J. Amer. Soc. Hort. Sci. 122(4):594–598. 1997.

Influence of Pigment Composition on Skin Color in a Wide Range of Fruit and Vegetables

Jane E. Lancaster[1] **and Carolyn E. Lister**[1]
New Zealand Institute for Crop and Food Research, Private Bag 4704, Christchurch, New Zealand

Peter F. Reay[1]
New Zealand Institute for Horticulture and Food Research, Private Bag 11030, Palmerston North, New Zealand

Christopher M. Triggs[2]
Department of Statistics, University of Auckland Private Bag 92019 Auckland, New Zealand

Additional index words. anthocyanins, carotenoids, chlorophyll, Hunter colorlab, *Malus domestica*, *Capsicum annuum*, *Brassica oleracea* var. Capitata, *Cucumis sativus*, *Vitis vinifera*, *Persea americana*, *Citrus limon*

Abstract. **The color of fruits and vegetables results from the presence of chlorophyll, carotenoid, and anthocyanin pigments. Instrumental measurements of color are used routinely in describing processes of changing color, such as fruit ripening. The applicability of using skin color measurements to predict changes in pigment composition was investigated using a wide range of fruit and vegetables. Skin color was measured using a Hunter Colorlab and represented as the coordinates X, Y, Z, L*, a*, b*, chroma (C*), and hue angle (h°). Identical skin samples were extracted and analyzed for chlorophyll, carotenoid, and anthocyanin concentration. Sets of pairwise scatter plots were generated for each set of color variables and for the chlorophyll, anthocyanin, and carotenoid pigments. There were linear relationships between h° and anthocyanin concentration and between L* and log [chlorophyll concentration]. Multiple regressions for each pigment variable and sets of color variables also were calculated. However, there was no unique linear combination of pigments that gave rise to a unique point in the color space. Conversely, a given set of coordinates in the color space can be accounted for by many combinations of pigments. Therefore, a given color measurement cannot be described in terms of a unique combination of pigments. Caution is urged in interpreting tristimulus color coordinates in terms of a simple change in pigment composition without prior knowledge of the pigment composition within the fruits and vegetables. The surface topography of fruits and vegetables may be of considerable significance in measuring color.**

Skin color of fruits and vegetables results from the pigments, chlorophyll, and carotenoids in the chloroplasts and chromoplasts and the phenolic pigments (anthocyanins, flavonols, and proanthocyanins) in the vacuole. The expression of pigment color is also influenced by physical factors, such as the presence of cuticular waxes, epidermal hairs, and the shape and orientation of cells in the epidermis and sub-epidermis. Pigments and surface topography selectively absorb and refract incident visible light to produce a reflectance spectrum characteristic of a particular fruit or vegetable skin.

To enable descriptions and comparisons of this reflective spectrum and, thus, color to be made, the spectrum is reduced to a numeric description of its red, green, and blue components as X, Y, Z tristimulus values. Between 1930 and 1976, ≈20 color scales were developed that converted X, Y, Z to visually meaningful attributes such as hue, saturation, depth, vividness, redness–greenness, and yellow–blueness (Hunter and Harold, 1987). The scale most used in measuring plant color was set by the Commission Internationale de L'Eclairage (CIELAB). This scale is based on measuring color in terms of a standard observer and standard illuminant. Any given color is located as a point in a three-dimensional space. The lightness coefficient L* ranges from black = 0 to white = 100, while the coordinates a* and b* locate the color on a rectangular-coordinate grid perpendicular to the L* axis. The color at the grid origin (a* = 0, b* = 0) is achromatic (gray). On the horizontal axis, positive a* indicates a hue of red–purple and negative a* of bluish–green. On the vertical axis, positive b* indicates yellow and negative b* blue.

Hue and chroma are measurements derived from a* and b* and correspond to the basic tint of a color (e.g., yellow, red) and the saturation or vividness of the color, respectively:

$$C^* = \sqrt{a^2 + b^2}$$

$$h^\circ = \tan^{-1}(b^*/a^*)$$

noting that, when a* < 0 and b* > 0, $h^\circ = 180^\circ + \tan^{-1}(b^*/a^*)$.

As C* increases, color becomes more intense. Thus, two fruit with a hue angle of 88 and a chroma of 34 and 56 would be described as dull orange–yellow and vivid orange–yellow, respectively. Because tristimulus color measurements are easy to obtain, they often are used as descriptors for changes in pigment composition in lieu of extracting and measuring pigments themselves. During a study of developmental changes of color in apple (*Malus* ×*domestica* Borkh.) skin, we found that tristimulus color measurements did not correlate well with pigment composition in a range of apple cultivars (Lister, 1994).

There are conflicting reports in the literature on the correlation between color measurements and pigment composition. In sweet potatoes *Ipomea batatas* L., Francis (1969) observed good correlations between pigment content and color, although correlation was poor for squash fruit (*Cucurbita maxima*). In kiwifruit (*Actinidia chinensis* Planch.), Lawes (1989) found no correlation between fruit color and chlorophyll and L*, a*, and b* measure-

Received for publication 26 Mar. 1996. Accepted for publication 20 Mar. 1997. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

[1]Research scientist.

[2]Senior lecturer.

ments. In dark-colored beverages, Eagerman et al. (1973) found that none of the color scales correlated well with pigment concentration. Singha et al. (1991) reported coefficients of determination for selected regression models relating measurements of color in apples to anthocyanin content. They found near-zero correlation between a* and anthocyanin levels ($R^2 = 0.10$).

Our study was undertaken to determine relationships between color measurements and pigment concentrations and to examine the usefulness of tristimulus color measurements as predictors of pigment composition so that these measurements can be used instead of tedious determinations of pigment composition. A wide range of fruits and vegetables, representing single and multiple combinations of anthocyanins, carotenoids, and chlorophyll, were used.

Materials and Methods

Plant material. The following fruits and vegetables were used: apple, avocado (*Persea americana* Mill), grape (*Vitis vinifera* L.), red cabbage (*Brassica oleracea* var. Capitata forma ruba L.), lime (*Citrus limon* L.), cucumber (*Cucumus sativus* L.), and pepper (*Capiscum annuum* L.).

Color measurements. Zones on each fruit (3 × 3 cm) representing a range of colors were labeled with a felt pen so that color measurements were made on the same area of skin used for pigment extraction. Color measurement were made using the head 15 mm in diameter of the Hunter Colorlab and were expressed in CIELAB units of X, Y, Z, L*, a*, and b*. Chroma (C*) and hue angle (h°) were computed from L*, a*, and b*. The Hunter Colorlab was calibrated using the manufacturers' standard white tile.

Analysis of pigment composition. Skin was cut from the fruit, and any underlying cortical cells were scraped off. Skin was weighed and extracted for analysis of anthocyanins, chlorophylls, and carotenoids. All pigment concentrations were expressed on a fresh-mass basis.

Chlorophyll extraction and estimation. The methods used for determining chlorophyll and total carotenoid content were essentially the same as those used by Knee (1972). Skin (0.5 to 1.0 g) was ground to a fine powder using liquid N_2 and was extracted with 15 mL cold acetone. The residue was re-extracted with 5-mL aliquots of 80% acetone until clear. The combined extracts were adjusted to 30 mL with 80% acetone and centrifuged at 5000 g_n for 10 min. Absorbance was measured at 645, 652, and 663 nm and a reading also was taken at 700 nm to correct for any turbidity. Chlorophyll content was calculated from the data using the equations of Maclachlan and Zalik (Holden, 1965).

Total carotenoid estimation. A 10-mL aliquot of the acetone extract from the previously mentioned procedure was taken, 10 mL of petroleum ether (40 to 60 °C) and 3 mL 50% saturated aqueous ammonium sulfate was added, and the mixture was stirred for 15 min. The upper phase was washed twice with 3 mL of ammonium sulfate, 1 mL of 25% (w/v) potassium hydroxide in methanol was added, and the mixture was stirred for 15 min. The upper phase was washed with 5 mL water until clear. The petroleum ether solution then was dried over sodium sulfate for 1 h before the absorbency was measured at 446 nm. A reading was also taken at 550 nm to correct for any turbidity (Goodwin, 1955). Total carotenoid content was calculated, assuming that extinction of 1% solution in a 1 cm light path optical cell ($E^{1\%}$ cm) is 2500.

Anthocyanin extraction and estimation. Skin (0.5 to 1.0 g) was ground to a fine powder using liquid N_2 and was extracted with 10 mL 15.0% (v/v) acetic acid in methanol. The residue was re-extracted at least twice to remove all of the pigments. The combined extracts were centrifuged at 5000 g_n for 10 min, and absorbency was measured at 530 nm. A reading was also taken at 600 nm to correct for interference. Total anthocyanin content was calculated as described by Siegelman and Hendricks (1958).

Statistical analysis. Sets of pairwise scatter plots were calculated for each of the pigments and the color variables (Figs. 1 and 2). Multiple regression relationships were calculated between pigment variables and the sets of color variables.

Results

Pigment content of fruit and vegetable skin. Fruits and vegetables were selected to represent a wide range of skin color as judged and described by a human observer, within a species and between species (Table 1). Levels of chlorophyll, anthocyanin, and carotenoid were analyzed in the skin of each fruit and vegetable. Chlorophyll was detectable in almost all the samples apart from light-purple red cabbage and red and yellow peppers. Chlorophyll content was highest in purple avocado, green cucumber, and black grape skin. Carotenoids were present in all skins, there being a 600-fold variation in levels between yellow apple skin (0.0003 mg·g^{-1}) and red pepper skin (0.187 mg·g^{-1}). Anthocyanins were present in the skin of apple, avocado, grape, and red cabbage but were absent from the skin of cucumber, lime, and pepper. Black grapes had the highest concentration of anthocyanins (3.120 mg·g^{-1}), followed by red cabbage skin (1.6 to 1.9 mg·g^{-1}).

Measurements of fruit and vegetable skin color. Skin color as measured by the Hunter Colorlab was represented in three scales: X, Y, Z; L*, a*, and b*; and C* and h° (Table 1). Lightness (L*) ranged from 73.7 (yellow limes) to a darker 21.8 (purple cabbage); a* values ranged from 39.3 for red apples to –11.4 for green peppers. All b* values were positive with the exception of black grapes and purple cabbage. Highest b* values were yellow cucumber skin and yellow pepper skin. In red cabbage, which had high anthocyanin concentration, no chlorophyll and negligible carotenoids, the a* values were lower than tissues containing anthocyanin concentration a tenth of that in cabbage.

Tissues in which a particular pigment predominates tended to have a particular color and fall within a range of hue values. Yellow- and red-skinned fruits and vegetables had hue angle values that were in the 0° to 90° quadrant of the color sphere. The green-skinned material (e.g., green apple, cucumber, avocado, green grapes, lime, and pepper) had hue angle values in the second quadrant, while black grapes and red cabbage leaves had values in the fourth quadrant. Values in the third quadrant (blue–green) are not found for fruit and vegetable skins.

Correlations between pigment composition and color. The relationships between pigments and color scales were investigated (Fig. 1). The pigment and color scale values from Table 1 were used to generate scatter plots for pairs of variables. Thus, the top right panel is a scatter plot of anthocyanin concentration (0.0 to 3.0 mg·g^{-1}) vs. h° (0° to 100°), while the bottom left panel is a plot of X vs. chlorophyll. There was no linear relationship between any of the pigments and any of the color scales. However, for tissues containing anthocyanin, there was a linear relationship between anthocyanin concentration and h° (Fig. 2a).

Scatter plots of the logs of the three pigments and the color variables were generated. A linear relationship between log (chlorophyll) and L* was apparent (Fig. 2b); doubling chlorophyll reduced L* by 0.04 units ($P < 0.001$). Similar relationships were not observed for the other pigments and color variables.

Multiple regressions of the pigments and color scales were

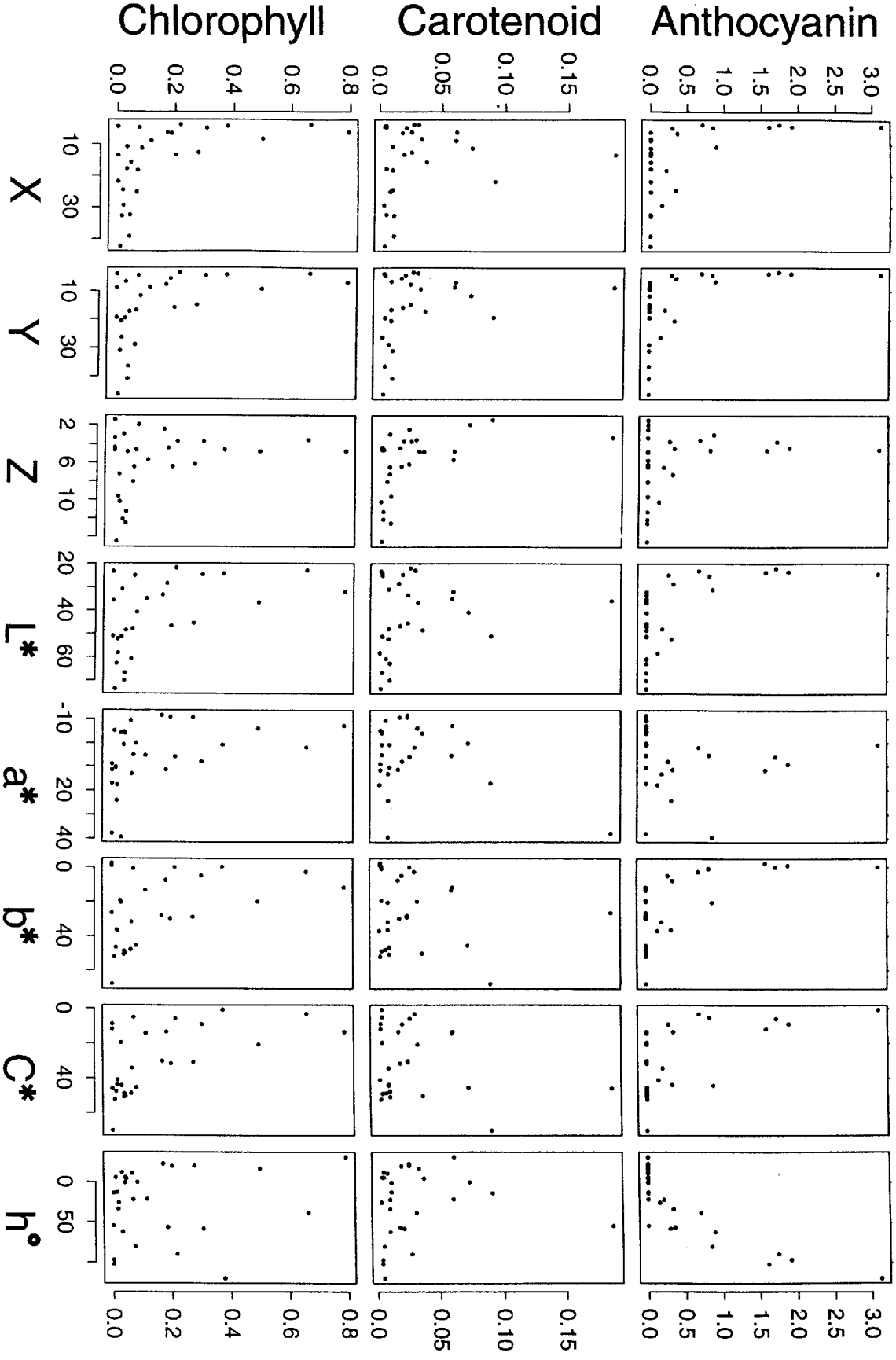

Fig 1. Relationships between pigments and color scales using scatter plots of pairs of variables. Units are mg·g^{-1} on a fresh mass basis for anthocyanin, carotenoids, and chlorophyll. All values used are from Table 1. The top right panel represents anthocyanin (0.0 to 3.0 mg·g^{-1}) vs. h° (0° to 100°). The bottom left panel represents X vs. chlorophyll.

computed (data not shown), but the regression coefficients were not easily interpretable and were different when calculated for the full data sets and separately for the fruit-based subsets of data.

In an additional analysis, each pigment concentration was multiply-regressed onto each set of color variables. Each multiple regression equation then was used to predict the pigment value for a given L*, C*, and h° value. For the complete data, the SEs for the predicted values gave wide confidence intervals. Thus, there is no unique linear combination of pigments that gives rise to a unique point in the color space. Equally, a given set of coordinates on the color space can be accounted for by many combinations of pigments.

Discussion

Tristimulus measurements of color are widely used in plant science to specify the color of a tissue and to identify tissues with the same color. Because pigments give rise to color, the reverse logic—that color can specify pigments—has been used as a basis for understanding pigment changes in biological processes such as ripening. This idea is appealing because measurements of pigments, per se, are tedious and destructive, and rapid, nondestructive measurements are preferable.

In plant material where two or more pigments are present, close examination of published data shows that there has not been good agreement between color measurements and pigment content. For ripening peaches [*Prunus persica* (L.) Batsch.], major changes in color were reflected by an increase in a* values but no change in b* values (Byrne et al., 1991; Delwiche and Baumgardner, 1985). However, the increased a* value was brought about by two pigment changes—a decrease in chlorophyll and an increase in anthocyanin. Similarly, for the surface of watermelons [*Citrullus lanatus* (Thumb. Matsum. & Nakai)], an increase in a* values during ripening is brought about by a loss of chlorophyll. The carotenoid levels and, thus, the b* value, is constant, but the fruit appear more orange because the chlorophyll loss unmasks the carotenoids (Corey and Schlimme, 1988). In papaya (*Carica papaya* L.) in which ripening is characterized by the fruit turning yellow and an increase in b* values, small green flecks considerably reduced the a* values (Peleg and Gomez Brito, 1975). In petals from tulip (*Tulipa gesnerana* L.) and chrysanthemum [*Dendranthema grandiflorum* (Ramet) Kitamura], carotenoids in the presence of anthocyanins modify the appearance to red–orange or bronze (Nieuhwhof et al., 1989; Teynor et al., 1989). High chlorophyll levels, in particular, modify the color measurement of fruits and vegetables with high anthocyanins. Apple genotypes, such as 'Oregon Red Delicious', which have high anthocyanin levels and, therefore, would be expected to have a higher a* value, also have high chlorophyll levels, which reduces the a* value (Lancaster et al., 1994). For eggplant (*Solanum melongena* L.) the darkest purple fruit had high levels of chlorophyll and anthocyanins (Nothmann et al., 1976).

In these results, similar L*, a*, b*, and C* and h° values are given by tissues with different combinations of pigments. Thus, each point on the three-dimensional color space does not correspond to a unique set of pigment concentrations per se but can be arrived at by many different quantitative combinations of pigments.

In simple systems, where one pigment predominates or where only one pigment is present, linear relationships between pigment and color may be significant. There has been good agreement between color measurements and red pigment content in tomato [*Lycopersicon esculentum* var. *cerasiforme* (Duval) A. Gray] and

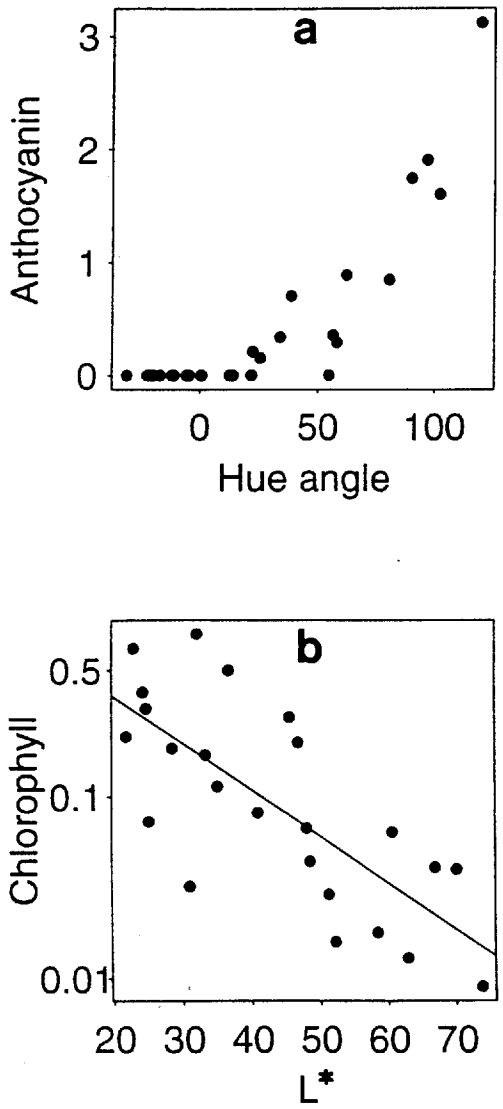

Fig 2. (**a**) Relationships between anthocyanin concentration (fresh mass basis, in $mg \cdot g^{-1}$) and h° and (**b**) log [chlorophyll concentration on a fresh mass basis in $mg \cdot g^{-1}$) and L*.

cranberry (*Vaccinium macro-carpon* Ait.) (Francis and Clydesdale, 1970; Larrigaudiere et al., 1991). For tomato, the a* value is sufficient to characterize maturity stages. We found a significant linear relationship between anthocyanin content (in tissues containing anthocyanin) and h°. The relationship was significant over a wide range of anthocyanin concentrations (0.15 to 3.120 $mg \cdot g^{-1}$). A relationship between anthocyanin and a* value was not evident. A significant linear relationship was detected between log (chlorophyll) and L*, indicating a logarithmic relationship between increasing chlorophyll and darkness of the material. No other significant linear relationships between color measurements and pigment concentrations were detected.

Our results indicate the need for caution in interpreting tristimulus color coordinates in terms of simple changes in pigment composition. A priori predictions of pigment composition are not valid because more than one combination of pigments can give rise to a given set of tristimulus coordinates. In simpler pigment systems, some predictive relationships do exist; however, knowledge of the pigments within the material are necessary to determine the extent of the relationship. Knee (1980) observed that evidence is lacking on the extent to which differences in pigment composition were

Table 1. Color measurements and pigment composition (on a fresh mass basis) of skin of fruits and vegetables.

Fruit–vegetable	Skin appearance	Anthocyanins ($mg \cdot g^{-1}$)	Carotenoids ($mg \cdot g^{-1}$)	Chlorophyll ($mg \cdot g^{-1}$)	X	Y	Z	L*	a*	b*	C*	h°
Apple	Yellow	0.156	0.003	0.018	29.4	26.4	10.2	58.4	17.6	37.1	41.2	25.4
	Red	0.892	0.010	0.032	10.7	6.6	3.0	30.9	39.3	20.6	44.4	62.3
	Green	0.000	0.008	0.064	25.2	28.9	8.1	60.7	–9.2	48.0	48.8	–10.8
	Bronze	0.214	0.010	0.067	18.3	16.8	6.5	48.0	13.1	31.8	34.4	22.3
	Yellow	0.000	0.011	0.013	32.6	31.0	9.6	62.9	10.4	46.6	47.8	12.6
	Red	0.339	0.010	0.016	24.6	20.5	7.2	52.2	24.2	36.5	44.0	33.6
Avocado	Green	0.000	0.033	0.498	8.2	9.3	4.8	36.6	–6.0	20.0	20.8	–16.7
	Red	0.358	0.018	0.184	6.3	5.6	4.4	28.4	11.4	7.5	13.6	56.6
	Purple	0.706	0.031	0.664	3.7	3.8	3.6	22.8	2.1	2.6	3.4	38.5
	Red	0.296	0.021	0.306	4.7	4.3	3.7	24.7	7.9	4.9	9.3	58.3
Grape	Green	0.000	0.005	0.029	17.7	19.5	12.1	51.3	–4.0	19.4	19.9	–11.7
	Red	0.848	0.005	0.073	4.6	4.5	4.6	25.1	5.2	0.9	5.3	80.7
	Black	3.120	0.005	0.378	4.1	4.2	4.6	24.2	0.8	–0.5	1.0	119.6
Red cabbage	Dark purple	1.742	0.027	0.215	3.7	3.5	3.7	21.8	6.0	–0.0	6.0	90.1
	Medium purple	1.907	0.004	0.000	4.3	3.9	4.4	23.3	8.9	–1.1	9.0	97.0
	Lighter purple	1.605	0.004	0.000	4.5	3.9	4.7	23.3	11.6	–2.5	11.9	102.1
Cucumber	Yellow	0.000	0.011	0.040	39.0	40.9	12.5	70.1	0.9	50.9	50.9	1.0
	Green	0.000	0.061	0.792	6.0	7.0	4.8	32.0	–7.0	11.7	13.6	–30.7
Lime	Yellow	0.000	0.005	0.041	32.3	36.6	11.2	66.9	–4.4	48.8	49.1	–5.2
	Green	0.000	0.025	0.276	12.5	14.9	6.2	45.5	–10.5	29.0	30.8	–20.0
	Yellow	0.000	0.004	0.009	42.3	46.3	14.5	73.7	–5.0	52.1	52.3	–5.4
	Green	0.000	0.019	0.199	13.3	15.8	6.4	46.7	–10.5	30.0	31.7	–19.3
Pepper	Yellow	0.000	0.091	0.000	21.8	19.4	1.4	51.1	17.0	68.2	70.4	14.0
	Green/yellow	0.000	0.073	0.082	11.2	11.8	2.0	40.8	0.4	45.5	45.5	0.4
	Green	0.000	0.025	0.170	6.1	7.7	2.5	33.2	–11.4	28.1	30.4	–22.1
	Red/green	0.000	0.060	0.114	8.8	8.6	5.7	35.0	5.4	13.5	14.5	21.7
	Red/yellow/green	0.000	0.037	0.044	15.6	17.2	4.9	48.5	–3.8	50.1	50.3	–4.3
	Red	0.000	0.187	0.000	13.4	8.9	3.3	35.7	37.7	26.6	46.1	54.8

apparent as color differences to the eye. Despite the numerous measurements of color and pigments, this conclusion remains the case.

In this paper, we did not include the optical significance of surface topography in modifying color measurement. The presence of cuticular waxes, epidermal hairs, and the orientation of cells in the epidermis and subepidermis may be of considerable significance to the measurement of color.

Literature Cited

Byrne, D.H., A.N. Nikolic, and E.E. Burns. 1991. Variability in sugars, acids, firmness, and color characteristics of 12 peach genotypes. J. Amer. Soc. Hort. Soc. 116:1004–1006.

Cory, K.A. and D.V. Schlimme. 1988. Relationship of rind gloss and groups of color to flesh quality of watermelon fruits during maturation. Scientia Hort. 34:211–218.

Delwiche, M.J. and R.A. Baumgardner. 1985. Ground color measurements of peach. J. Amer. Soc. Hort. Sci. 108:1012–1016.

Eagerman, B.A., F.M. Clydesdale, and F.J. Francis. 1973. Comparison of color scales for dark colored beverages. J. Food Sci. 38:1051–1055.

Francis, F.J. 1969. Pigment composition and color in fruits and vegetables. Food Technol. 23:32–36.

Francis, F.J. and F.M. Clydesdale. 1970. Cranberry products. Food Prod. Dev. 4:54, 56, 60–62, 83, 86.

Goodwin, T.W. 1955. Carotenoids, p. 272–311. In: K.Peach and M.V. Tracey (eds.). Modern methods in plant analyses. vol. 3. Springer-Verlag, Heidelberg.

Holden, M. 1965. Chlorophylls, p. 461–488. In: T.W. Goodwin (ed). Chemistry and biochemistry of plant pigments. Academic, London.

Hunter, R.S. and R.W. Harold. 1987. The measurement of appearance. Wiley Interscience, New York.

Knee, M. 1980. Methods of measuring green color and chlorophyll content of apple fruit. J. Food Tech. 15:493–500.

Knee, M. 1972. Anthocyanin, carotenoid, and chlorophyll changes in the peel of Cox's Orange Pippin apples during ripening on and off the tree. J. Expt. Bot. 23:184–196.

Lancaster, J.E., J.E. Grant, C.E. Lister, and M.C. Taylor. 1994. Skin color in apples—Influence of copigmentation and plastid pigments on shade and darkness of red color in five genotypes. J. Amer. Soc. Hort. Sci. 119:63–69.

Larrigaudière, C.A., J.C. Peach, and C. Triantaphylides. 1991. Relationship between stress ethylene production induced by gamma irradiation and ripening of cherry tomatoes. J. Amer. Soc. Hort. Sci. 116:1000–1003.

Lawes, G.S. 1989. The effect of shading on the chlorophyll content of "Hayward" kiwifruit. N.Z. J. Crop and Hort. Sci. 17:245–249.

Lister, C.E. 1994. Biochemistry of fruit colour in apples (*Malus pumila* mill.) PhD Thesis, Univ. of Canterbury, Christchurch, New Zealand

Nieuwhof, M., J.P. Van Eiijk, and W. Eikelboom. 1989. Relation between flower color and pigment composition of tulip (*Tulipa* L.). Netherlands J. Agr. Sci. 37:365–370.

Nothmann, J., I. Rylski, and M. Spigelman. 1976. Color and variations in color intensity of fruit of eggplant cultivars. Scientia Hort. 4:191–197.

Peleg, M. and L. Gomez Brito. 1975. The red component of the external color as a maturity index of Papaya fruits. J. Food Sci. 40:1105–1106.

Siegelman, H.W. and S.B. Hendricks. 1958. Photocontrol of anthocyanin synthesis in apple skin. Plant Physiol. 33:185–190.

Singha, S., T.A. Baugher, E.C. Townsend, and M.C. D'Souza. 1991. Anthocyanin distribution in 'Delicious' apples and the relationship between anthocyanin concentration and chromaticity values. J. Amer. Soc. Hort. Sci. 116:497–499.

Teynor, T.M., P.D. Ascher, R.D. Widmer, and J.J. Luby. 1989. Inheritance of flower color in *Dendranthema grandiflora* Tzvelev. (*Chrysanthemum morifolium* Ramat) using cultivars in inbreds. I. Plastid pigmentation. Euphytica 42:199–207.