Seasonal Changes of Carotenoid Pigments and Color in Hamlin, Earlygold, and Budd Blood Orange Juices

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The developmental patterns of carotenoids in Hamlin, Earlygold (an early-maturing selection), and Budd Blood sweet orange juices were studied during the September to mid-January period of the 1996−97 and 1997−98 seasons. The carotenoid concentration of Earlygold increased by as much as 4.9 times during the color development compared to 3.9 times in Hamlin and 4.5 times in Budd Blood juice in the same period. For the profiles of carotenoid pigment, dramatic changes occurred among the pigments that were present in high concentrations at the beginning of the season, with lutein and violaxanthin noted as the predominant pigments in Hamlin fruit. A marked increase in the percentage of β-cryptoxanthin allowed it to become a major pigment in the late stage of maturation. The color development in the new cultivar Earlygold was especially notable, reaching the 36 color number, which is grade A, by late October to mid-November whereas Hamlin juice did not reach this grade A color number until January. Budd Blood juice was similar in carotenoid pigment content and seasonal changes to Hamlin juice, but also, the development of red anthocyanin pigment in January significantly increased juice color.

Keywords: *Carotenoids; Hamlin; Earlygold; Budd Blood; color number; ripening; citrus*

INTRODUCTION

In the citrus juice industry, Hamlin (*C. sinensis*) is the principal early-season (November−January) sweet orange in Florida. This cultivar has the advantage of superior yield capacity, but its juice color, flavor, and overall quality are relatively poor. For these reasons, juice from Hamlin is blended with high-colored, better quality juice from other sources to improve the color score and flavor. Overcoming the Hamlin color limitation would substantially aid the pasteurized orange juice segment of the Florida citrus juice industry.

Because color is one of the most important and complex attributes of orange products and is largely due to the presence of diverse carotenoid pigments, considerable attention has been directed toward characterization of carotenoid pigments (*1*−*6*) and their relationship to color development (*7* and *8*). Understanding color development and color components can be a benefit in the identification of sweet orange cultivars and deliberate efforts to manipulate them for enhanced juice color.

A broad range of sweet orange selections has been under evaluation in a commercial grove for about 10 years (*9*). The study is part of a cooperative scion cultivar trial to identify sweet orange selections with juice quality superior to Hamlin, specifically emphasizing flavor and color. One selection, Earlygold, is particularly promising in that it matures earlier than Hamlin and its juice achieves a satisfactory color in November for stand-alone use in pasteurized products. Budd Blood was noteworthy for its excellent orange flesh color and the consistent appearance of red coloration. The fruit did not develop the deep red coloration of blood oranges grown in cool climates, but the peel was usually the typical yellow to orange color of sweet oranges. Therefore, our objective in this study was to characterize and compare the seasonal changes in juice carotenoids and color of these three cultivars.

MATERIALS AND METHODS

Fruit Sampling. The field trial was located in St. Cloud, Florida and consisted of pairs of trees replicated 4 times, all on Swingle citrumelo (C. paradisi Macf. x Poncirus trifoliati (L.) Raf.) rootstock. The trees were planted in 1989 in east−west rows. The Hamlin trees were normal nursery trees, i.e., propagated using a mature line source of buds. The Earlygold and Budd Blood trees were propagated using young or juvenile sources of buds. Samples of 10 fruit each were collected about every 2 to 4 weeks from 3 replications of each selection during the periods from September 1996 to January 1997 and from August 1997 to January 1998. Each sample was collected to represent all canopy positions.

Juice Preparation. Juice was extracted with a household-type electric hand reamer (Waring, New Hartford, CT) from all fruit in each sample, filtered through cheesecloth, pasteurized (90 °C for 30 s), cooled, and then kept frozen (−20 °C) until analyzed.

Juice Color Analysis. Juice color development (CIE L*a*b*) was measured on duplicate juice samples in test tubes (25 mm × 20 cm o.d.). The CIE L*, a*, and b* values were measured with a Macbeth Color-EYE 3100 spectrophotometer (Kollmorgan Instruments Corp., Newburgh, NY) with Optiview software package in the reflectance mode, with illuminant C and 2° observer angle. Orange juice color number (CN) was measured using a Hunter Model D-45 Citrus Colorimeter (Hunter Associates Laboratories, Inc., Reston, VA).

Carotenoid Extraction. An aliquot of orange juice (25 mL) was homogenized (30 s at speed 4) in an Omni mixer homogenizer (Warrenton, VA) with 50 mL of extracting solvent

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(hexane/acetone/ethanol, 50:25:25, v/v) and centrifuged for 5 min at 6500 rpm at 5 °C (IEC, Needham, MA). The top layer of hexane containing the color was recovered and transferred to a 25-mL volumetric flask. The volume of recovered hexane was then adjusted to 25 mL with hexane.

Saponification. Saponification was carried out according to Noga and Lenz (*2*) with modification. The hexane extract was concentrated to dryness utilizing a rotary evaporator, redissolved with 2 mL of methyl *tert*-butyl ether (MTBE), and placed in a 15-mL culture tube to which was added 2 mL of 10% methanolic KOH. The sample was shaken and wrapped with aluminum foil to protect it from light. Prior to capping, the tube was gently blanketed with nitrogen, then closed, and placed in the dark overnight at room temperature. Sample was removed from the dark and transferred to a separatory funnel to which was added 5 mL of water and 2 mL of 0.1% BHT/MTBE, and the aqueous layer was removed. Three additional water rinses were carried out, draining the aqueous layer after each rinse. The MTBE layer was then filtered through an Isolute sodium sulfate drying cartridge (Intl. Sorbent Technol. Ltd., UK), transferred into a 15-mL centrifuge tube, concentrated by evaporation with nitrogen, and the volume was adjusted with 0.1% BHT/MTBE to 1.0 mL. Sample was filtered through a Millipore FHLC 0.5-μm filter (Bedford, MA) before injection to HPLC. The experiment was conducted under dimmed light and all samples were wrapped in foil.

Total Carotenoids. Total carotenoid determination was carried out on an aliquot of the hexane extract (described previously in carotenoid extraction) by measuring the absorbance at 450 nm in a Genesis-5 Spectronic spectrophotometer (Rochester, NY). Total carotenoids were calculated according to Ritter and Purcell (*10*) using an extinction coefficient of β-carotene, $E^{1\%} = 2505$.

HPLC Analysis of Carotenoids. Carotenoid pigments were analyzed by reversed-phase high-performance liquid chromotography (RPHPLC) using ternary gradient elution with modification from a previous work (*4*). Chromatography was carried out with a Waters liquid chromatography system equipped with a model 600E solvent delivery system, a model 996 photodiode array detector, a model 717 plus autosampler, and Millennium Chromatography Manager. A Carotenoid C_{30} column (150 × 4.6 mm i.d., 3 μm) from YMC Inc. (Wilmington, NC) was used with MeOH/ACN (25:75, v/v, eluent A), 100% MTBE (eluent B), and water (eluent C) as mobile phases. Eluent contained 0.01% BHT and 0.05% triethylamine as modifiers (*6*). Flow rate was 1 mL/min, column temperature was set at 25 °C, and injection volume was 10 μL. A gradient program was performed: initial condition was 95% A/ 5% C; 0−10 min, 95% A/5% B; 10−19 min, 86% A/14% B; 19−29 min, 75% A/25% B; 29−54 min, 50% A/50% B; 54−66 min, 26% A/74% B and back to the initial condition for reequilibration. Analysis was conducted under subdued light to avoid carotenoid degradation during analysis. Reproducibility of analysis and characterization of carotenoids are presented in Lee et al. (*11*).

The β-carotene and α-carotene standards were obtained from Sigma (St. Louis, MO). β-cryptoxanthin, lutein, and zeaxanthin were obtained from Extrasynthese (Genay, France). One mixture of standards obtained from Henkel Corp. (Kankakee, IL) consisted of lutein, zeaxanthin, β-cryptoxanthin, β-carotene, and α-carotene.

Statistical Analysis. The data were subjected to analysis of variance (ANOVA), and all possible correlation coefficients between color parameters and carotenoid pigments, and multiple mean comparison (Duncan's method) were computed using the SigmaStat software from Jandel Scientific Software (San Rafael, CA). Trends were considered significant when means of compared sets differed at $P < 0.05$.

RESULTS AND DISCUSSION

Color Changes. A gradual increase in juice color number (CN) with fruit maturity occurred with cultivars in each season (Table 1). USDA grade A orange juice requires a minimum color number (CN) of 35.5. The color number of Hamlin orange juice during late October to early November usually runs between 33 and 34 as occurred in this study. Thus, Earlygold color development was especially notable, reaching the grade A 36 color number in mid-October. Hamlin did not obtain its characteristic pigmentation until late season, reaching a 36 color number only in January. Hamlin oranges, which are usually commercially harvested from November to January, did not develop the desired color. The Budd Blood cultivar followed the same trend of gradual increase through most of the season, however, in January, a significant increase in color number (Table 1) was noted for both seasons, probably due to the development of red anthocyanin pigments.

Table 1. Seasonal Changes in Juice Color of Hamlin, Earlygold, and Budd Blood Sweet Oranges

	L*	a*	b*	hue	chroma	CN[a]
Hamlin						
17-Sep-96	50.3	−6.5	16.2	111.9	17.5	31.2
16-Oct-96	46.4	−5.3	16.1	108.4	16.9	33.2
1-Nov-96	47.6	−4.7	18.2	104.4	18.8	33.8
15-Nov-96	47.2	−4.4	18.8	103.2	19.4	34.3
2-Dec-96	46.9	−3.9	20.4	100.8	20.8	35.2
13-Dec-96	48.0	−3.2	20.1	99.2	20.4	35.1
13-Jan-97	49.3	−3.0	23.3	97.4	23.5	36.0
25-Aug-97	45.4	−5.6	11.7	115.7	13.0	31.5
19-Sep-97	44.7	−5.1	13.3	111.1	14.2	32.5
17-Oct-97	45.3	−4.5	14.4	107.2	15.1	33.2
31-Oct-97	45.3	−4.1	14.5	105.6	15.0	33.5
25-Nov-97	45.6	−4.1	18.6	102.5	19.0	35.1
19-Jan-98	45.8	−2.3	20.8	96.2	20.9	36.7
Earlygold						
17-Sep-96	49.3	−5.0	19.8	104.1	20.4	33.0
16-Oct-96	46.1	−3.0	20.0	98.6	20.2	35.9
1-Nov-96	48.4	−2.8	25.7	96.1	25.9	36.5
15-Nov-96	46.5	−1.4	23.0	93.7	23.0	37.6
2-Dec-96	47.6	−2.8	29.6	95.4	29.7	37.6
13-Dec-96	47.2	−0.2	26.9	90.4	26.9	38.8
13-Jan-97	47.8	1.0	27.1	87.9	27.1	37.7
25-Aug-97	47.8	−5.4	15.9	108.8	16.8	32.8
19-Sep-97	46.7	−4.3	17.4	103.9	17.9	34.1
17-Oct-97	44.0	−2.4	17.5	97.8	17.7	36.0
31-Oct-97	45.6	−1.4	20.0	93.9	20.1	36.6
25-Nov-97	44.7	−1.6	22.1	94.0	22.2	37.8
19-Jan-98	43.4	0.9	22.9	87.8	22.9	39.9
Budd Blood						
17-Sep-96	47.3	−6.0	16.1	110.5	17.2	31.6
16-Oct-96	45.9	−5.4	19.7	105.3	20.4	34.4
1-Nov-96	46.5	−4.3	20.2	102.1	20.7	35.2
15-Nov-96	47.0	−3.7	21.4	99.7	21.7	35.9
2-Dec-96	45.7	−0.8	23.1	92.1	23.2	37.9
13-Dec-96	46.0	2.5	20.9	82.6	21.2	39.8
13-Jan-97	39.9	10.6	14.4	53.7	18.0	46.1
25-Aug-97	47.5	−6.4	15.2	112.8	16.5	32.2
19-Sep-97	46.6	−5.7	16.7	109.0	17.7	32.8
17-Oct-97	46.3	−4.0	17.0	103.3	17.4	34.4
31-Oct-97	46.5	−3.3	17.8	100.5	18.1	34.8
25-Nov-97	45.1	0.4	17.8	88.7	17.8	38.0
19-Jan-98	37.8	9.8	12.4	51.7	15.8	46.0

[a] CN is orange juice color number based on the Hunter Citrus Colorimeter.

Changes in CIE color parameters (L*, a*, b*) in juice were significant ($P < 0.05$) as a function of fruit maturity. Furthermore, the chroma diagram (Figure 1), which is based on changes of CIE a* and CIE b* values (Δa* versus Δb*) with fruit maturity, clearly illustrates that color changes became progressively more pronounced for both seasons as the values gradually

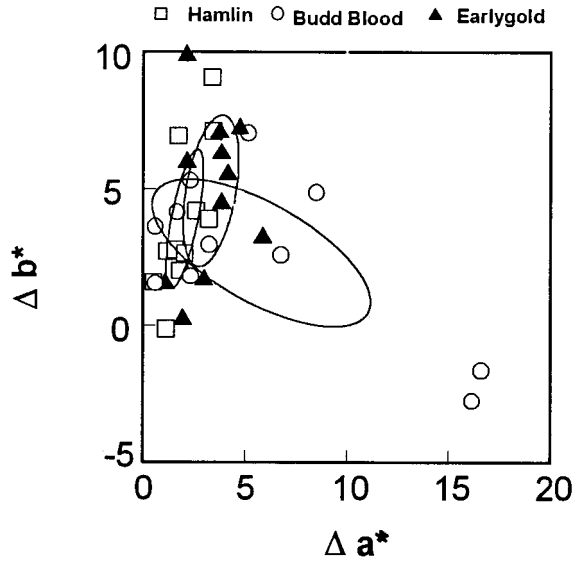

Figure 1. Changes in juice color (Δa* versus Δb*) during 2 growing seasons.

departed from zero on both Δa* and Δb* variables in Figure 1. The confidence ellipse ($P < 0.05$) is centered on the values of the x (Δ a*) and y (Δ b*) variables for each cultivar. Especially large changes were found in the CIE b* values of Hamlin and Budd Blood which suggested that color changes in juice were more related to changes in CIE b* value. Yellowing of juices was the most noticeable visual change developed by fruit ripening. A color shift toward positive Δb* and Δa* directions (Figure 1) indicated more desirable deep color development in the juices from fully matured fruits. However, the changes in CIE a* and b* in the Budd Blood cultivar were opposite of the color changes observed in both Hamlin and Earlygold (Figure 1). This is probably due to the appreciable development of red anthocyanin pigments in Budd Blood in January.

Correlations between the development of total carotenoid content and changes in color parameters (CIE a*, b*, Munsell hue, and Munsell chroma) were significant ($P < 0.05$) in this study, especially with hue angle ($r = -0.94$). The hue angle appeared to be valuable for following maturation phases, estimated at 111.89−115.73 for early stage of maturation (August−September), and stabilized at 96.19−97.42 for late stage (January) for juices from Hamlin in both seasons (Table 1). Also, the color parameters (CIE a* and b*) and chromatic attributes (chroma and hue) were significantly different ($P < 0.05$) between the three cultivars. CIE L* value (brightness), however, was not significantly different between the three cultivars, and was poorly correlated ($P > 0.05$) to juice carotenoid content.

Total Juice Carotenoids. Total juice carotenoids, expressed as the sum of carotenoid concentrations measured at 450 nm as β-carotene, increased for Earlygold and Budd Blood sweet oranges as the fruit matured in both seasons (Figure 2). The carotenoid concentration in Hamlin juice was lower throughout the season than in the Earlygold juice, but increased about 3- to 4-fold from 1 to 3.2 μg/mL in the 1996−97 season, and from 1 to 3.9 μg/mL in the 1997−98 season. The carotenoid concentration in Earlygold juice started each season at about 1.8 μg/mL and increased at greater rates than those in the Hamlin juice, to concentrations between 8 and 9 μg/mL in January. Budd Blood carotenoid concentration increased throughout each season, 1.2 to 6.1 μg/mL in the 1996−97 season, and 1.2 to 5.4 μg/mL in the 1997−98 season.

Higher pigment concentrations were generally observed for these sweet oranges in the 1997−98 season, however, there was no statistically significant seasonal difference ($p > 0.05$).

Juice Pigment Profile. The major carotenoids typically found in sweet orange include antheraxanthin, cis-antheraxanthin, α-carotene, β-carotene, α-cryptoxanthin, β-cryptoxanthin, lutein, violaxanthin, and cis-violaxanthin (*12*). These pigments were quantified for our study. Identification was based on authentic standards, on-line spectral data, and chromatographic retention as discussed in previous works (*2*, *4*, *5*, and *13*−*15*). The percentages representing neoxanthin, violaxanthin, and luteoxanthin (Table 2) are the sums of two respective isomers.

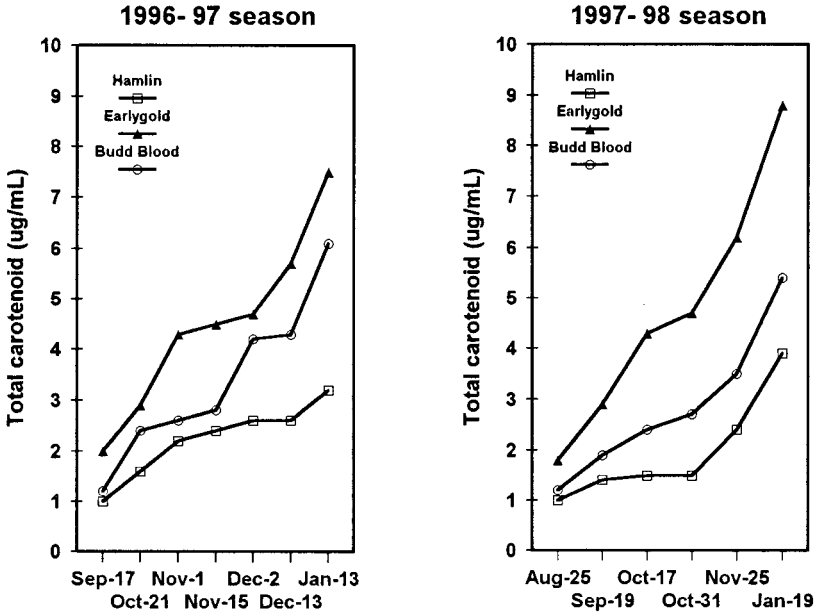

Figure 2. Changes in total carotenoid contents (μg/mL) in juice as a function of fruit ripening (□, Hamlin; ○, Budd Blood; ▲, Earlygold).

Table 2. Seasonal Quantitative Carotenoid Changes in the Juice of Hamlin, Earlygold, and Budd Blood Sweet Orange as a Percentage of Total Carotenoids

Hamlin												
date	neo-xanthin[a]	viola-xanthin[a]	luteo-xanthin[a]	anthera-xanthin	mutato-xanthin	lutein	isolutein	zea-xanthin	α-crypto-xanthin	β-crypto-xanthin	α-carotene	β-carotene
17-Sep-96	2.8	9.9	9.9	8.0	8.2	36.0	3.3	7.5	0.7	3.6	1.3	1.42
21-Oct-96	3.0	19.3	12.1	9.0	3.9	23.7	8.5	6.0	1.0	5.5	1.2	1.04
1-Nov-96	3.0	18.1	10.5	9.1	3.5	21.1	9.2	7.6	1.4	7.4	1.2	1.26
15-Nov-96	2.9	18.5	12.0	8.8	4.1	19.2	8.3	7.5	1.5	7.9	1.2	1.33
2-Dec-96	3.0	17.9	12.6	9.2	3.5	18.4	8.3	8.1	1.7	8.7	1.3	1.33
13-Dec-96	2.8	16.7	11.9	9.2	3.6	18.0	8.5	9.0	1.8	10.3	1.3	1.50
13-Jan-97	2.6	16.2	10.9	9.6	3.4	16.2	9.1	11.4	2.1	11.7	1.3	1.70
25-Aug-97	2.9	15.7	13.2	10.3	8.9	28.5	6.2	4.7	0.7	2.2	1.0	1.06
19-Sep-97	2.4	19.1	12.6	9.7	7.3	25.0	7.8	5.5	0.8	3.2	1.0	0.91
17-Oct-97	2.2	13.9	11.3	9.1	4.8	23.5	10.5	9.3	1.4	6.8	1.3	1.45
31-Oct-97	2.1	14.4	9.7	8.6	4.5	22.8	9.8	9.9	1.9	9.0	1.2	1.43
25-Nov-97	2.6	15.8	11.8	7.9	4.7	19.7	9.1	9.4	2.3	9.4	1.4	1.40
19-Jan-98	2.9	20.3	12.0	8.9	3.3	13.9	9.1	9.8	2.5	10.5	1.2	1.34

Earlygold												
date	neo-xanthin[a]	viola-xanthin[a]	luteo-xanthin[a]	anthera-xanthin	mutato-xanthin	lutein	isolutein	zea-xanthin	α-crypto-xanthin	β-crypto-xanthin	α-carotene	β-carotene
17-Sep-96	2.2	28.1	12.4	11.7	4.3	20.6	5.7	5.7	0.5	2.6	0.8	0.98
21-Oct-96	2.2	34.1	11.1	10.3	2.6	15.0	8.3	5.2	0.8	5.4	0.7	0.85
1-Nov-96	2.3	30.5	12.1	10.5	3.0	14.8	8.5	5.8	1.0	6.0	0.9	1.08
15-Nov-96	2.3	30.5	10.4	10.6	2.3	13.2	8.2	6.5	1.1	9.8	0.8	1.11
2-Dec-96	2.5	19.1	12.6	9.5	4.4	15.4	8.3	7.6	1.7	11.5	1.0	1.35
13-Dec-96	2.8	24.4	13.4	10.6	2.8	13.9	7.4	7.2	1.9	10.5	1.0	1.31
13-Jan-97	2.7	19.5	11.0	9.2	4.1	16.4	7.5	9.1	2.3	10.3	1.1	1.50
25-Aug-97	1.8	22.7	11.6	11.5	4.8	21.4	6.5	6.9	0.4	6.4	0.8	1.07
19-Sep-97	1.8	30.5	10.9	11.6	3.4	17.9	8.1	6.3	0.6	4.0	0.8	0.94
17-Oct-97	2.0	31.4	11.0	11.1	2.7	14.2	9.4	6.5	0.9	6.2	0.8	0.95
31-Oct-97	2.0	31.4	10.7	10.8	2.6	14.3	8.8	6.6	1.3	6.1	0.9	1.02
25-Nov-97	2.2	26.2	11.7	10.3	3.1	13.9	8.3	7.5	1.6	9.8	0.9	1.20
19-Jan-98	2.3	25.5	11.9	10.1	2.6	12.1	7.3	7.8	2.5	11.9	1.1	1.38

Budd Blood												
date	neo-xanthin[a]	viola-xanthin[a]	luteo-xanthin[a]	anthera-xanthin	mutato-xanthin	lutein	isolutein	zea-xanthin	α-crypto-xanthin	β-crypto-xanthin	α-carotene	β-carotene
17-Sep-96	2.9	10.5	9.4	6.1	7.4	37.5	2.8	7.0	0.6	4.6	1.4	1.4
21-Oct-96	2.3	12.5	10.2	7.4	5.2	27.6	6.9	7.7	1.2	8.8	1.0	1.1
1-Nov-96	2.3	13.5	11.1	7.6	4.9	23.7	7.4	8.1	1.6	9.1	1.1	1.0
15-Nov-96	2.5	12.4	8.9	7.2	6.1	25.0	6.8	8.9	1.9	10.6	1.5	1.6
2-Dec-96	2.2	15.2	9.8	7.6	4.3	21.2	8.3	8.4	2.5	12.1	1.3	1.2
13-Dec-96	2.5	12.5	10.0	7.9	4.6	21.4	7.0	8.9	2.7	13.2	1.2	1.3
13-Jan-97	2.2	11.0	9.0	7.7	4.1	19.0	7.3	11.1	3.1	17.0	1.3	1.5
25-Aug-97	2.2	11.0	10.0	7.0	7.1	35.8	6.3	8.8	0.4	4.0	0.8	0.8
19-Sep-97	2.4	13.0	9.8	8.1	4.9	32.7	7.6	7.9	0.8	4.4	1.0	0.9
17-Oct-97	2.5	17.5	11.5	9.6	4.0	24.5	9.5	7.0	1.4	5.2	1.1	0.8
31-Oct-97	2.3	15.6	9.8	8.7	5.0	25.1	8.2	7.9	1.9	7.5	1.1	1.0
25-Nov-97	2.4	16.4	9.6	8.1	4.1	21.1	8.1	7.7	2.5	11.6	1.1	1.2
19-Jan-98	2.5	17.5	9.7	8.4	3.1	16.7	9.2	9.3	3.8	12.6	1.4	1.3

[a] Sum of two respective isomers.

Hamlin. Juice pigmentation of Hamlin fruit at the beginning of the two seasons was primarily represented by lutein, violaxanthin, luteoxanthin, mutatoxanthin, antheraxanthin, and zeaxanthin. Their combined total was nearly 80% of the total carotenoids. The seasonal changes among these were relatively small compared to lutein, the pigment present in the largest quantity (36%) in the early stage of the 1996−97 season. It declined throughout the season while still maintaining the highest percentage of total pigment content. Lutein is a typical basic chloroplast pigment, which predominates in unripe fruits (*16*), and is probably converted to epoxy carotenoids as described by John et al. (*17*) as ripening proceeds.

The second most common juice pigment was violaxanthin. Its synthesis pattern appears to be complex. Violaxanthin showed an early increase as a percentage of the total carotenoids, followed by a gradual decline. With the onset of peel color development, the gradual synthesis of new pigments begins, in particular, β-cryptoxanthin, zeaxanthin, and antheraxanthin. β-Cryptoxanthin increased more than 4-fold, from 2.2 to 10.5%, to eventually become the third most predominant pigment in the juice (1997−98 season). β-Cryptoxanthin is an orange-colored carotenoid having visible absorption maxima of 481, 452, and 429 nm. Increasing amounts of this pigment in juice imparts a desirable bright, deep-orange color. The percentage of zeaxanthin also increased gradually then leveled off. As the biosynthesis proceeds, it is transformed further into the other xanthophylls through epoxidation according to the pathway proposed by Gross et al. (*16*).

Hydrocarbon carotenoids such as α- and β-carotene, and the more saturated delta carotene, were present in small amounts at the early stage of maturation and gradually increased, but remained at low levels. It is probably due to the synthesis of orange and yellow xanthophylls in orange juice via hydroxylation and epoxidation of hydrocarbon carotenoids (*18*).

Earlygold. The primary pigments in the early

stage of maturity were violaxanthin followed by lutein, luteoxanthin, and antheraxanthin. The high percentage of violaxanthin, more than 28% in early stage of Earlygold compared to 9.9% in Hamlin (1996−97 season), was a noticeable difference in their respective pigmentation. A similar trend was also observed in the 1997−98 season. The relatively large percentage of violaxanthin in Earlygold might be responsible for the higher juice color score of CIE b* values compared to those of Hamlin (Table 1). Violaxanthin is a yellow pigment with the main cis-form in citrus (*16*) having visible absorption maxima at 466, 437, and 414 nm.

Violaxanthin is known to form from epoxidation of zeaxanthin via antheraxanthin (*16*), and is a precursor of neoxanthin and luteoxanthin. Thus, it is speculated that differences in the rates of biosynthesis of violaxanthin, or the rates of rearrangement of the epoxide groups from violaxanthin to other xanthophylls such as neoxanthin or luteoxanthin during fruit ripening, appear to exist between Hamlin and Earlygold.

Lutein was the second most predominant pigment in Earlygold, but it declined as a percentage of the total with fruit maturity. The percentage of β-cryptoxanthin showed a steady increase throughout the study to become one of the predominant pigments as the fruit matured. In the 1997−98 season, the percentage of cryptoxanthin ($\alpha + \beta$) exceeded the percentage of lutein in mature fruits of Earlygold. Accumulation of β-cryptoxanthin, which is the main source of provitamin A in sweet orange juice, was comparable to that of the Hamlin cultivar.

Budd Blood. The primary carotenoid pigments in the early stage of ripening were lutein and violaxanthin. Lutein was again the most significant pigment, but the percentage of the total declined steadily with maturity. The percentage of β-cryptoxanthin showed a steady increase throughout the study, becoming the predominant pigment in the late stage of ripening. Violaxanthin, as in the Hamlin juice, showed an increase at first with fruit maturity, but then declined as a percentage of the total carotenoids.

The development of anthocyanin pigment in Budd Blood was not significant (less than 2 μg/mL) until mid-January. The total anthocyanin content, by the pH differential method (*19*), in juice was 12.5 μg/mL for the 1996−97 season and 11.0 μg/mL for the 1997−98 season. However, the additional development of red anthocyanin pigment significantly increased the juice color number, especially in mid-January (Table 1). A relatively high percentage of β-cryptoxanthin and the development of red anthocyanins in Budd Blood could be the major differences compared to the pigmentation in Hamlin.

CONCLUSIONS

Earlygold had the highest total juice carotenoid content among the cultivars studied, followed by Budd Blood. Hamlin had the least amount of total carotenoid content.

Changes in carotenoids are so complicated in citrus, a schematization is hardly possible, but the trend in these sweet oranges indicates that typical basic chloroplast pigments decrease during ripening. A marked increase in the percentage of β-cryptoxanthin allowed it to become a primary pigment in late season. Increased biosynthesis of β-cryptoxanthin appears to be one of the principal events occurring during ripening of Hamlin orange. The carotenoids which were present in small amounts (such as α− and β-carotene, isolutein, and zeaxanthin) at the beginning of the season gradually increased but remained at low levels. A relatively high accumulation of violaxanthin in Earlygold was found to be one of the major differences in pigmentation compared to that of standard Hamlin orange. In two growing seasons, pigmentation in these three cultivars developed a similar trend during both seasons, but the 1997−98 season provided slightly more pigmentation than that of the 1996−97 season.

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