Correlation of Lycopene Measured by HPLC with the L^* , a^* , b^* Color Readings of a Hydroponic Tomato and the Relationship of Maturity with Color and Lycopene Content

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Tomatoes (*Lycopersicon esculentum* cv. Laura) were separated, according to the ripening stage, by a sensory panel into seven groups, and color was measured on the tomato surface with a Minolta Chroma meter. The L^* , a^* , b^* , hue, chroma, and lycopene content were plotted against the maturity stages of the tomatoes, and several good correlations were found. The a^*/b^* ratio and the lycopene content were the parameters that allowed six of seven maturity groups in the tomato to be statistically distinguished. The lycopene content, measured by HPLC, was also correlated with the color measurements, and the a^* , a^*/b^* , and $(a^*/b^*)^2$ color factors produced the best regressions. An estimation of the lycopene content in tomatoes can be achieved by using a portable chroma meter, with a possible field usage application. Equations to calculate the lycopene content of tomatoes based on the color readings are reported.

Keywords: *Lycopersicon esculentum cv. Laura; lycopene; maturity; color; colorimeter; L*, a*, b*; correlation; HPLC*

INTRODUCTION

The color of tomatoes is a very important marketing factor that affects the buying decision of the consumer (Garrett et al., 1960) and is also a very important quality attribute for the tomato industry (Gould, 1974; Stevens and Rick, 1986). Chlorophyll and carotenoids are responsible for the color of tomatoes. In the early stages of development the chlorophyll imparts a green color, and when the tomato starts the ripening process, the chlorophyll is degraded and carotenoids are synthesized (Hobson and Davies, 1971).

The carotenoids are important not only because of the color they impart but also because of the recognized health benefits associated with their presence. Carotenoid intake reduces the risk of certain types of cancer, atherosclerosis, and cataract formation, and it is wellknown to play a role in disease prevention (Sandstrom et al., 1994; Weisburger, 1998). There are two main carotenoids in the tomato, lycopene (ψ , ψ -carotene), which is the major carotenoid and imparts the red color to the tomato, and β -carotene, which is \sim 7% of the total carotenoid content (Gould, 1974). β -Carotene presents provitamin A activity, and lycopene acts as an antioxidant, anticarcinogenic, and antimutagenic agent (Pfander, 1992). The lycopene concentration augments with the maturity of the tomatoes when the chloroplasts change to chromoplasts (Kirk, 1978) and the synthesis of lycopene increases, causing the development of red color.

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Due to the convenience and ease of using color measurements instead of long, tedious, and costly chemical methods, several studies have correlated the color with the pigment content of different food systems. Paprika (Ramakrishnan and Francis, 1973), parsley leaves (Berset and Caniaux, 1983), blueberries (Francis, 1985), salmon (Skrede and Storenakken, 1986; Ando et al., 1992), red pepper (Reeves, 1987), grapes (Watada and Abbott, 1975), peaches (Morrison, 1990), sweet potatoes (Ameny and Wilson, 1997; Takahata et al., 1993), carrots (Ling et al., 1996; Chen and Tang, 1998), meat (Ling et al., 1996), Swiss chard (Ihl, 1994), and squash, cranberries, and wines (Francis, 1969) are some of the food systems in which the color has been correlated with the pigment content.

Tomato pigments have been correlated to color readings as well. Watada et al. (1976) found a relationship between the chlorophyll and carotenoid contents, measured with a spectrophotometer, with the absorbance at certain wavelengths of tomato samples. The samples were classified according to color, and absorbance was read at 510, 600, and 690 nm with a photometer. A spectrophotometer was also used for this purpose at a wavelength range of 390–880 nm. Light reflectance was measured with a Hunter color meter, and the L^* , a^* , b^* , X, Y, and Z values were correlated with the lycopene content. The L^* factor was the best correlated parameter ($R^2 = 0.94$). The lycopene content was measured by spectroscopic methods at a wavelength of 502 nm.

Later, D'Souza et al. (1992) related the concentration of lycopene with the chromaticity values of tomatoes. The lycopene concentration was calculated on the basis of the equation by Beerh and Siddappa (1959) after the sample absorbance had ben read at 503 nm. Quantification of lycopene by spectrophotometric methods in pericarp and skin disk samples was performed. The best

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correlation found between lycopene and color ($R^2 = 0.83$) was with the $(a/b)^2$ factor.

The absorbance of tomatoes is attributed to several carotenoids, and although lycopene is the major one, the rest of the pigments, which absorb in near wavelengths, may influence absorbance as well, especially in varieties having reduced lycopene concentration. HPLC can provide an accurate separation and quantification of the several carotenoids present in tomatoes, and their isomers, and provide a more accurate estimation of their concentration. The objectives of this study were to establish the correlation between lycopene content, measured by HPLC, and the chromaticity values of the tomato surface measured with a portable Minolta chroma meter and to study the relationship between the maturity stages of the tomato and the color readings and lycopene content. We used the whole-homogenized tomato to calculate the average lycopene content of the entire fruit after the color readings were taken in the equatorial area of the intact tomato. The use of a portable chroma meter is an easy, convenient, and nondestructive method that may provide a good estimation of the lycopene content of tomatoes and inform about their maturity stages. This portable chroma meter can be transported and used in the field or greenhouse and is a nondestructive method, so there is no need to harvest the tomatoes to determine their lycopene content; therefore, this method can be used to assess the lycopene content of tomatoes, on the basis of their chromaticity values, in a very simple and easy way in any place.

MATERIALS AND METHODS

Laura tomatoes (*Lycopersicon esculentum* Mill cv. Laura) were grown in a greenhouse located at Cook College, Rutgers University, under a flood hydroponic system. The seeds were raised in a 3 in. rock wool cube and transferred to a production bench on day 35. They were placed on a rayon polyester material at a spacing of 12 in. \times 12 in. All plants were topped at a height of 36 in., and the side shoots were pruned regularly.

The tomatoes were harvested at different ripening stages, varying from mature green to intense red color, and sorted according to the color stage by visual evaluation performed by a two-member sensory panel. The selected tomatoes generally presented a homogeneous color and were separated into seven different groups according to the maturity stages: green (G), yellowish with some pinkish regions (Y), orange (O), light red (LR), red (R), intense red firm (IRF), and intense red soft (IRS). Five to eight tomatoes per group were analyzed for color and lycopene individually.

The tomatoes were washed with distilled water and dried thoroughly. Color measurements were performed on the surface of the tomatoes, around the equatorial region. The color was measured at least 11 times, with a maximum of 18, depending on the tomato size, with an average of 14 times for each tomato. A Minolta Chroma Meter CR-200 (Minolta Camera Co. Ltd., Osaka, Japan) tristimulus color analyzer, consisting of a head with an 8 mm diameter measuring area and a diffuse illumination/0° viewing, was used. The chroma meter was first calibrated with a white tile and checked for recalibration between measurements, although no adjustments were necessary. Readings are reported in the L^* , a^* , b^* system. The chroma and hue were also calculated on the basis of the following equations:

chroma =
$$\sqrt{(a^{*2} + b^{*2})}$$

 $H^{\circ} = \tan^{-1} (b^{*}/a^{*})$ when $a^{*} > 0$ and $b^{*} \ge 0$

$$H^{\circ} = 180 + \tan^{-1} (b^*/a^*)$$
 when $a^* < 0$

After the color measurements, the samples were homogenized for 3 min in a blender. Water content was measured by the constant weight method in a vacuum oven at 70 °C.

The carotenoids were extracted from the homogenized samples with a mixture of hexane, acetone, and ethanol (Fisher Scientific, Springfield, NJ) (50:25:25) while stirring for 15 min. All solvents were of HPLC grade. Water was added, and stirring was set for 15 min to help the phase separation; the mixture was then filtered, and the polar and nonpolar layers were separated (Sadler and Dezman, 1990). An aliquot of the nonpolar phase was filtered with a 0.45 μ m nylon filter membrane (Fisher Scientific) and injected into the HPLC without further treatment. The extraction was performed under dark conditions to avoid lycopene isomerization and degradation.

The analysis, separation, and quantification of lycopene $(\psi,\psi$ -carotene) were accomplished by HPLC. We used a 4.6 \times 250 mm, 5 μ m, polymeric carotenoid C₃₀ column (Emenhiser et al., 1995) (YMC, Inc., Wilmington, NC), under an isocratic mobile phase of methyl alcohol (Fisher Scientific) and methyl tert-butyl ether (Sigma Chemical Co., St. Louis, MO) in a ratio of 3:7. The lycopene standard used was 95% pure (Sigma Chemical Co.) and showed a retention time of 9 min. A calibration curve was made with the standard to quantify the lycopene, on the basis of the retention time of the peak. The HPLC grade solvents were filtered using 0.45 μ m nylon filter membranes and degassed with helium gas. The HPLC system consisted of a Waters 600 E system controller, a Waters 991 photodiode array detector, a Waters U 6 K injector system, and a Waters 600 multisolvent delivery system (Millipore, Milford, MA). The wavelength range used was 420-530 nm, and 471 nm was used to analyze the lycopene peak.

The lycopene standard and the extracted lycopene from the tomato samples were covered with aluminum foil to avoid direct contact with light. The analyses were conducted at ambient temperature at a flow rate of 1 mL/min.

The results were analyzed by ANOVA at a probability level of ≤ 0.05 and correlated using the Pearson product moment correlation method.

RESULTS AND DISCUSSION

Maturity Stages of the Tomatoes and Color Evaluation. The sensory evaluation of the tomatoes correlated with most of the color readings and the lycopene content of the tomatoes at different maturity stages (Figure 1). The human perception of color has previously been reported to have a good correlation with the color readings of Hunter and Munsell color disks (Mavis and Gould, 1954) and with the light transmittance and reflectance of tomatoes during ripening (Jahn, 1975). Edan et al. (1997) also correlated maturity and hue of tomatoes using a chroma meter and sensory evaluation.

The a^* value showed a linear correlation with the ripening stages of the tomatoes, although the intense red firm and the intense red firm—intense red soft groups were superimposed. The a^* value increased from -10.37 to 29.25 (Figure 1) as a consequence of the synthesis of lycopene and depletion of chlorophyll, representing the color change from green to red.

The lightness factor, L^* , decreased during the five first ripening stages and then remained constant, producing a fair correlation (Table 1) with the ripening stages, although it did not distinguish between the red, intense red firm, and intense red soft maturity groups. The decrease of L^* with maturity reflects the darkening



Figure 1. Color readings of Laura tomatoes at different maturity stages in the L^* , a^* , b^* system. The x-axis shows the maturity stages of the tomatoes: green (G), yellow (Y), orange (O), light red (LR), red (R), intense red firm (IRF), and intense red soft (IRS). The bars represent the confidence interval (P = 0.95).

Table 1. Summary of the Linear Regression and PearsonCorrelations between the Color Readings and theLycopene Content and Maturity Stages of the Tomatoes

factor	linear regression R ²	correlation coefficient	probability level
L^*	0.90	-0.95	< 0.0001
<i>a</i> *	0.87	0.93	< 0.0001
b^*	0.09	0.22	< 0.05
a*/b*	0.90	0.95	< 0.0001
$(a^*/b^*)^2$	0.86	0.92	< 0.0001
hue	0.44	0.67	< 0.0001
chroma	0.67	0.82	< 0.0001
lycopene	0.91	0.94	< 0.0001

of the tomatoes with carotenoid synthesis and the loss of greenness. Shewfelt et al. (1988) reported the same trend.

The b^* value increased through the first four maturity stages of the tomatoes and then decreased, reflecting the synthesis of β -carotene (the second most important carotenoid of tomatoes) and its subsequent masking with the increase of lycopene concentration. The b^* value produced a low correlation coefficient with the maturity groups of the tomatoes.

The a^*/b^* ratio is often used as an indicator of color development in tomatoes and redder hue (Worthington et al., 1969; Hall, 1964; Koskitalo and Ormrod, 1972). This ratio produced a good linear regression with the maturity stages of the tomatoes (Figure 2). The statistical analyses of this ratio distinguished six of the seven maturity groups; the intense red firm and intense red soft groups were similar. We obtained a very good correlation coefficient, 0.95, between the a^*/b^* ratio and the maturity classification of the tomatoes (Table 1). Hall (1964) also reported a good correlation between the a^*/b^* ratio and tomato ripening.

The $(a^*/b^*)^2$ ratio is also used as a maturation index and produced a good correlation with ripening stages; however, as with the a^* factor, the intense red firm and the intense red firm–intense red soft groups were superimposed.

The hue, which is the actual color (for example, red, yellow, blue, etc.) (Clydesdale, 1969), decreased from 180.58° to -1.44° . A hue of 180° represents pure green and a hue of 0°, pure red (Shewfelt, 1988). The dramatic decrease of the hue was from the green to the orange stage and then remained constant. The hue and the



Figure 2. a^*/b^* color ratio correlation with the ripening of Laura tomatoes. The *x*-axis shows the maturity stages of the tomatoes: green (G), yellow (Y), orange (O), light red (LR), red (R), intense red firm (IRF), and intense red soft (IRS). The bars represent the confidence interval (P = 0.95) of the linear plot.

chroma did not show a good correlation with the ripening stages of the tomatoes (Table 1).

Lycopene and Color Readings. The HPLC analysis of lycopene showed a spectrum with three main peaks, at 446, 471, and 505 nm, the major absorption of lycopene being at 471 nm.

The average lycopene content and color readings corresponding to the sensorial maturity classification of tomatoes are shown in Table 2. The average lycopene content was 0.11 mg/100 g for the green stage, and the highest concentration was 12.20 mg/100 g for the intense red maturity stage. The lycopene content varies according to tomato variety. Sadler et al. (1990) reported an average lycopene content of 15.8 ± 1.9 mg/100 g for the Michigan-Ohio variety, whereas Yamaguchi et al. (1960) reported 10.77 mg/100 g for the Pearson variety. Nguyen and Schwartz (1999) also reported that the lycopene content of tomatoes varies from 3.1 to 40 mg/100 g according to the variety.

Several correlations were also found between the lycopene content and the color evaluations performed with the portable Minolta chroma meter, as well as with the sensory classification of the tomatoes at different maturity stages.

The lycopene content and the sensory classification of the ripening stages (Figure 3) produced a good linear regression and an even better fit when an exponential regression was used (Table 1). The lycopene content increased from 0.0062 to 16.067 mg/100 g, and this increase was mainly through the first six ripening stages, which were significantly different. Although the intense red soft group was riper than the intense red firm, the lycopene contents were not different, suggesting that the synthesis of lycopene occurs up to the intense red firm stage, with no further synthesis of lycopene and an evident senescence.

The L^* and lycopene content produced a very good correlation coefficient (Table 3). While the lycopene concentration increased, L^* decreased and the tomatoes changed from a light to a dark color. The panel perceived these changes in lightness as well.

The b^* value did not produce a good correlation and cannot be used to predict the lycopene content of tomatoes.

 Table 2. Average and Confidence Intervals (0.05) of Lycopene Content and Color Readings of Laura Tomatoes at Different Maturity Stages

maturity stage	lycopene (mg/100 g)	L^*	<i>a</i> *	b^*	a*/b*	$(a^{*}/b^{*})^{2}$	chroma	hue
green	0.116 (0.07)	57.454 (0.71)	-9.647 (0.33)	19.804 (0.64)	-0.487 (0.007)	0.238 (0.007)	22.034 (0.72)	180.58 (0.04)
yellow	1.445 (0.34)	55.102 (1.24)	3.361 (1.35)	23.587 (1.22)	0.141 (0.05)	0.040 (0.01)	24.045 (1.20)	29.54 (0.44)
orange	3.406 (0.50)	51.152 (0.98)	10.483 (0.47)	29.501 (0.99)	0.352 (0.02)	0.158 (0.01)	31.269 (0.90)	-1.441(0.80)
light red	4.950 (0.46)	46.506 (0.74)	20.992 (0.80)	31.133 (0.97)	0.682 (0.02)	0.475 (0.03)	37.640 (1.08)	0.083 (0.05)
red	9.257 (0.75)	41.844 (0.60)	24.225 (0.78)	26.140 (1.02)	0.942 (0.04)	0.904 (0.07)	35.722 (1.06)	0.555 (0.05)
intense red firm	12.208 (0.84)	40.619 (0.34)	25.932 (0.58)	24.826 (0.72)	1.053 (0.01)	1.117 (0.03)	35.93 (0.89)	0.718 (0.02)
intense red soft	11.996 (1.07)	40.602 (0.47)	26.910 (0.84)	24.384 (0.71)	1.111 (0.01)	1.242 (0.03)	36.343 (1.07)	0.787 (0.02)



Figure 3. Lycopene content of Laura tomatoes at different maturity stages. The *x*-axis shows the maturity stages of the tomatoes: green (G), yellow (Y), orange (O), light red (LR), red (R), intense red firm (IRF), and intense red soft (IRS). The bars represent the confidence interval (P = 0.95).

Table 3. Summary of the Pearson Correlations andRegressions of the Color Readings with the LycopeneContent of Laura Tomatoes

factor	linear regression <i>R</i> ²	exponential regression <i>R</i> ²	correlation coefficient	probability level
L^*	0.84	0.93	-0.92	< 0.0001
a*	0.82	0.96	0.87	< 0.0001
b^*	0.05		0.12	0.26
a*/b*	0.88	0.96	0.93	< 0.0001
$(a^*/b^*)^2$	0.90	0.85	0.95	< 0.0001
hue	0.55	0.80	0.74	< 0.0001
chroma	0.35	0.88	0.28	0.78

The development of lycopene with ripening in tomatoes coincides with the literature data (Meredith and Purcell, 1966; Yamaguchi et al., 1960; Forbus at al., 1985). The increase of a^* is directly associated with lycopene synthesis. This relation can be described as an exponential rise ($R^2 = 0.96$) with saturation at the latest maturity stages, where a^* is an exponential function of lycopene (Figure 4); correspondingly, lycopene will be a logarithmic function of a^* :

lycopene, mg/100 g =
$$3.0845 \text{ Ln}\{1 - [(37.3536)/(a^* + 10.4137)]\}$$

The linear regression of a^* and lycopene produced a fair fit ($R^2 = 0.82$) for a range between 0.01 and 10.35 mg/100 g and from -10.37 to 29.25 of lycopene and a^* , respectively. This range corresponds to the changes from green to red maturity stages; however, in commercial practice, the lycopene content may be higher than the former, as in the case of processing tomatoes, which are processed in the intense red stage.

The a^*/b^* and $(a^*/b^*)^2$ ratios were also correlated with the lycopene content (Table 3). Both linear correlations were similar, although the a^*/b^* ratio and the lycopene



Figure 4. Exponential regression of the a^* factor with the lycopene content of Laura tomatoes produced a good fit ($R^2 = 0.96$).



Figure 5. Lycopene content and a^*/b^* color ratio linear regression.

exponential regression produced a better fit. The linear range for the lycopene and the a^*/b^* ratio was between 1.13 and 14.32 mg/100 g and from 0.12 to 1.17, respectively. This wider range may be more useful for commercial applications, because industry and consumers frequently use tomatoes within this range of color, which corresponds to the yellowish to the intense red ripening stages of tomatoes. Figure 5 shows the exponential rise ($R^2 = 0.96$) of the lycopene and a^*/b^* ratio, and the following equations show the lycopene as a logarithmic function of a^*/b^* and their linear correlation:

lycopene, mg/100 g = 3.6101 Ln{1 - [$(1.5913)/(a^*/b^* + 0.4767)$]} lycopene, mg/100 g = 11.848 (a^*/b^*) + 1.5471

The linear regression of the $(a^*/b^*)^2$ ratio was between 0.01 and 1.36 of the ratio and from 1.13 to 14.32 mg/



Figure 6. Linear regression of lycopene content and the $(a^*/b^*)^2$ ratio.

100 g of lycopene and is shown in Figure 6. The next equation was obtained from the linear regression ($R^2 = 0.905$):

lycopene, mg/100 g =
$$8.7073(a^*/b^*)^2 = 1.5212$$

We observed a clear exponential behavior in the a^* , a^*/b^* , and $(a^*/b^*)^2$ color readings with the lycopene content not reported before. These high correlations may be due to the low dispersion of the readings obtained.

The chroma, which produced a reasonable exponential fit with the lycopene content (Table 3), increased up to a level of \sim 6 mg/100 g of lycopene and then remained steady while lycopene continued increasing. D'Souza (1992) reported a very low linear correlation between the chroma and the lycopene content. We did not find a good linear correlation either.

As with the chroma, the hue and lycopene content did not produce a good correlation.

CONCLUSION

The visual evaluation of the tomatoes and the maturity stages showed a good correlation with the objective measurements of lycopene and color performed by HPLC and with the Minolta chroma meter, respectively.

The tomatoes were classified into six maturity groups, based on the color readings $a^{*/b^{*}}$ obtained with a Minolta chroma meter and lycopene content, as follows: green, yellowish with some pinkish regions, orange, light red, red, and intense red. Although the intense red soft group was at a more advanced ripening stage than the intense red firm group, the a^{*} and $a^{*/b^{*}}$ values were not significantly different for both maturity groups.

The prediction of lycopene content, measured by HPLC, with the objective color readings produced better fits with the a^* , $(a^*/b^*)^2$, and a^*/b^* factors, but because the a^*/b^* ratio was significantly different for six of the seven groups, we recommend its usage to predict the lycopene content in Laura tomatoes.

In summary, the lycopene content of tomatoes can be accurately predicted with the use of a portable chroma meter camera. The sensory classification of the ripening stages of tomatoes correlated with the objective color readings and with the lycopene content.

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