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Article *in* Journal of the Science of Food and Agriculture · August 2001

Impact Factor: 1.71 · DOI: 10.1002/jsfa.880

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J Sci Food Agric **81**:983–987 (online: 2001)
DOI: 10.1002/jsfa.880

Lycopene content differs among red-fleshed watermelon cultivars†

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Abstract: Lycopene, a carotenoid, has antioxidant properties that may reduce the incidence of certain cancers. Watermelon (*Citrullus lanatus* (Thunb) Matsum & Nakai) is a natural source of lycopene, with a reported average content of 48.7 μg g^{-1} fresh weight based on samples taken from retail produce. This study demonstrated the variability of lycopene content in 11 red-fleshed watermelon cultivars grown at one location, representing seedless, open-pollinated and hybrid types, and in commercially shipped hybrid and seedless melons, representing seasonal production periods. Tristimulus colorimeter a* and chroma values were positively correlated with lycopene values, but linear or quadratic regressions of colorimeter data against lycopene values were not significant. Tristimulus colorimeter readings from cut melons were compared to amounts of lycopene extracted from the same melons. Lycopene content varied widely among cultivars, with four cultivars having mean values greater than 65.0 μg g^{-1} fresh weight. Seedless types sampled tended to have higher amounts of lycopene (>50.0 μg g^{-1} fresh weight) than seeded types. Watermelon lycopene content changed for some cultivars with production season.
Published in 2001 for SCI by John Wiley & Sons, Ltd.

Keywords: carotenoids; functional foods; colour

INTRODUCTION

Numerous epidemiological studies have concluded that a diet rich in fruits and vegetables reduces the incidence of heart disease and certain cancers in humans.[1] Use of carotenoids such as *β*-carotene and lycopene in the diet has been positively correlated with reduced cancer incidence.[2–5] Lycopene is a red pigment and occurs naturally only in plant and algal tissues. It is a highly effective antioxidant owing to its ability to act as a free radical scavenger, and has the highest singlet oxygen-quenching rate of all carotenoids tested from biological systems.[6]

Most medical research studies with lycopene have used tomato as the food source. This choice is due to the wide use of tomatoes in the Western Hemisphere and to the high concentrations of lycopene in processed tomato products such as tomato paste and ketchup.[7,8] Other common sources of lycopene are watermelon, grapefruit and guava. Watermelon consumption in the USA is 2.3×10^9 kg year^{-1},[9] while it accounts for as much as 50% of dietary lycopene intake in Spanish diets.[10]

The lycopene content in commercial watermelons has been reported to be 45.1–53.2 μg g^{-1} fresh weight (FW).[8,11–13] These values are 60% higher than those reported for fresh tomatoes (mean value of 30.2 μg g^{-1} FW).[8] Lycopene values for watermelon were based on large composite samples obtained from retail markets. However, the cultivars used and the environmental conditions during production have not been reported.

Measurement of lycopene content requires use of specialised laboratory equipment and environmentally hazardous solvents. In contrast, tristimulus colorimeters, which measure visible colour, are relatively inexpensive and easy to use. Tristimulus colorimeter values have been positively correlated with lycopene content in tomatoes[14,15] and grapefruit juice.[16] Pardo *et al*[17] found that the formula $1000a^*/(b^* + L)$ generated from tristimulus colorimeter values was highly correlated with visual colour rankings of watermelons. However, no correlations have been reported between colorimeter measurements and lycopene content in watermelon. This study was done to determine the variability of lycopene content among

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Contract/grant sponsor: National Watermelon Promotion Board, Orlando, FL, USA
(*Received 5 September 2000; revised version received 2 February 2001; accepted 2 March 2001*)

watermelon types (seedless triploid and diploid seeded hybrid) and among commercial shipments of cultivars, and to determine the ability of tristimulus colorimetry to effectively predict lycopene content in watermelon.

MATERIALS AND METHODS

Plant material

Two independent studies, consisting of cultivar comparisons and of commercial shipments, were conducted in 1999. Melons used for the cultivar study were grown at USDA research plots, Lane, OK under standard cultural practices following Oklahoma State Extension Service guidelines.[18] A total of 13 cultivars, including 11 red- and two yellow-fleshed ('Solid Gold' and 'Hopi Yellow'), were evaluated, representing seedless triploids and diploid hybrid or open-pollinated types (see Table 1). For the commercial shipment study, ripe watermelons were sent to Lane, OK by commercial growers in Oklahoma, Texas and Mexico in February, May, July and December 1999, representing winter, spring, summer and autumn growing seasons respectively (see Table 2). All melons were transported at temperatures of 15–25 °C to Lane, OK within 2 days of harvest, and processed upon arrival.

Colour and soluble solids determination

Melons were weighed and cut transversely, about 5 cm off-centre. The percentage soluble solids concentration (SSC) was measured by cutting a wedge of flesh from the heart tissue and locular tissues and squeezing juice onto a digital refractometer (Atago PR-100, NSG Precision Cells, Inc, Farmingdale, NY) calibrated with a 10% sucrose solution. To measure colour, a tristimulus colorimeter (Minolta CR-200, Ramsey, NJ) with an 8.5 cm aperture and diffuse illumination (D/65 illuminant, 0° viewing angle and CIELAB colour space) was calibrated with a white tile. Readings were taken at two sites (heart) on commercial fruit and at five sites (three locular, two heart) on fruit grown at Lane, OK (Fig 1). Sites were numbered consecutively, starting with the ground spot and moving in a counter-clockwise direction. Chroma and hue were calculated, where $\text{hue} = \tan^{-1}(b^*/a^*)$ and $\text{chroma} = (a^{*2} + b^{*2})^{1/2}$.[19] Colorimeter values of heart and locular tissue did not differ significantly; means of colorimeter values were therefore compared to extracted lycopene.

Lycopene determination

Two 100 g tissue samples were removed from the centre of the slice (heart) for lycopene measurements and stored at −80 °C until used. Total lycopene content was measured spectrophotometrically using the methods of Sadler *et al*[20] with modifications. A 20 g sample of frozen watermelon tissue was ground with a mortar and pestle. A 2 g portion of the puree was added to a mixture consisting of 50 ml of hexane, 25 ml of acetone, 25 ml of ethanol and 0.05% (w/v) butylated hydroxytoluene (BHT), then stoppered and agitated at 5 °C for 10 min using a wrist action shaker. Cold doubly distilled water (15 ml) was added and the suspension was agitated for an additional 5 min. The solution was allowed to stand for 15 min for separation of polar and non-polar layers. Duplicates of 1 ml each were removed from the hexane layer and measured using a Shimazdu UV 160 spectrophotometer (Shimazdu, Columbia, MD). Lycopene standards were prepared using purified lycopene from Sigma (L-9879), and calculations were made using an extinction coefficient of $17.2 \times 10^4 \text{mol cm}^{-1}$.[21] A wavelength of 503 nm was selected for measurements, since, of the carotenoids known to be in watermelon, only lycopene appears at this wavelength. Lycopene values were

Table 1. Percentage soluble solids concentration (SSC), lycopene content and tristimulus colorimeter CIE *L*, *a**, *b**, hue and chroma values for watermelons (10 melons sampled per cultivar) grown at Lane, OK in 1999. The values given indicate mean ± (standard error)

			Locular and heart colour				
Cultivar	*SSC (%)*	*Lycopene (μg g⁻¹ FW)*	L	a*	b*	*Hue*	*Chroma*
Seedless types							
King of Hearts	10.8 (0.1)	57.4 (2.4)	38.9 (1.5)	25.8 (0.8)	12.2 (0.6)	25.2 (0.8)	28.6 (0.9)
Tri-X-313	10.9 (0.3)	65.7 (2.3)	38.1 (1.5)	26.3 (0.8)	12.2 (0.7)	24.8 (0.8)	29.0 (1.0)
Summer Sweet 5244	11.3 (0.3)	66.4 (6.9)	36.4 (0.9)	27.2 (0.7)	12.3 (0.6)	24.2 (0.6)	29.9 (0.9)
Crimson Trio	11.0 (0.2)	71.2 (3.3)	35.0 (0.5)	27.3 (1.0)	11.5 (0.7)	22.6 (0.7)	29.6 (1.1)
Hybrid seeded types							
Royal Sweet	10.5 (0.2)	38.6 (2.7)	45.3 (1.2)	20.8 (0.7)	12.1 (0.3)	30.3 (1.1)	24.1 (0.7)
Dumara	10.8 (0.1)	45.6 (2.0)	39.8 (1.5)	23.2 (0.5)	11.1 (0.4)	25.5 (0.7)	25.7 (0.6)
Sangria	11.0 (0.2)	46.1 (2.0)	38.9 (0.9)	24.3 (0.7)	11.5 (0.5)	25.3 (0.7)	26.9 (0.7)
Heirloom (open-pollinated) seeded types							
Black Diamond	9.7 (0.2)	37.9 (2.2)	42.4 (1.6)	20.8 (0.4)	10.7 (0.5)	27.1 (1.0)	23.4 (0.5)
Calhoun Gray	9.7 (0.2)	40.0 (2.2)	38.0 (1.9)	20.4 (0.9)	10.6 (1.0)	27.1 (1.5)	23.0 (1.2)
Crimson Sweet	10.6 (0.4)	36.5 (1.7)	38.1 (1.3)	20.0 (1.2)	9.4 (0.6)	25.4 (1.6)	22.1 (1.2)
Dixie Lee	10.2 (0.4)	69.2 (3.1)	33.4 (1.0)	25.8 (1.0)	11.2 (0.8)	23.3 (0.9)	28.1 (1.2)

Mean value for lycopene from watermelon is 48.7 μg g^{-1} F W.[8]

Table 2. Percentage soluble solids concentration (SSC), lycopene content and tristimulus colorimeter CIE *L*, *a**, *b**, hue and chroma values for commercial shipments of watermelons harvested in 1999 from Oklahoma (OK), Texas (TX) and Mexico (Mex) (20 melons sampled per cultivar per harvest date). The values given indicate mean ± (standard error)

						Locular and heart colour				
Cultivar	Type	Harvest date	Source	SSC (%)	Lycopene ($\mu g\,g^{-1}$ FW)	L	a*	b*	Hue	Chroma
Sangria	Hybrid	5 Feb	Jalisco, Mex	9.1 (0.3)	68.6 (2.0)	43.5 (1.0)	28.2 (0.4)	14.4 (0.4)	27.1 (0.6)	31.7 (0.5)
		6 May	Edinburg, TX	9.3 (0.2)	56.2 (1.2)	38.2 (0.9)	25.2 (0.5)	13.4 (0.7)	27.7 (0.9)	28.6 (0.7)
		14 Jul	Ryan, OK	11.8 (0.2)	51.2 (2.4)	42.4 (1.0)	24.0 (0.6)	11.2 (0.4)	25.1 (0.5)	26.6 (0.7)
		7 Dec	Oberon, Mex	10.4 (0.2)	47.6 (1.0)	42.4 (0.5)	25.6 (0.6)	11.8 (0.3)	24.7 (0.3)	28.2 (0.6)
Summer Sweet 5244	Seedless	5 Feb	Jalisco, Mex	9.6 (0.4)	47.3 (2.7)	41.8 (0.9)	26.0 (0.9)	13.2 (0.5)	26.9 (0.5)	29.2 (1.0)
		14 Jul	Ryan, OK	11.8 (0.1)	59.7 (2.1)	40.2 (1.0)	25.2 (0.6)	12.8 (0.3)	26.6 (0.3)	28.5 (0.7)
		7 Dec	Oberon, Mex	10.3 (0.2)	50.2 (2.4)	40.3 (0.6)	25.2 (0.6)	12.3 (0.3)	25.8 (0.3)	28.3 (0.7)

verified by HPLC with a photodiode array detector (Hewlett Packard 100, Wilmington, DE) and a Microsorb MV-C-18 column (Ranin Instrument Co, Walnut Creek, CA). Samples were extracted in tetrahydrofuran and partitioned into dichloromethane using the method of Tonucci *et al.* [22]

Statistical analysis

The experiment was designed as a completely randomised study. Comparisons were made among cultivars in the cultivar study and within cultivars in the commercial study using the general linear means procedure, and means and standard errors were calculated. Correlations were made using Pearson's correlation coefficient, and regression was done at linear and quadratic levels (SAS V 7.0, Cary, NC).

RESULTS

Lycopene content among cultivars grown at Lane, OK

Lycopene content varied widely among cultivars sampled (Table 1). Of the cultivars evaluated in this study, three seedless types ('Tri-X-313', 'Crimson Trio' and 'Summer Sweet 5244') and one heirloom (open-pollinated) seeded type ('Dixie Lee') had levels of lycopene higher than the current reported mean value for watermelon (65.7–71.2 vs 48.7 $\mu g\,g^{-1}$ FW).[8]

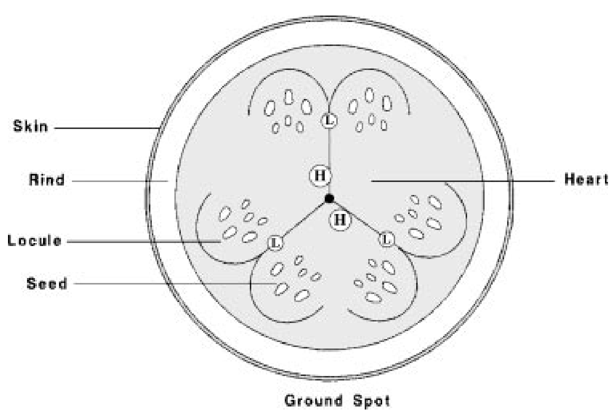

Figure 1. Transverse section of watermelon showing areas of sampling for SSC (in locular and heart areas), lycopene (in heart area) and colour (heart from areas with circled H and locular from areas with circled L).

Other red-fleshed cultivars had levels similar to the mean reported value. The two yellow-fleshed varieties ('Hopi Yellow' and 'Solid Gold') had $<5\,\mu g\,g^{-1}$ lycopene (data not shown). Tomes *et al*[23] also found lycopene variability among red-fleshed watermelon varieties (ranging from 12.2 to 52.5 $\mu g\,g^{-1}$ FW).

Lycopene content in commercial shipments

'Sangria' (a hybrid seeded type) and 'Summer Sweet 5244' (a seedless type) fruits were commercially shipped and assayed for lycopene (Table 2). Interaction of cultivar with harvest date was significant. 'Sangria' melons had decreased lycopene content as Julian date advanced from days with increasing day length to days with decreasing day length. 'Sangria' melons grown in Mexico and sampled from the winter harvest (5 February) had a higher lycopene content than melons sampled from the summer harvest (14 July), whereas the opposite was observed in 'Summer Sweet 5244' melons (Table 2).

Our results with the commercial shipments of watermelon were somewhat similar to those of tomato. Heinonen *et al*[11] found that the lycopene content of tomatoes available at retail stores could be widely affected by season of production. Harvest maturity, vine health, soil fertility, irrigation, light intensity and day/night temperatures could all conceivably affect lycopene formation in watermelon, but, except for air temperature, little is known about the interactive effect of these variables on carotenoid synthesis. High day temperatures (>37 °C) during fruit growth and ripening inhibit lycopene formation in tomato but were reported to have no effect on watermelon.[24]

Lycopene content and colorimeter values

For all red-fleshed melons the colorimeter readings a^*, chroma and $1000a^*/(b^*+L)$ were best correlated with levels of extracted lycopene for melons from cultivar and commercial experiments (Tables 3 and 4). Chroma measures colour saturation or intensity, while a^* measures the degree of red ($+a^*$) or green ($-a^*$) colour.[19]

Plotting a^* against lycopene content of red-fleshed melons of the cultivar study yielded an r^2 of 0.504 for linear regression (Fig 2A) and 0.543 for quadratic

Table 3. Correlation coefficients for percentage soluble solids concentration (SSC) and total lycopene content with tristimulus colorimeter measurements of watermelon cultivars grown at Lane, OK

Variable	*SSC*	*Lycopene*
L	−0.028	−0.354**
*a**	0.320**	0.723**
*b**	0.092	0.303**
Hue	−0.203*	−0.387**
Chroma	0.290**	0.678**
*a**+*b**	0.261**	0.627**
a/b	0.186*	0.357**
b/a	−0.205*	−0.392**
ab	0.200*	0.566**
$(a/b)^2$	0.170	0.332**
1000a*/(*b**+*L*)	0.301**	0.666**
Lycopene	0.374**	—

* and ** indicate significance at 0.05 and 0.01 level respectively (Pearson's correlation coefficient).

regression (data not shown). Plotting |*a**| against lycopene content for all red-fleshed watermelons in commercial shipments yielded an r^2 of 0.313 for linear regression (Fig 2B) and 0.336 for quadratic regression (data not shown). Separation by cultivar did not improve the r^2 values in either study (data not shown). The higher r^2 for the cultivar study versus the commercially shipped fruit may be due to less variability in melon-to-melon lycopene values. Regression of other colorimeter values, such as chroma, *a**/*b** or 1000*a**/(*b**+*L*), yielded r^2 values that were less than those with *a** in both cultivar and commercial shipment studies (data not shown).

The hue angle measured by tristimulus colorimeters is frequently used to discriminate among subtle visual colour differences in flower-breeding programmes.[19] A hue angle of 26.7° is considered a pure red colour, while a hue angle of 28–30° is a reddish-yellow colour and 22–24° a garnet-red/purplish-red colour.[19] In our study the hue angle of the red watermelon cultivars varied from about 22 to 30° (Tables 1 and 2). 'Royal Sweet' watermelons had a hue angle of 30.3°, indicating a more reddish-yellow colour, while 'Crimson Trio' had a hue angle of 22.6°, indicating a garnet-red colour (Table 1). Although hue is usually well correlated with visual colour,[19] it did not differ significantly among watermelon cultivars in our study and was much less correlated to lycopene concentration than chroma or *a** values (Tables 3 and 4). Hue and lycopene content in tomatoes were also found to be poorly correlated using Minolta colorimeters.[14,15]

Chroma measures colour intensity or saturation, with a lower chroma value indicating less colour saturation.[19] In our study, 'Crimson Sweet' melons had the lowest colour saturation values and, correspondingly, the lowest lycopene concentrations (Table 1). The commercial shipments tended to have high chroma values (26.2–31.7), indicating high colour saturation (Table 2). D'Souza *et al*[15] obtained an r^2 of 0.77 when the lycopene content of tomatoes was regressed against $(a/b)^2$, while Lee[16] reported that the lycopene content of grapefruit juice was highly correlated (0.96) with CIE *a** values. In our experi-

Table 4. Correlation coefficients for percentage soluble solids concentration (%SSC) and total lycopene content with tristimulus colorimeter measurements of watermelons used in commercial shipment study

Variable	*SSC*	*Lycopene*
L	0.210	−0.27**
*a**	−0.125	0.468**
*b**	0.109	0.400**
Hue	−0.060	0.183*
Chroma	−0.127	0.477**
*a**+*b**	−0.126	0.472**
a/b	0.062	−0.123
b/a	−0.062	0.152
ab	−0.116	0.471**
$(a/b)^2$	0.040	−0.185
1000*a**/(*b**+*L*)	−0.110	0.451**
Lycopene	0.119	—

* and ** indicate significance at 0.05 and 0.01 level respectively (Pearson's correlation coefficient).

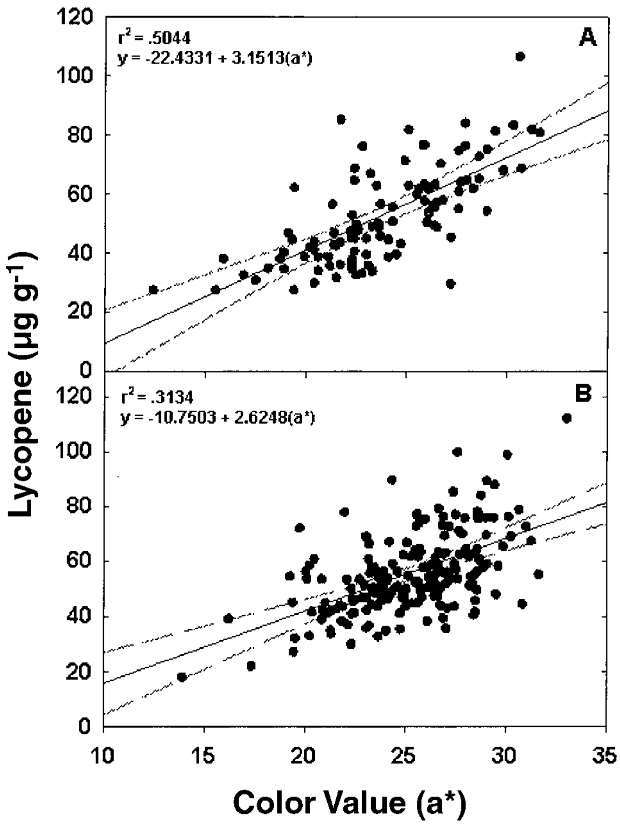

Figure 2. Regression of average tristimulus colorimeter values (from heart and/or locular tissue) against total lycopene content for melons from cultivar study, $n=110$ (A), and commercial shipments, $n=140$ (B). Broken lines represent confidence intervals, $P \leq 0.90$.

ment, a^* was better correlated than other colorimeter values to the lycopene content of watermelons in both cultivar and commercial studies (Tables 3 and 4).

In our study the tristimulus colorimeter for surface measurements of flesh colour was not a reliable tool for prediction of watermelon lycopene content. Arias *et al*,[14] using tomatoes at different ripeness stages, found a strong quadratic relationship ($r^2 = 0.96$) between a^* or a^*/b^* values and lycopene content when tomatoes ($n = 60$) ranged from 0 to 160 $\mu g\,g^{-1}$ FW in lycopene content. The most linear part of the curve fell between 0 and 40 $\mu g\,g^{-1}$. In our experiment we were interested in using tristimulus colorimetery to predict lycopene values in a large sample size (>100) of fully ripe, red-fleshed watermelons. Lycopene values for individual melons ranged from 25 to 100 $\mu g\,g^{-1}$ FW. When compared to similar values for tomatoes, watermelon lycopene values were located in a relatively flat phase of the quadratic curve.[14] Plotting lycopene values from watermelon versus a^*/b^* or $(a/b)^2$ colorimeter values failed to yield the highly significant quadratic and linear regressions respectively shown for tomatoes by Arias *et al.*[14] Watermelons with a^* values of 22–30 fell outside 90% confidence intervals (Figs 2A and 2B). For example, watermelons with an a^* value of 27 had lycopene contents ranging from 30 to 100 $\mu g\,g^{-1}$ FW, a range too great to be useful for predicting colour–lycopene relationships. The use of watermelon colorimetery with a more homogenous mix, such as juice, may prove to be more accurate predictor of lycopene content, although less rapid and more destructive than colour measurement of the surface of cut watermelons.

CONCLUSIONS

The data in our study incorporated a large sample size and indicated strong variability in lycopene content among watermelon germplasm. Additionally, the development and stability of the lycopene pigment may be affected by environmental conditions associated with production season or location. These results underscore the need to provide as many horticultural data as possible when analysing the composition of fresh fruits and vegetables. Colorimeter values of cut watermelons could not be used as a reliable indicator of lycopene content; other techniques need to be evaluated. More research needs to be conducted to determine lycopene biosynthesis and regulation in watermelon and to determine the health functional properties of this high-lycopene-containing fruit.

ACKNOWLEDGEMENTS

The authors thank Bobbie Ceriotti, Shelia Magby, Ron Marble, Bill Anderson and Wyatt O'Hern for their technical assistance, and Keith Hall, Bob Dyer for melons. This work was supported in part by a grant from the National Watermelon Promotion Board, Orlando, FL, USA.

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