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ESTIMATION OF CHLOROPHYLL AND CAROTENOID CONTENTS OF WHOLE TOMATO BY LIGHT ABSORBANCE TECHNIQUE

ABSTRACT

The relationship between optical measurements of whole tomato and pigment content was studied. The difference between the absorbances (A) at 710 and 780 nm [ΔA (710–780 nm)] correlated significantly (r = 0.98) with chlorophyll contents of 0.3–13.4 μg/g fresh weight (gfw). The ΔA (570–780 nm) correlated significantly (r = 0.97) with lycopene contents of 0.2–46.7 μg/gfw. The ΔA (550–580 nm) correlated significantly with the β-carotene content (r = 0.95). The reflectance measurements correlated with the chlorophyll and lycopene contents, but the coefficients were lower and the standard errors were larger than those observed with the absorbance measurements.

INTRODUCTION

WORTHINGTON (1974) estimated the ripeness of externally green and red tomatoes on the basis of absorbances (A) at 510, 600 and 690 nm. (A = log 1/T, where T is the transmittance of the sample. Absorbance as defined here includes the loss from absorption, reflectance and scatter of light.) Light absorption was assumed to be due to chlorophyll in green tomatoes and carotenoids in red tomatoes. If this assumption is correct, pigment content could be estimated from absorbance measurements of a whole fruit. Also, pigment content or change of content might be used as an index of physiological age of fruit that cannot be sacrificed for chemical analyses of the pigments.

In this study, pigment contents were correlated with absorbances at wavelengths specified by Worthington (1974). Other wavelengths were also evaluated so that the best correlation might be found.

Additionally, since light reflectance measurements are used universally to describe tomato color, the relationships of light reflectance to absorbance and to pigment content were determined.

EXPERIMENTAL

FRESHLY HARVESTED green tomatoes (cv. Walter) ranging from 5.7–6.6 cm diameter were air shipped from Miami, Fla. to Beltsville, Md. Fruit were held at 15°C in the dark until they reached the appropriate stage of ripeness for analyses. The stages were determined on the basis of criteria developed by Worthington (1974). Tomatoes in stage 1 (Table 1), termed "immature-green," lacked red internal color and gel type of placental tissue. Those in stages 2–4, termed "mature-green," had increased amounts of red internal color and gel type of placental tissue. Those in stage 5, termed "breakers," showed incipient ripening externally. Tomatoes in the remaining five stages had increased amounts of red color.

Fruit were measured optically and sorted into three lots of five fruit each for each stage of ripeness. The five fruit of similar absorbance were

Table 1—Chlorophyll and carotenoid compositions of "Walter" Tomato fruit categorized by absorbance (A) readings[a]

	Δ (nm)		Pigment content[b]						
Stages	510–600	600–690	Total chlorophyll μg/gfw	Lycopene μg/gfw	β-carotene μg/gfw	γ-carotene μg/gfw	ε-carotene μg/gfw	ζ-carotene μg/gfw	β-zeacarotene μg/gfw
1	0.02	+0.18	13.4a	–	0.7a	0.03a	0.04a	0.05a	0.02a
2	0.20	–0.30	11.2b	–	0.7a	0.03a	0.04ab	0.05a	0.03a
3	0.61	–0.63	8.6c	0.2a	1.0ab	0.04a	0.05ab	0.03a	0.03a
4	1.08	–0.93	5.9cd	0.6a	1.3ab	0.12ab	0.09ab	0.04a	0.05ab
5	1.52	–1.08	4.2d	2.5a	1.8b	0.26b	0.07ab	0.05a	0.04ab
6	1.56	–0.60	1.0e	10.1b	3.1c	0.54c	0.15b	0.08a	0.06ab
7	1.02	0.22	0.4e	16.7c	3.3c	0.87d	0.12ab	0.17ab	0.12bc
8	0.71	0.62	0.3e	22.8d	3.7c	0.90d	0.12ab	0.27bc	0.18cd
9	0.32	1.10	0.3e	34.8e	3.1c	0.97d	0.08ab	0.42c	0.21d
10	0.01	1.51	0.3e	46.7f	2.9c	1.13e	0.11ab	0.89d	0.29e

[a] Average of three samples, each containing five tomatoes.
[b] Values within a column not followed by a common letter are significantly different ($P < 0.01$) (Duncan, 1955).

analyzed together for pigment content. The average optical measurement of these fruit was compared with the pigment content.

Two instruments were used for measuring the absorbance. A four-filter photometer was used for wavelengths reported by Worthington (1974). A high-intensity spectrophotometer (Massie and Norris, 1975) was used for the other wavelengths evaluated.

Four-filter photometer

The four-filter photometer measures absorbance at four wavelengths on samples having absorbance as high as 9.

The absorbances of the whole tomato were measured at 510, 600 and 690 nm as described by Worthington (1974). The ripeness stages of green fruit and breaker were based on the difference between absorbances at 510 and 600 nm [ΔA (510–600 nm)]; where as the ripeness stages of red fruit were based on ΔA (600–690 nm) (Table 1).

High-intensity spectrophotometer

Tomatoes were held in the innertube-venturi-type holder (Massie and Norris, 1975) and the absorbances were measured at 50 wavelengths spaced 10 nm apart from 390 to 880 nm. Data were stored on magnetic tape and later compared with chemical analyses of the pigments to determine the best single wavelength and best combination of two wavelengths to predict pigment content.

Reflectance

Light reflectance was measured with a Hunter D25 Color Difference Meter, standardized with a white standard tile. The blossom-end of the fruit was placed on a 5-cm diameter aperture. The L, a_L and b_L values were recorded and used to calculate tristimulus X, Y and Z (Wysecki and Stiles, 1967).

Pigments

Chlorophylls a and b were analyzed and calculated as described by Sweeney and Martin (1961), except that the initial maceration with acetone contained 3g $MgCO_3$ per 150g tomato sample.

The carotenoids were analyzed as described by Tomes (1963) with a few modifications. A 50-g aliquot of the tomato acetone-$MgCO_3$ macerate that was prepared for chlorophyll analysis was homogenized with 75 ml acetone-hexane (30:45 v/v) in a Virtis homogenizer for 7 min. The chromatographic column of MgO-Hyflo Supercel was washed with 0.5% acetone in hexane to minimize isomeration of carotenoids (Wiseman et al., 1952). ε-Carotene, β-carotene and ζ-carotene were eluted from the column with increasing concentrations of acetone in hexane. β-Zeacarotene, γ-carotene and lycopene were eluted from slices of the extruded column. The absorbtion spectrum of each eluate was measured with a Bausch & Lomb recording spectrophotometer and the concentration was calculated by using the extinction coefficient reported by Davies (1965).

RESULTS & DISCUSSION

Pigments

The average chlorophyll content decreased from 13.4 μg/g fresh weight (gfw) in immature-green (stage 1) fruit to 0.3 μg/gfw in partially ripe (stage 8) fruit (Table 1). Changes in the chlorophyll content after stage 6 were not significant and a small amount (0.3 μg) of chlorophyll was still present in the table ripe (stage 10) fruit. The ratio of chlorophyll "a" to "b" was 2 in the immature fruit and approached 1 as the fruit ripened.

Carotenoid accumulation differed with time. Lycopene content at stage 3 was 0.2 μg/gfw and increased at each successive stage to 46.7 μg/gfw (Table 1); however, the increase from stages 3 to 5 was not significant. β-Carotene was 0.7 μg/gfw at stage 1 and accumulated to a maximum of 3.7 at stage 8. Only the increase from stages 5 to 6 was significant. γ-Carotene was 0.03 μg/gfw at stage 1 and increased to 1.13 by stage 10. The increases from stages 1 to 4 and from stages 7 to 9 were not significant. The quantitative increases of ε-carotene, ζ-carotene, and β-zeacarotene were small, and the total level of the three was 1.29 μg/gfw at stage 10.

Four-filter photometer

The measured absorbance at 510 nm changed only slightly with each successive stage of ripening. The absorbance at 690 nm decreased continuously from stages 1 to 10. The absorbance at 600 nm decreased from stages 1 to 5 and then increased from stages 6 to 10. Thus the ΔA (510–600 nm) increased up to stage 6 and then decreased, and the ΔA (600–690 nm) decreased up to stage 5 and then increased (Table 1). Because of the reversal of ΔA between stages 5 and 6, Worthington (1974) used ΔA (510–600 nm) to measure maturity of green fruit and those showing incipient ripe color, and ΔA (600–690 nm) for those showing ripe color.

The chlorophyll content correlated significantly with the ΔA (510–600 nm) of fruit at stages 1 to 5 (r = 0.91), but the coefficient was better (r = 0.97) when the log of chlorophyll content was correlated with the ΔA values (Fig. 1). However, the chlorophyll content of only fruit at stages 2 to 5 could be estimated satisfactorily with these absorbance values. The average chlorophyll content of fruit at stage 1 fit the regression line, but the replicate values differed widely. After stage 5 the relationship was not logarithmic.

The lycopene content correlated significantly with the ΔA (600–690 nm) of fruits at stages 6 to 10 (r = 0.94), but the coefficient was better (r = 0.98) when the log of lycopene content was correlated with the ΔA values (Fig. 2). The lycopene contents of only fruit at stages 7 to 10 could be estimated satisfactorily with ΔA (600–690 nm). The ΔA at stage 6 deviated from the line due to absorption at 690 nm by the chlorophyll (data not shown). The chlorophyll contents at

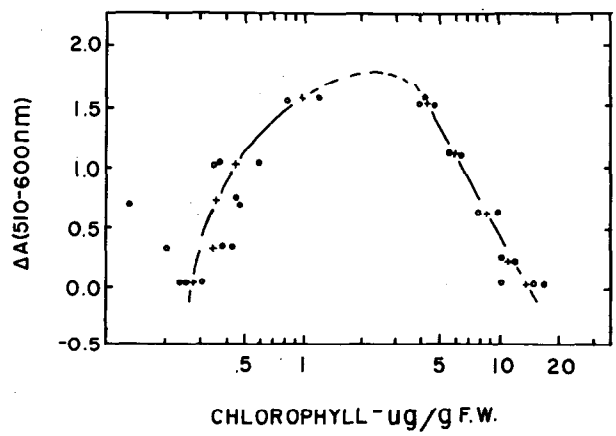

Fig. 1—Correlation between total chlorophyll content and ΔA (510–600 nm) (four-filter photometer) of whole "Walter" tomatoes.

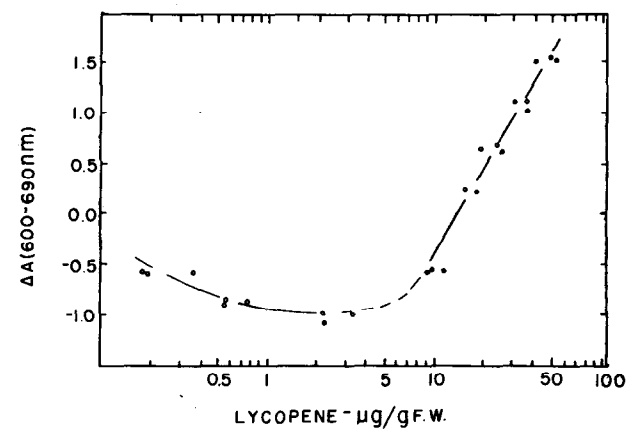

Fig. 2—Correlation between lycopene content and ΔA (600–690 nm) (four-filter photometer) of whole "Walter" tomatoes.

stages 6 and 7 were not different statistically, but the difference did affect the absorbance.

High-intensity spectrophotometer

Typical spectra of fruit at different stages of ripeness (Fig. 3) show the changes in absorbance with ripening. The absorbance in the 600–710 nm region decreased during stages 1 to 6 with the loss of chlorophyll. With continued ripening of the fruit the absorbance from about 500–620 nm increased, probably due to changes in the carotenoids, particularly lycopene. Thus, from about 560–620 nm the absorbance decreased during stages 1 to 5 and then increased during stages 6 to 10. This pattern of change at 600 nm was also observed with the four-filter photometer. Absorbance values below 500 nm were not valid due to interference by filter stray light (Massie and Norris, 1975).

For the selection of wavelength pairs that correlated best with chlorophyll content, two sets of criteria were used. One was that 690 nm be used as one of the wavelengths because it was used in the four-filter photometer and by Sidwell et al. (1961) for estimating chlorophyll content. The other was that the best linear correlation be sought.

When 690 nm was one of the wavelength pair, any wavelength from 730–830 nm was a satisfactory companion, but the linear correlation was the highest with 780 nm (r = 0.96). In using ΔA (690–780 nm) to estimate chlorophyll content, the calculated value was higher than the actual value when the ΔA was from 0.4–1.1 (Fig. 4). At high chlorophyll levels, the actual values deviated widely because of fluorescence (Massie and Norris, 1975). Thus 690 and 780 nm did not appear to be the best wavelength pair for estimating chlorophyll.

The ΔA of wavelength pair, 710–780 nm, gave the highest linear correlation with the chlorophyll content (Fig. 5). The coefficient was significant (0.98), and the linear regression equation was:

$$\Delta A\ (710–780\ nm) = 0.0178 + 0.0389\ \text{chlorophyll}$$

The ΔA (710–780 nm) of about −0.05 to 0.40 could be used to estimate the chlorophyll content of fruits at stages 2 through 10. The content could not be estimated satisfactorily at stage 1 because the replicates differed widely as observed with other measurements.

The lycopene content correlated linearly and significantly (r = 0.97) with ΔA (570–780 nm) (Fig. 6). The regression equation of the linear line was:

$$\Delta A\ (570–780\ nm) = 0.604 + 0.0431\ \text{lycopene}$$

The ΔA (570–780 nm) could be used to estimate the lycopene content of fruit at any of the 10 stages in contrast to only 4 stages that were covered when ΔA (600–690 nm) was used.

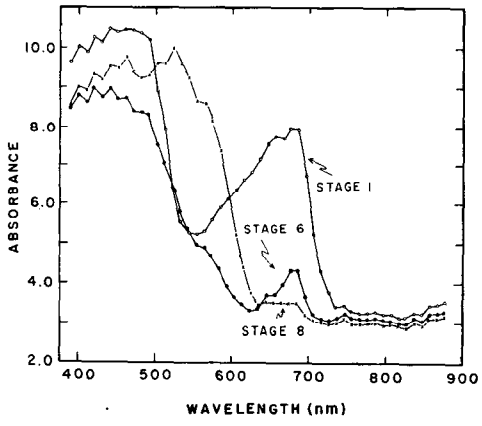

Fig. 3—Absorption spectra of green (stage 1), partially ripe (stage 6) and ripe (stage 8) tomatoes.

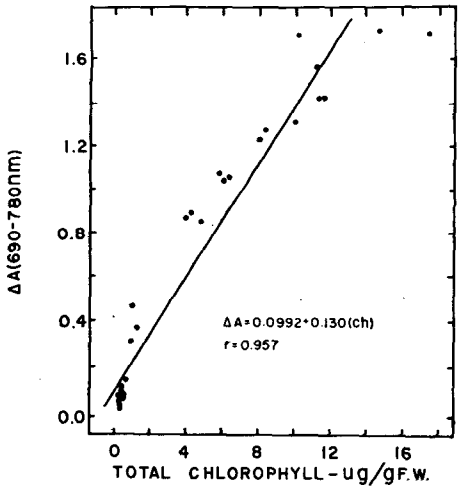

Fig. 4—Correlation between total chlorophyll content and ΔA (690–780 nm) (high-intensity spectrophotometer) of whole "Walter" tomatoes.

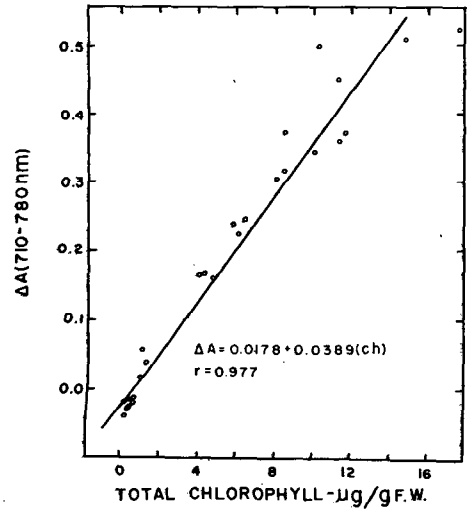

Fig. 5—Correlation between total chlorophyll content and ΔA (710–780 nm) (high-intensity spectrophotometer) of whole "Walter" tomatoes.

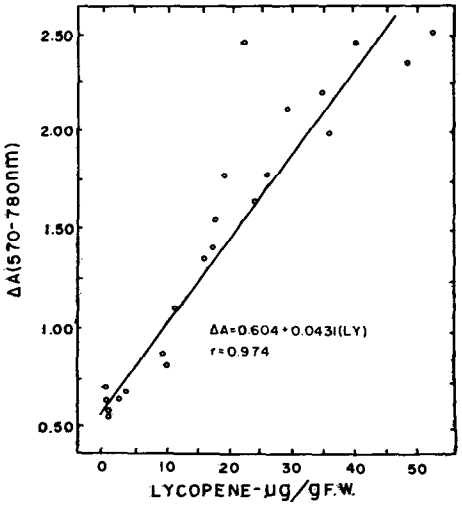

Fig. 6—Correlation between lycopene content and ΔA (570–780 nm) (high-intensity spectrophotometer) of whole "Walter" tomatoes.

The β-carotene content correlated significantly [r = 0.95 with ΔA (550–580 nm)] (Fig. 7). The linear regression equation was:

$$\Delta A = -0.433 + 0.297\ \beta\text{-carotene}$$

The deviation of the predicted from the actual value was large. Thus additional study is needed to reassess this relationship.

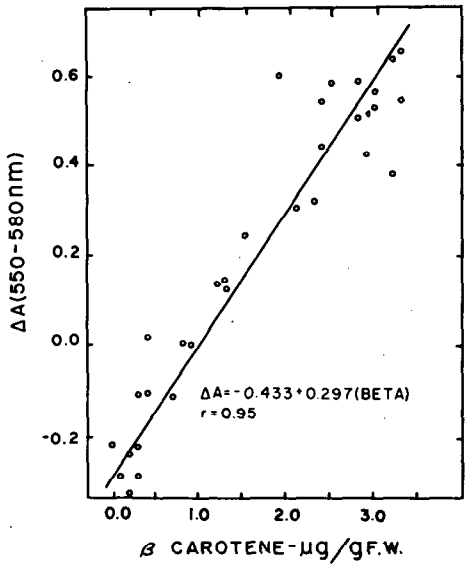

Fig. 7–Correlation between β-carotene content and ΔA (550–580 nm) (high-intensity spectrophotometer) of whole "Walter" tomatoes.

Table 2–L, a_L and b_L values (Hunterlab color difference meter) of "Walter" tomatoes categorized on the basis of absorbance (ΔA) measurements[a]

	ΔA (nm)		Hunterlab color difference meter readings[b]		
Stage	510–600	600–690	L	a_L	b_L
1	0.02	+0.18	55.58a	−10.89a	21.23c
2	0.20	−0.30	55.80a	−11.19a	22.04bc
3	0.61	−0.63	56.22a	− 9.78ab	22.94ab
4	1.08	−0.93	56.31a	− 7.57b	23.24a
5	1.52	−1.10	57.00a	− 3.64c	23.61a
6	1.56	−0.60	52.49b	9.28d	21.73c
7	1.02	0.22	47.31c	20.31e	19.49d
8	0.71	0.62	45.00c	24.90f	17.28e
9	0.32	1.10	41.34d	27.84g	15.87f
10	0.01	1.51	38.38e	30.27h	15.76f

[a] Average of three samples, each containing five tomatoes
[b] Values within a column not followed by a common letter are significantly different (P < 0.01) (Duncan, 1955).

Reflectance

The "L" value, which indicates lightness or darkness, increased as the green color disappeared, then decreased as the red color increased after stage 6 (Table 2). The "a_L," which indicates greenness or redness, approached zero from a negative value as the green color disappeared, then increased positive with red color formation. The "b_L" which indicates blueness or yellowness, increased up to stage 5 and then decreased.

Some of the light reflectance values correlated with the pigment content. The "L," "a_L" and tristimulus "Y" and "Z" correlated significantly with the chlorophyll content. The linear correlation coefficients were 0.69, −0.85, 0.70 and 0.76, respectively. All the measurements correlated significantly with the lycopene content. The coefficients of the linear correlation were −0.96, 0.92, −0.93, −0.72, −0.95 and −0.92, respectively, for L, a_L, b_L, X, Y and Z. Although the coefficients of L and Y approached the correlation between ΔA (570–780 nm) and lycopene (r = 0.97), the standard errors (SE) of L and Y (4.62 and 5.10, respectively) were larger than the SE of ΔA (3.76). The close correlation between carotenoid content and reflectance measurements of tomatoes have been reported by others (Hall, 1963; Koskitalo and Ormrod, 1972).

CONCLUSION

THE CHLOROPHYLL and lycopene contents in tomatoes could be estimated rapidly by nondestructive optical methods. The pigment contents in tomatoes of a limited range of maturities could be estimated when absorbance at wavelengths described by Worthington (1974) were used. A wider range of maturities was covered when ΔA (710–780 nm) and ΔA (570–780 nm), were used for chlorophyll and lycopene respectively. The light reflectance values could be used to estimate pigment content, but the correlation coefficients were lower and the standard errors were greater than those observed with the ΔA values.

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