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Color Development in 'Golden Delicious' Apples[1]

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Abstract. **The concentrations of chlorophylls, carotenoids, and flavonoids were measured in 'Golden Delicious' apple (*Malus domestica* Borkh.) skin during maturation, ripening, and storage. Chlorophyll was found to be the most important color determinant. Yellow coloration was not visible unless chlorophyll concentration was less than 0.15-0.20 μg/cm^2 of skin. The minimum amount of carotenoids necessary for yellow color was found to be 0.3-0.4 μg/cm^2 of skin. Flavonoids play a secondary role in yellow coloration. A decrease in total carotenoids occurred in September, followed by an increase to the original level. The flavonoid concentration was relatively constant during ripening.**

The color of apples is an important selling-price determinant on the American market. The preferred colors are red or yellow, with green fruits selling poorly. Development of attractive yellow coloration is a problem with 'Golden Delicious' and 'Grimes Golden' in some areas (15) as these cultivars are too green or "white" in some seasons.

Fruit color depends upon the amount of pigments in the skin and the type of illumination. The pigments responsible for 'Golden Delicious' skin color are chlorophyll (green) as well as carotenoids and flavonoids (yellow). During ripening chlorophyll disappears, making the yellow pigments visible (9, 11, 15).

There is disagreement in the literature on the changes in and importance of the various yellow pigments. Several workers have reported increases in total carotenoids, but they disagree as to whether the carotene fraction (5) or the xanthophyll fraction (9, 15) is more important. It was reported that others found the carotenoid content of apples relatively constant during ripening (9). Flavonoids may also play a role in the development of yellow colors (12, 13). We had observed a high correlation between the level of flavonoids in apple skin and yellow color. However, Workman (15) has suggested that flavonoids play a secondary role.

The objectives of this study were to determine changes in the relative and absolute levels of the various pigments during the ripening process and storage, and the roles of the individual pigments in color development.

Materials and Methods

Source of fruit. Fruit was obtained from 10-year-old 'Golden Delicious' trees on seedling rootstocks in the Cornell University Orchard at Ithaca, NY. About 200 fruits were covered with paper bags immediately after petal fall in late May. An additional 200 fruits were covered with black polyethylene bags on August 4, 1975. All of these fruits were harvested on Oct. 15. At this time, control apples were harvested from the same trees and divided visually into 2 groups, yellow and green. Fruits from each group were stored in polyethylene bags at 4^{o}C.

Pigment analysis. Ten randomly selected fruits from each treatment were analyzed for chlorophyll, flavans, flavonols, total carotenoids, and individual carotenoids. Fifteen discs, 1 cm in diam, were cut from random points on the skin of each apple. Chlorophyll (8), flavonol (2), and flavan (3) in methanol extracts from these discs were determined. Carotenoids were determined using a modification of the AOAC method (1). The discs were homogenized in 30 ml of 10 hexane:7 acetone:6 absolute alcohol:7 toluene (v/v) in a VirTis 45, and left in the dark for 1 hr. The extract was then filtered through glass wool into a 50 ml volumetric flask. The residue was washed three times with hexane. Two ml of 40% methanolic KOH was pipetted into the flask, which was then swirled for 1 min, and placed in the dark for 15 min. The flask was filled to 50 ml with hexane; the contents were transferred to a 100 ml volumetric flask and 10 ml of hexane was added. The flask was diluted to volume with 10% Na_2SO_4 and shaken for 1 min. After 1 hr, the absorbance of the organic phase was measured at 445 nm on a Beckman DK-2A spectrophotometer. The total carotenoid content was calculated from the extinction coefficient of Goodwin (7).

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Separation and estimation of individual carotenoids. Ten ml of the organic phase (upper layer) from the analysis for total carotenoids was washed with a rapid drip of distilled water to remove acetone and alcohol. The individual carotenoids were separated on a magnesium oxide plus Celite 545 column and measured according to the methods of Friend and Nakayama (6). The individual carotenoids were identified by comparing their spectra in hexane and ethanol with those given in the literature (4), and by R_f using TLC (9).

Color measurement. Color was measured using the "Techwest" Apple Color Meter Model GD3. Table 17 from the *Dictionary of Color* (10) was used for optical color differentiation tests.

Results and Discussion

Results of testing the ability of the Apple Color Meter to differentiate known color chips revealed that this instrument only registers differences in the amount of green pigment. The color index (CI) determined was very well correlated with the chlorophyll content of apple skin (Fig. 1). This instrument reads on an arbitrary scale of 2 to 8: 2-4 is "green," 4-5 is "turning," 5-6 is "yellow," 6-7 is "fancy," and 7-8 is "extra fancy," but in fact these descriptions apply only if yellow pigments happen to be present.

Tests were conducted to study the preception by the human eye of the colors yellow, green and combinations of yellow and green in warm white fluorescent light. Over 30 human subjects were presented with 2 color chips (10), one dense green pigment, and one the same amount of pigment plus an equal quantity of yellow pigment. When asked to indicate the greener of the two, 96% of the subjects selected the second square. The subjects were then presented with a range of 13 squares, each with the same high density of yellow pigment but with added green pigment varying from none to an equal amount. Subjects were asked which square was yellow enough to call yellow. Green plus yellow appeared greener to the human eye than pure green. The human eye is very sensitive to the presence of green color: 90% of the subjects rejected as yellow those mixtures of yellow and green containing more than 9% green pigment.

Contribution of individual pigments to fruit color. Apples which were covered with black bags in August had low levels of both chlorophyll and flavonoids when sampled October 13 (Table 1). The bags were then removed and the fruits were left on the trees for two days. The flavonoid content increased rapidly, while the levels of chlorophyll and carotenoids did not change appreciably (Table 1). The increase in flavonoid content caused no changes in the color of these apples as measured by CI or by visual appearance. Thus the role of flavonoids in color development in apple skin is secondary.

During storage of detached "green" fruits the color changed from green to yellow. Although carotenoids did not increase, chlorophyll levels fell continuously (Fig. 2). These data show that the carotenoid content of these apples was high enough to provide a yellow color, but this yellow was masked at harvest by the presence of chlorophyll.

On Nov. 4, apples covered with paper bags throughout their development (harvested Oct. 15) had very low levels of both chlorophyll and carotenoids; they were typical examples of colorless or "white" fruit (Table 2). After several months of storage in air at 4°C these apples were yellow in color. The constant flavonoid level confirms the secondary role of these pigments in yellow color development, while the increase in carotenoids levels and yellowness shows the correlation of carotenoids with color.

Quantitative and qualitative changes in pigments. Analysis of pigments from control fruits (harvested Oct. 15) showed the presence of the following carotenoids: β-carotene, violaxanthin,

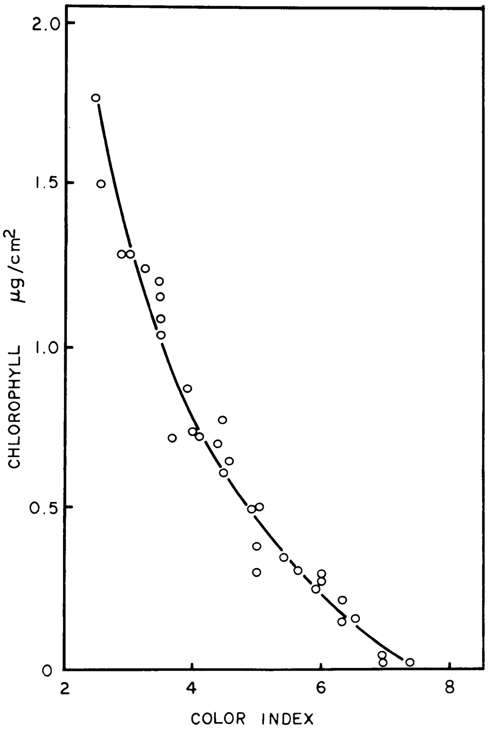

Fig. 1. Color index from "Techwest" Apple Color Meter versus chlorophyll content of 'Golden Delicious' apple skin. Each point is the mean value for 10 fruits. Data fitted to hyperbolic curve (correlation coefficient, 0.982).

Table 1. Color index, and pigment and flavonoid concn of 'Golden Delicious' apple skin following removal (Oct. 13) of black polyethylene bags from attached fruit.

Date	Color index	Concn (µg/cm² skin)			
		Chlorophyll	Carotenoids	Flavonols	Flavans
Oct. 13	6.5	0.15	0.64	27	68
Oct. 15	6.5	0.14	0.63	51	92

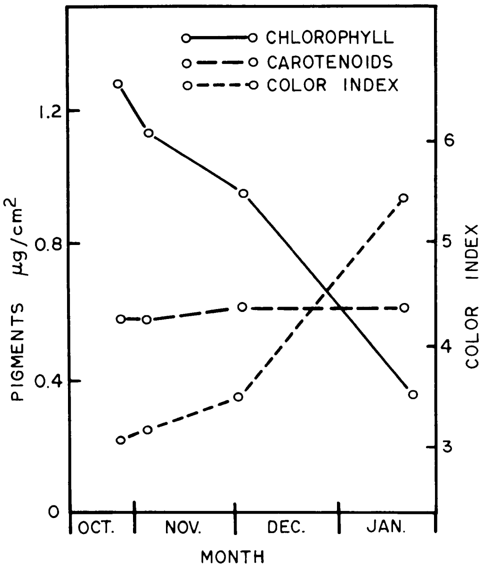

Fig. 2. Chlorophyll and carotenoid content of 'Golden Delicious' apple skin and color index of "green" fruit during storage. Fruits harvested on Oct. 15 and stored at 4°C.

Table 3. Changes in individual carotenoids and flavonoids in 'Golden Delicious' apple skin (control fruits) during maturation, ripening, and storage. Harvested Oct. 15, stored at 4°C.

	Concn (μg/cm^2)							
Date	β-carotene	xanthophyll dihydroxy esters	xanthophyll monohydroxy esters	violaxanthin	lutein	neoxanthin	flavonols	flavans
July 25	–	–	–	–	–	–	67	110
Aug. 14	–	–	–	–	–	–	25	110
Aug. 18	.10	.09	.02	.18	.17	.07	–	–
Aug. 28	–	–	–	–	–	–	24	63
Sept. 8	.10	.07	.04	.11	.10	.07	–	–
Sept. 12	–	–	–	–	–	–	25	63
Sept. 23	.09	.05	.06	.09	.07	.05	27	64
Oct. 6	.08	.12	.08	.07	.06	.05	32	64
Oct. 13	.09	.18	.11	.06	.05	.04	–	–
Oct. 20	.09	.20	.14	.07	.05	.04	39	74
Oct. 27	.08	.23	.16	.06	.05	.04	47	74
Dec. 2	.07	.25	.18	.08	.06	.04	70	80

Table 2. Changes in pigment content and color in 'Golden Delicious' apples during storage. Attached fruits covered with paper bags in late May, harvested on Oct. 15, and stored at 4°C. Apples were "white" when sampled on Nov. 4, and yellow on Jan. 21, 1976.

Date	Pigment content of apple skin (μg/cm^2)				Apple
analyzed	Chlorophyll	Carotenoids	Flavonols	Flavans	color
Nov. 4, 1975	.18	.20	5	55	"white"
Jan. 21, 1976	.05	.40	5	55	yellow

lutein, neoxanthin, and two further pigments which, on the basis of spectral and TLC analysis, appear to be identical with the xanthophyll di-esters and xanthophyll mono-esters identified in 'Cox's Orange Pippin' (8). No auroxanthin was found, although Valadon and Mummery (14) considered this to be a major pigment.

A decrease in total carotenoids and chlorophyll occurred in Sept., followed by an increase in carotenoids to the original level (Fig. 3). The quantitative changes in total carotenoids are caused by the disappearance of carotenoids typical of actively photosynthesizing tissues (β-carotene, lutein, violaxanthin, neoxanthin), and the subsequent synthesis of mono- and dihydroxy xanthophylls (Table 3). The same changes occur, but more rapidly, in black polyethylene bags.

The flavonol and flavan concentration was relatively constant during ripening. An increase in both these pigments was observed in storage (Table 3).

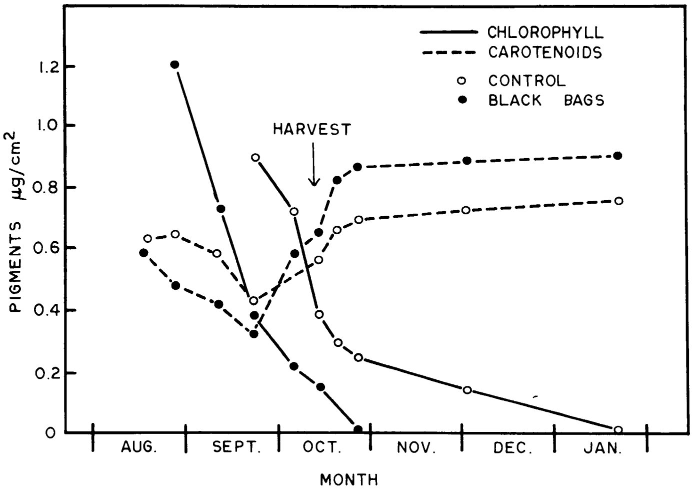

Fig. 3. Chlorophyll and carotenoid content of 'Golden Delicious' apple skin during maturation, ripening and storage. Attached fruits covered with black polyethylene bags in August, and control apples. Both harvested Oct. 15 and stored at 4°C.

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