in the chromoplasts leading to lycopene loss, followed by extensive membrane damage, water soaking, electrolyte leakage, altered respiration, and visible rind pitting.

Total lycopene in watermelons stored at 21 °C showed an increase, with a gain of as much as 40% seen in some cultivars compared to fresh watermelons (Table 2). This occurred for all three types of fruit used in the study, although the increase

was statistically significant only for Sugar Shack and Summer Flavor 800. Red tomatoes, also a high-lycopene fruit, gained 50% more red color after being held at 21 °C for 8 days (26).

The increased lycopene,  $\beta$ -carotene, and phytofluene contents of fruit held at 21 °C but not at 5 °C indicate temperature sensitivity and enhancement of carotenoid pathway enzymes in watermelon. Carotenoid synthesis in tomato depends on phytoene synthase, phytoene desaturase, and *a* and *b* cyclases (32, 33). The major limiting steps are thought to be the conversion of geranyl-geranyl diphosphate (GGPP) to phytoene by phytoene synthase and the conversion of phytoene to phytofluene,  $\zeta$ -carotene, and lycopene by phytoene desaturase (34). Lycopene is cyclized to  $\alpha$ -carotene and  $\beta$ -carotene by *a* and *b* cyclases, and then  $\alpha$ -carotene is hydroxylated to form lutein. Several forms of phytoene synthase have been found in tomato, and it is possible a similar situation occurs in watermelon. In watermelon held at 21 °C, there may have been an increased pool of GGPP for conversion to lycopene, or the enzymes phytoene synthase and phytoene desaturase may have increased in activity, or isomers of these enzymes may have been activated. As watermelons held at 21 °C increased in  $\beta$ -carotene, the *b* cyclase enzyme may have increased in activity. Conversely, enzyme activity in watermelons held at 5 °C may have had decreased activity. Our results indicate that the carotenoid synthesis in watermelons continues to function long after harvest, and the system is enhanced by storage at 21 °C and inhibited by storage at 5 °C.

In conclusion, of the three watermelon cultivars examined in this study, fruit of two cultivars held at 21 °C significantly increased in lycopene content and red color relative to nonstored watermelons. The increase in puree pH indicates involvement of ripening or other physiological activity. Our evidence indicates that the increased levels of phytofluene, lycopene, and  $\beta$ -carotene in watermelons held at 21 °C may reflect increased enzyme activity, such as those involved in the carotenoid pathway. The ability of some watermelon cultivars to accumulate lycopene and  $\beta$ -carotene when held at 21 °C after harvest may be useful for processors wishing to obtain these carotenoids for the natural products market.

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#### LITERATURE CITED

- Chen, L.; Stacewicz-Sapuntzakis, M.; Duncan, C.; Sharifi, R.; Ghosh, L.; van Breemen, R.; Ashton, D.; Bowen, P. E. Oxidative DNA damage in prostate cancer patients consuming tomato sauce-based entrees as a whole-food intervention. *J. Natl. Cancer Inst.* **2001**, *93*, 1872–1879.
- Hadley, C. W.; Miller, E. C.; Schwartz, S. J.; Clinton, S. K. Tomatoes, lycopene, and prostate cancer: progress and promise. *Exp. Biol. Med. (Maywood)* **2002**, *227*, 869–880.
- Hadley, C. W.; Clinton, S. K.; Schwartz, S. J. The consumption of processed tomato products enhances plasma lycopene concentrations in association with a reduced lipoprotein sensitivity to oxidative damage. *J. Nutr.* **2003**, *133*, 727–732.
- Andreassi, M.; Stanghellini, E.; Ettore, A.; Di Stefano, A.; Andreassi, L. Antioxidant activity of topically applied lycopene. *J. Eur. Acad. Dermatol. Venereol.* **2004**, *18*, 52–55.
- Stahl, W.; Heinrich, U.; Wiseman, S.; Eichler, O.; Sies, H.; Tronnier, H. Dietary tomato paste protects against ultraviolet light-induced erythema in humans. *J. Nutr.* **2001**, *131*, 1449–1451.

- (6) Wattanapenpaiboon, N.; Lukito, W.; Wahlqvist, M. L.; Strauss, B. J. Dietary carotenoid intake as a predictor of bone mineral density. *Asia Pac. J. Clin. Nutr.* **2003**, *12*, 467–473.
- (7) Gupta, N. P.; Kumar, R. Lycopene therapy in idiopathic male infertility—a preliminary report. *Int. Urol. Nephrol.* **2002**, *34*, 369–372.
- (8) Edwards, A. J.; Vinyard, B. T.; Wiley, E. R.; Brown, E. D.; Collins, J. K.; Perkins-Veazie, P.; Baker, R. A.; Clevidence, B. A. Consumption of watermelon juice increases plasma concentrations of lycopene and  $\beta$ -carotene in humans. *J. Nutr.* **2003**, *133*, 1043–1050.
- (9) Tadmor, Y.; King, S.; Levi, A.; Davis, A.; Meir, A.; Wasserman, B.; Hirschberg, J.; Lewinsohn, E. Comparative fruit colouration in watermelon and tomato. *Food Res. Int.* **2005**, *38*, 837–841.
- (10) Tomes, M. L.; Johnson, K. W.; Hess, M. The carotene pigment content of certain red fleshed watermelons. *Proc. Am. Soc. Hortic. Sci.* **1963**, *82*, 460–464.
- (11) Perkins-Veazie, P.; Collins, J. K.; Pair, S.; Roberts, W. Lycopene content differs among red-fleshed watermelon cultivars. *J. Sci. Food Agric.* **2001**, *81*, 983–987.
- (12) Perkins-Veazie, P.; Collins, J. K.; Davis, A. R.; Roberts, W. Carotenoid content of 50 watermelon cultivars. *J. Agric. Food Chem.* **2006**, *54*, 2593–2597.
- (13) Leskovar, D. I.; Bang, H.; Crosby, K. M.; Maness, N.; Franco, J. A.; Perkins-Veazie, P. Lycopene, carbohydrates, ascorbic acid and yield components of diploid and triploid watermelon cultivars are affected by deficit irrigation. *J. Hortic. Sci. Biotechnol.* **2004**, *79*, 75–81.
- (14) Perkins-Veazie, P.; Collins, J. K. Flesh quality and lycopene stability of fresh-cut watermelon. *Postharvest Biol. Technol.* **2004**, *31*, 159–166.
- (15) Rushing, J. W.; Fonseca, J. M.; Keinath, A. P. Harvesting and postharvest handling of watermelons. *Watermelons*; Maynard, D. N., Ed.; ASHS Press: Alexandria, VA, 2001; pp 156–165.
- (16) Risse, L. A.; Brecht, J. K.; Sargent, S. A.; Locasio, S. J.; Crall, J. M.; Elmstrom, G. W.; Maynard, D. N. Storage characteristics of small watermelon cultivars. *J. Am. Soc. Hortic. Sci.* **1990**, *115*, 440–443.
- (17) Showalter, R. K. Watermelon color as affected by maturity and storage. *Proc. Fla. State Hortic. Soc.* **1960**, *73*, 289–293.
- (18) Showalter, R. K.; Harmon, S. A.; Brantley, B. B.; Newson, D. W.; Pittman, J. F. Changes in Congo watermelons after harvest. *Proc. Assoc. Southern Agric. Workers* **1955**, *52*, 136–137.
- (19) Risse, L. A.; Maynard, D. N. Evaluation of selected seedless watermelon cultivars during storage. *Proc. Fla. State Hortic. Soc.* **1990**, *103*, 288–291.
- (20) Gonnet, J. P. CIE lab measurement a precise communication in flower colour: an example with carnation (*Dianthus caryophyllus* cultivars). *J. Hortic. Sci.* **1993**, *68*, 499–510.
- (21) Davis, A. R.; Fish, W.; Perkins-Veazie, P. A rapid hexane-free method for analyzing lycopene content in watermelon. *J. Food Sci.* **2003**, *68*, 328–332.
- (22) Fish, W.; Perkins-Veazie, P.; Collins, J. K. A quantitative assay for lycopene that utilizes reduced volumes of organic solvents. *J. Food Compos. Anal.* **2002**, *15*, 309–317.
- (23) Craft, N. Chromatographic techniques for carotenoid separation. *Curr. Protocols Food Anal. Chem.* **2001**, F2.3.1–F2.3.15.
- (24) Corey, K. A.; Schlimme, D. V. Relationship of rind gloss and groundspot color to flesh quality of watermelon fruits during maturation. *Sci. Hortic.* **1988**, *34*, 211–218.
- (25) Chisholm, D. N.; Picha, D. Effect of storage temperature on sugar and organic acid contents of watermelon. *HortScience* **1986**, *21*, 1031–1033.
- (26) Hall, C. The effect of low storage temperature on the color, carotenoid pigments, shelf life and firmness of ripened tomatoes. *Proc. Am. Soc. Hortic. Sci.* **1961**, *78*, 480–487.
- (27) Elkashif, M. E.; Huber, D. J. Electrolyte leakage, firmness, and scanning electron microscopic studies of watermelon fruit treated with ethylene. *J. Am. Soc. Hortic. Sci.* **1988**, *113*, 378–381.
- (28) Karakurt, Y.; Huber, D. J. Ethylene-induced gene expression, enzyme activities, and water soaking in immature and ripe watermelon (*Citrullus lanatus*) fruit. *J. Plant Physiol.* **2004**, *161*, 381–388.
- (29) Karakurt, Y.; Huber, D. J. Cell wall-degrading enzymes and pectin solubility and depolymerization in immature and ripe watermelon (*Citrullus lanatus*) fruit in response to exogenous ethylene. *Physiol. Plant.* **2002**, *116*, 398–405.
- (30) Hobson, G. E. Low-temperature injury and the storage of ripening tomatoes. *J. Hortic. Sci.* **1987**, *62*, 55–62.
- (31) Marangoni, A. G.; Smith, A. K.; Yada, R. Y.; Stanley, D. W. Ultrastructural changes associated with chilling injury in mature-green tomato fruit. *J. Am. Soc. Hortic. Sci.* **1989**, *114*, 958–962.
- (32) Fraser, P. D.; Romer, S.; Shipton, C. A.; Mills, P. B.; Kiano, J. W.; Misawa, N.; Drake, R. G.; Schuch, W.; Bramley, P. M. Evaluation of transgenic tomato plants expressing an additional phytoene synthase in a fruit-specific manner. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 1092–1097.
- (33) Ronen, G.; Carmel-Goren, L.; Zamir, D.; Hirschberg, J. An alternative pathway to  $\beta$ -carotene formation in plant chromoplasts discovered by map-based cloning of  $\beta$  and old-gold color mutations in tomato. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 11102–11107.
- (34) Fraser, P. D.; Truesdale, M. R.; Bird, C. R.; Schuch, W.; Bramley, P. M. Carotenoid biosynthesis during tomato fruit development (evidence for tissue-specific gene expression). *Plant Physiol.* **1994**, *105*, 405–413.

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