# Objective Measurement of Red Grapefruit Juice Color

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The color properties (CIE $L^*a^*b^*$, hue, and chroma) of 90 red grapefruit juices were studied by tristimulus reflectance measurement for two growing seasons. Juices were prepared from six red grapefruit cultivars including Ruby Red, Rio Red, Star Ruby, Ray Ruby, Flame, and Marsh Red grown in Florida. Very wide varietal and seasonal variations on juice color were observed, and most notably, CIE $a^*$ values varied widely from −1.41 to 9.06 (CV = 82.6%). Lycopene is the major colored pigment in red cultivars with lesser amounts of β-carotene as determined by HPLC on a carotenoid $C_{30}$ column with gradient elution using MeOH and MTBE. The highest correlation coefficients ($r$ = 0.963) found between CIE $a^*$ values and lycopene contents in juices from all cultivars combined suggest the possibility that the CIE $a^*$ value would be a good indicator for pigmentation in red grapefruit juice.

**Keywords:** *Red grapefruit juice; lycopene; β-carotene; color; carotenoid*

## INTRODUCTION

Color is one of the critical quality factors in grading citrus products, as is flavor, and has been used in judging variety and maturity for citrus fruits. The red or pink color in pigmented cultivars of grapefruit (*Citrus paradisi* Macf.) is associated with carotenoids. Lycopene and β-carotene are the principal carotenoids in the pulp accompanied by lesser amounts of phytofluene, phytoene, and ζ-carotene (Khachik et al., 1989; Rouseff et al., 1992). Traces of α- and γ-carotene and xanthophylls (<1%) are also reported, but the characteristic carotenoid pattern varies according to fruit parts such as in flavedo, pulp, and peel (Gross, 1987). Red grapefruit often yields a juice product with a color that is neither distinctive nor pleasing, and the juice can develop a muddy, brown, unappetizing color during processing and subsequent storage. Generally, the color change in pigmented grapefruit juice products is probably due to carotenoid degradation, principally due to considerable loss of lycopene (Shaw and Nagy, 1993).

The USDA has recognized the importance of color as a quality factor in grapefruit juice. A grade scale from 0 to 100 points was developed to ascertain the grade of grapefruit juice products. A value of 20 points was assigned for canned single-strength grapefruit juice and 40 points for the color of frozen concentrated grapefruit juice (Huggart and Petrus, 1976). Color can be one of the important characteristics consumers use when selecting and purchasing product, and the consumer may react negatively to a product if its color does not meet expectations. The importance of color in consumer preference is often emphasized in citrus juices (Huggart et al., 1977, 1979; Tepper, 1993). In practice, because the consumer generally associates the pleasant flavor with an attractive color, the attractive pink or red color of grapefruit has been used in marketing through transparent packages and the use of sliced fruit in advertising. Pink grapefruit juice beverages are notably one of the most popular drink items in the market (Labell, 1993).

The USDA has also established a standard for the grading of the colors of orange juice using six plastic color tubes, and the citrus industry is required to use a colorimeter acceptable to the USDA for measuring orange juice color (Huggart and Petrus, 1976). There are, however, no officially accepted objective methods of measuring color in either white or red/pink grapefruit juice products processed in Florida. As such, subjective methods are widely used procedures for determining the color scores of grapefruit juices. Color, however, is a matter of perception. Different people interpret expressions of color in many different ways. Thus, subjective expression of color may not be accurate enough to communicate the color. Objective approaches in color measurement and expression would help to minimize color-related problems, and color communication between processors and buyers would be much simpler and exact. The HunterLab citrus colorimeter, which was developed primarily for visual judgment of orange juices, is often applied to grapefruit juices (Huggart et al., 1979; Lee, 1997). However, there are not sufficient data available to evaluate its success for red/pink grapefruit, and the range of color for pink and red grapefruit juices appears to be wider than the color range of orange juice presented using CR (citrus red) or CY (citrus yellow) scales. Furthermore, the HunterLab citrus colorimeter is no longer manufactured for the citrus industry. This study presents the objective evaluation of color function in red grapefruit juices by using tristimulus CIELAB parameters and their correlation to pigmentation in juice.

## MATERIALS AND METHODS

**Red Grapefruit Juices.** Red grapefruit cultivars (*Citrus paradisi* Macf.) harvested from growing regions in coastal areas (Gulf and Indian River) and in the central interior of Florida were used. The juices were prepared using commercial FMC juice extractors using standard settings and finished in

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**Table 1. Descriptive Statistics[a] on Color Parameters and Pigments in Red Grapefruit Juices**

| | CIE $L^*$ | CIE $a^*$ | CIE $b^*$ | hue ($h^*$) | chroma ($c^*$) | $\beta$-carotene (ppm) | lycopene (ppm) | lycopene/$\beta$-carotene ratio |
|---|---|---|---|---|---|---|---|---|
| Marsh Red ($n$ = 16) | | | | | | | | |
| min | 37.15 | −1.41 | 6.73 | 62.57 | 7.03 | 0.80 | 0.80 | 0.7 |
| max | 42.13 | 5.64 | 10.86 | 98.77 | 12.24 | 4.43 | 9.57 | 5.4 |
| mean | 39.08 | 1.53 | 8.53 | 80.63 | 8.87 | 1.60 | 3.82 | 2.5 |
| SD | 1.50 | 2.11 | 1.18 | 12.40 | 1.43 | 0.99 | 2.60 | 1.5 |
| Ruby Red ($n$ = 17) | | | | | | | | |
| min | 38.27 | −1.39 | 6.73 | 80.91 | 6.74 | 0.82 | 0.68 | 0.7 |
| max | 42.24 | 1.18 | 11.17 | 98.57 | 11.17 | 1.80 | 2.80 | 2.4 |
| mean | 40.12 | −0.18 | 8.56 | 90.81 | 8.61 | 1.19 | 1.51 | 1.3 |
| SD | 1.18 | 0.84 | 1.09 | 5.69 | 1.09 | 0.26 | 0.70 | 0.6 |
| Flame ($n$ = 19) | | | | | | | | |
| min | 34.89 | 3.39 | 7.41 | 43.24 | 9.35 | 1.80 | 5.78 | 1.4 |
| max | 38.32 | 9.06 | 11.80 | 73.09 | 12.44 | 4.50 | 18.60 | 10.3 |
| mean | 36.56 | 5.47 | 9.41 | 59.74 | 11.04 | 3.26 | 9.55 | 3.4 |
| SD | 0.95 | 1.65 | 1.28 | 9.83 | 0.86 | 0.91 | 3.55 | 2.3 |
| Star Ruby ($n$ = 19) | | | | | | | | |
| min | 33.61 | 2.67 | 6.41 | 38.80 | 8.72 | 1.46 | 5.80 | 2.8 |
| max | 38.47 | 8.84 | 10.35 | 75.56 | 12.05 | 5.31 | 20.49 | 9.7 |
| mean | 35.13 | 6.54 | 8.13 | 51.41 | 10.58 | 2.76 | 13.62 | 5.3 |
| SD | 1.40 | 1.69 | 1.15 | 9.87 | 1.01 | 1.01 | 4.23 | 1.9 |
| Ray Ruby ($n$ = 9) | | | | | | | | |
| min | 36.97 | 3.15 | 6.51 | 56.14 | 7.57 | 0.89 | 5.71 | 3.5 |
| max | 38.54 | 5.48 | 9.51 | 70.33 | 10.30 | 1.90 | 11.13 | 8.4 |
| mean | 37.64 | 4.02 | 7.96 | 63.03 | 8.95 | 1.39 | 7.84 | 5.9 |
| SD | 0.56 | 0.79 | 1.10 | 5.32 | 1.05 | 0.38 | 1.85 | 1.7 |
| Rio Red ($n$=10) | | | | | | | | |
| min | 34.28 | 3.63 | 6.76 | 38.48 | 9.06 | 1.60 | 7.40 | 4.1 |
| max | 38.54 | 8.50 | 11.21 | 72.05 | 12.39 | 2.00 | 14.20 | 8.9 |
| mean | 36.25 | 6.02 | 8.66 | 54.88 | 10.74 | 1.80 | 11.27 | 6.3 |
| SD | 1.71 | 1.86 | 1.92 | 11.88 | 1.40 | 0.14 | 2.52 | 1.8 |
| total ($n$ = 90) | | | | | | | | |
| min | 33.61 | −1.41 | 6.41 | 38.48 | 6.74 | 0.80 | 0.68 | 0.7 |
| max | 42.24 | 9.06 | 11.80 | 98.77 | 12.44 | 5.31 | 20.49 | 10.3 |
| mean | 37.52 | 3.71 | 8.61 | 68.21 | 9.82 | 2.16 | 7.69 | 3.6 |
| SD | 2.23 | 3.06 | 1.28 | 17.81 | 1.50 | 1.14 | 5.40 | 2.4 |

[a] Minimum, maximum, mean, and standard deviation.

an FMC juice finisher in the pilot plant at the Citrus Research and Education Center (CREC, University of Florida, Lake Alfred, FL). Thermal pasteurization (91 °C, 10 s) was performed using an APV (Tonawanda, NY) plate heat exchanger. The same treatment was applied to the juices through the two processing seasons from October 1996 to May 1997 and from October 1997 to May 1998.

**Color Analysis.** Color was measured on duplicate samples in test tubes (25 cm × 20 cm o.d.). The CIE $L^*a^*b^*$ values were measured with a Macbeth Color-Eye 3100 spectrophotometer (Kollmorgen Instruments Corp., Newburgh, NY) with an Optiview software package in the reflectance mode, with illuminant *C* and 2° observer angle. From CIE $a^*$ and $b^*$ values the chroma $[(a^{*2} + b^{*2})^{1/2}]$ and hue angle ($\tan^{-1} b^*/a^*$) were calculated. Reproducibility of color measurement was <1% CV.

**Carotenoid Analysis by HPLC.** Carotenoid pigment analysis was conducted using an HPLC method (Lee and Coates, 1999). The HPLC system consisted of a Waters 600E gradient pump and a 717 plus autosampler equipped with a chiller (Waters Associates, Milford, MA) and a Spectra 200 UV−visible detector (Thermo Separation Products, San Jose, CA). A Waters 996 photodiode array detector was also used for on-line spectral acquisition. Analysis was carried out using a YMC (Wilmington, NC) $C_{30}$ column (4.6 mm × 15 cm, 3 $\mu$m), oven temperature at 25 °C, using binary gradient elution. The eluents were methanol (A) and methyl *tert*-butyl ether (B). Both eluents contained 0.05% triethylamine and 0.01% BHT. The gradient program and data acquisition were performed as described in a previous work (Lee and Coates, 1999). All data were duplicate analyses, and mean values are reported. The $\beta$-carotene and lycopene standards were obtained from Sigma (St. Louis, MO).

**Statistical Analysis.** Statistical analyses (descriptive, ANOVA, regression and discriminant analysis) were conducted using SYSTAT 8.0 software from SPSS, Inc. (Chicago, IL). Trends were considered significant when means of compared sets differed at $P < 0.05$.

## RESULTS AND DISCUSSION

**Juice Color and Pigments.** Table 1 presents the descriptive statistics on color parameters (CIE $L^*a^*b^*$) for 90 samples representing 6 different varieties of red grapefruits grown in Florida during the 1996−1997 and 1997−1998 seasons. Pigmented grapefruit juice has two major carotenoid pigments (lycopene and $\beta$-carotene). The red color in juice is primarily due to lycopene, which makes up the largest percentage of pigment (Table 1).

As an objective evaluation of red grapefruit juices, two color parameters appear to be important and sensitive to color evaluation in the pigmented grapefruit juice. One is the CIE $a^*$ value, which indicates the redness (positive $a^*$) and greenness (negative $a^*$). As shown in Table 1, the average $a^*$ value for 90 samples was 3.71 with a wide range from −1.41 to 9.06 (CV = 82.6%). Ruby Red juice had the lowest mean $a^*$ value of −0.18. A lower CIE $a^*$ value in Ruby Red juice could be attributed to a lower content of red pigments, specifically lycopene, as presented in Table 1. Marsh Red also had a relatively low mean value of $a^*$ of <2. Ruby Red is the most widely grown colored grapefruit in Florida; therefore, the color of Marsh Red, which is based on randomly mixed available fruit, probably reflects the poor color of the majority, Ruby Red. Among juices prepared during two processing seasons, one-third of the samples had a CIE $a^*$ value of <2. The CIE $L^*$ value, which indicates the lightness of juice, did not appear to

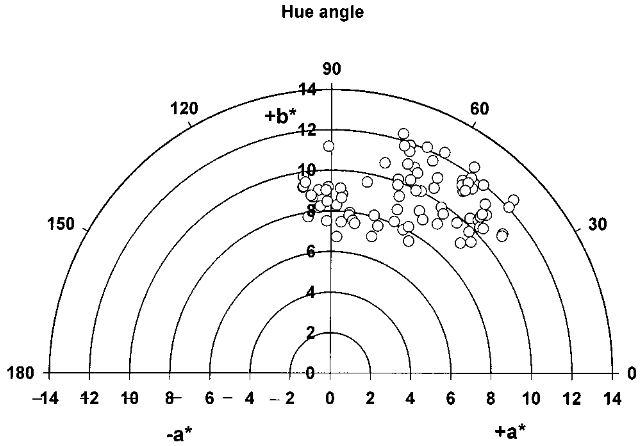


**Figure 1.** Plots of red grapefruit juice color in chromaticity diagram.

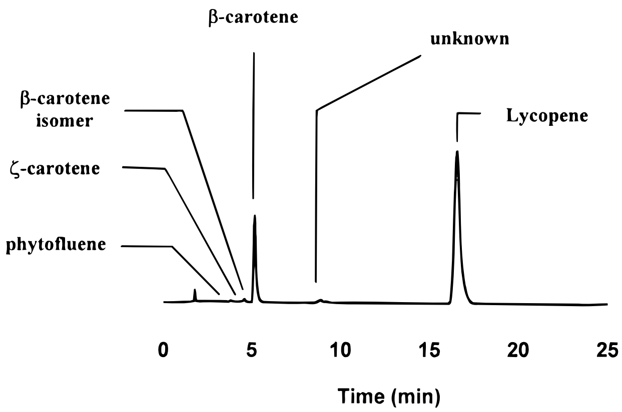


**Figure 2.** HPLC chromatogram of carotenoids from Star Ruby grapefruit juice using $C_{30}$ column and visible detection at 450 nm.

be as variable (CV = 5.9%) compared to other color parameters in juices. Thus, lightness of juice appears not to be affected by variations in pigmentation in juice.

The other important color parameter in red grapefruit juice is hue ($h$*) angle, which is the attribute of color that is perceived. Figure 1 illustrates the wide distribution of juice color on the CIE chromaticity diagram, which shows proportions of tristimulus values in bidimensional space. In the diagram, a majority of the juices were between 45° and 75° in hue angle, which can be described as orange to orange-yellow color, and only 8 of 90 samples had a hue of <45°, which can be described as red or red-orange. Less than one-fifth of juice samples showed >90° in hue, which could be perceived as off-yellow or dull brown. Ruby Red juices, visibly less colored compared to the juices of Star Ruby or Rio Red, are perceived as having a dull off-color. Most of the juices from Flame, Star Ruby, and Rio Red showed higher chroma values than Ruby Red as presented in Table 1.

Figure 2 shows the HPLC chromatogram of carotenoids in Star Ruby grapefruit juice analyzed with a methanol/methyl *tert*-butyl ether (MeOH/MTBE) gradient elution on a $C_{30}$ column. It was interesting to observe that the elution order for major carotenoids was reversed from the previous HPLC works with isocratic elution on $C_{18}$ columns using a mixture of methanol/acetonitrile/methylene chloride (Khachik et al., 1989; Rouseff et al., 1992) or aqueous methanol/THF (Sadler et al., 1990). Lycopene was retained longer than $\beta$-carotene and $\zeta$-carotene in this study. The $C_{30}$ bonded phase is known to provide different selectivity and enhanced retention of nonpolar hydrocarbons compared to the $C_{18}$ phase (Sander et al., 1994). Identifications of minor carotenoids such as phytofluene ($t_R$ = 3.61), $\zeta$-carotene ($t_R$ = 4.29),and isomers of lycopene ($t_R$ = 16.59) and $\beta$-carotene ($t_R$ = 5.21) were tentatively done using spectral information from previous works with red/pink grapefruit carotenoids (Khachik et al., 1989; Rouseff et al., 1992). Substantial quantities of phytoene were reported in pigmented grapefruit (Rouseff et al., 1992). However, phytoene is a colorless carotenoid and only seen from chromatography under 290 nm; thus, its analysis was not attempted.

Table 1 also shows the lycopene and $\beta$-carotene contents found in red grapefruit juices commonly processed in Florida. Lycopene was the major colored pigment in red varieties grown in Florida with lesser amounts of $\beta$-carotene except in Ruby Red. In Ruby Red, small quantities of lycopene were found compared to $\beta$-carotene, which is in good agreement with previous work on hydrocarbon carotenoids in red grapefruit cultivars (Rouseff et al., 1992). A wide range from 0.68 ppm in Ruby Red to 20.49 ppm in Star Ruby for lycopene was obtained from 90 samples representing 6 commercial cultivars in Florida (Table 1). Phytofluene ($\lambda_{max}$ = 321.0, 343.4, 366.3 nm) is an essentially colorless carotenoid, and there is only a trace of $\zeta$-carotene ($\lambda_{max}$ = 380.0, 399.3, 424.4 nm) in juice; therefore, the intense juice color of grapefruit depends on the quantities of lycopene ($\lambda_{max}$ = 446.2, 472.6, 503.3 nm) and $\beta$-carotene ($\lambda_{max}$ = 452.4, 431.4 nm) as well as their ratio, as presented in Table 1. Besides the quantity of carotenoids, the ratio of lycopene and $\beta$-carotene is presented to express the pigmentation in fruit as a function of maturity (Curl and Bailey, 1957). The high ratio of lycopene to $\beta$-carotene appears to be responsible for the attractive red appearance in the flesh of pigmented grapefruit as discussed by Curl and Bailey (1957). The mean value for the ratio of lycopene to $\beta$-carotene from two growing seasons is 3.6. Furthermore, differences in tinctorial strength as well as different absorption coefficients of lycopene ($E^{1\%}$ = 3450; Bauernfeind, 1981) and $\beta$-carotene ($E^{1\%}$ = 2592; Bauernfeind, 1981) probably have effects on different visual perceptions of juice color, too.

Lycopene, which is the carotenoid responsible for the pleasant red hue in grapefruit juice, appears to be very important for color perception of red/pink grapefruit juice products. In an attempt to relate carotenoid pigment to color parameters, the highest correlation was found between lycopene content and CIE $a$* value (a measure of redness) in juices from all cultivars. A linear regression of lycopene content on CIE $a$* value provided a correlation coefficient value >0.96, which is higher than all of the remaining objective parameters, suggesting that the color of red grapefruit juices is dominated by lycopene. Hue angle calculated at $\theta = \tan^{-1} b^*/a^*$ also exhibited good negative correlation with lycopene content ($r = -0.924$) as well as with CIE $a$* value ($r = -0.934$). Generally, the carotenoid content correlated well with tristimulus $a$* value in fruit and vegetables, and most of the variations in the correlations of color and pigment content are due to the chemical analysis (Francis, 1969). The $\beta$-carotene in pink and red grapefruit is important as a precursor of vitamin A carotenoid but had a poor correlation ($r = -0.06$) to hue. Its contribution to visual color in red grapefruit juice

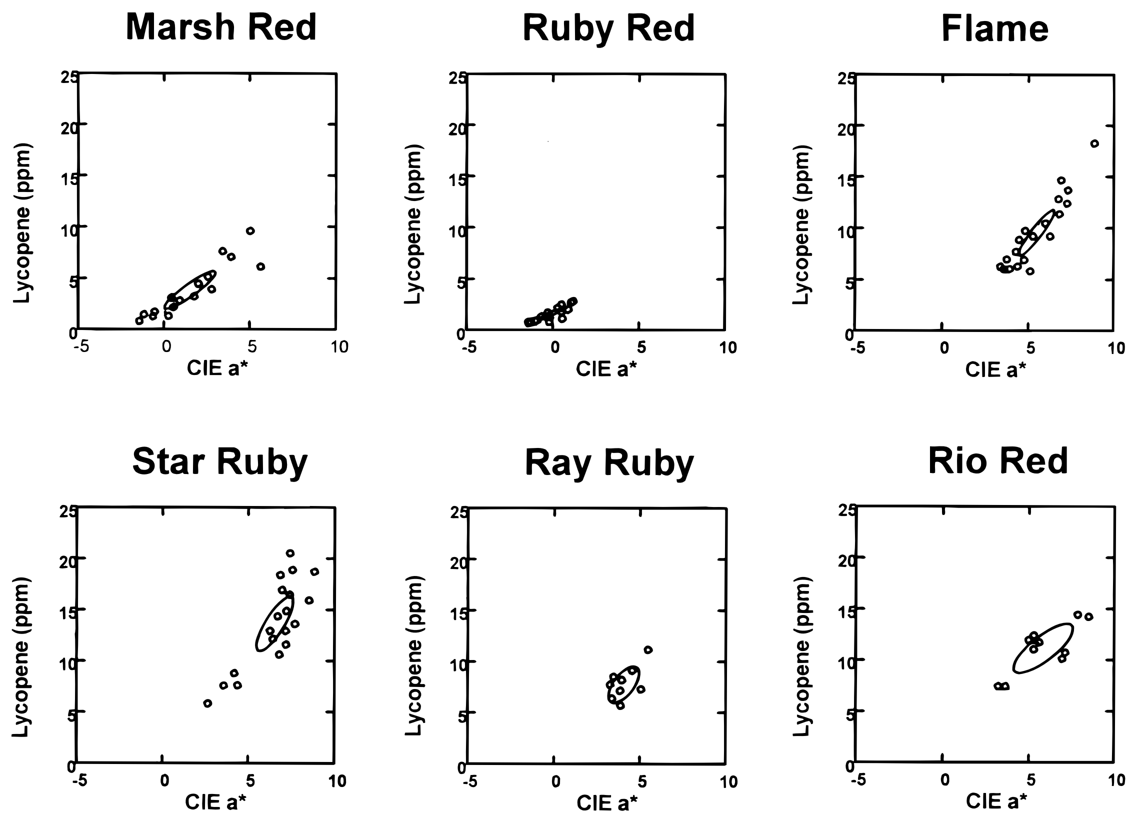


**Figure 3.** Scatter plot of differences in lycopene (parts per million) and color score (CIE $a^*$) among red grapefruit cultivars.

does not appear to be as significant as lycopene, which is probably due to the lower proportion of $\beta$-carotene to the total pigment contents (Table 1).

Previously, it was suggested that the objective color readings could be a more accurate index of total carotenoid content than the visual estimates of color (Francis, 1969). Because color measurement basically consists of the specifications of a point in three-dimensional space, it would be logical to utilize three coordinates. Under normal circumstances, however, such as in quality control laboratories of processing plants of grapefruit juice, it would probably be advantageous to use only one or two variables rather than three. As an example for orange juice, calculation of color number (CN) or equivalent color score is practiced for color grading of processed orange juice in the citrus industry. Because the CIE $a^*$ value is highly correlated to lycopene content and is easier to measure than lycopene or hue value, CIE $a^*$ values alone could be used as a quick and objective color score in pink/red grapefruit juices.

**Effects of Variety and Maturity on Juice Color.** Fruit variety, maturity, growing conditions, and season of the year can affect the carotenoid contents in juice. Scatter plots in Figure 3 illustrate the differences in pigmentation and color among six red grapefruit cultivars. The confidence ellipse ($p < 0.95$) is centered on the sample means of the $x$ (CIE $a^*$) and $y$ (lycopene) variables. Star Ruby juice clearly shows the most intense pigmentation among cultivars. Star Ruby has ~9 times more lycopene than Ruby Red, which is the most widely grown pigmented grapefruit, especially in Florida's Indian River area. The flesh color of Rio Red is known to be about the same as that of Flame, which is slightly darker than Ray Ruby but not as dark as Star Ruby (Tucker et al., 1998). There were statistically significant differences ($p < 0.05$) in redness (CIE $a^*$) of juice among cultivars.

Ruby Red also showed the lowest value of lycopene/$\beta$-carotene ratio, 1.3, which is comparable to the early study with Ruby Red grown in Texas. The ratio of lycopene to $\beta$-carotene was ~1.5 for both pulp and peel carotenoids (Curl and Bailey, 1957). The intensely colored Rio Red, Ray Ruby, and Star Ruby showed mean values >5.3. The highest ratio was found in Rio Red, but it is probably due to the small sample sizes compared to other highly pigmented varieties. There was a significant variation ($p < 0.05$) in the lycopene contents as well as the ratio of lycopene/$\beta$-carotene between cultivars.

Fruit maturity has a profound effect on carotenoid concentration. Optimum color of pigmented grapefruit develops early in the harvest season, but it fades as the season progresses (Cruse et al., 1979). Thus, there are obvious changes in juice color as a function of fruit maturity during the processing season. Typically, lycopene content decreases with advancing maturity, whereas the $\beta$-carotene content increases to a maximum and then falls in late maturity. Variation in amounts of carotenoids, especially lycopene, appeared to decline as the season progressed from October to May in this study and affected the juice color. Figure 4 shows the trends of the decline of juice color as a function of processing season. In Figure 4, color scores (CIE $a^*$) in Flame juices processed during the 1997−1998 season are plotted using a linear equation and show the decline of juice color scores to about half in late season (May). The equation that best fit the color change in Flame juice yielded a curved line, $\ln(\text{CIE } a^* \text{ value}) = 2.2025 - 0.0057x^2 \ln x$, where $x$ is the month. A similar trend could be seen from other cultivars but with more variations. However, there are no statistically signifi-

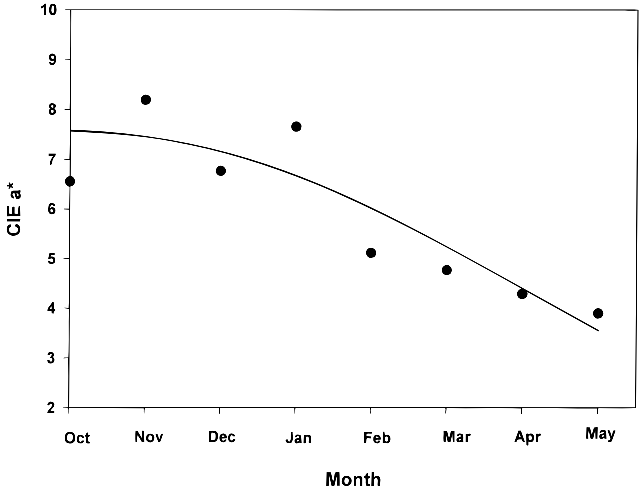


**Figure 4.** Changes in color value (CIE $a^*$) in Flame grapefruit juice as a function of processing season. ln(CIE $a^*$ value) = $2.2025 - 0.0057x^2 \ln x$, where $x$ is the month.

cant differences ($p > 0.05$) in color parameters as well as in pigment contents among juices processed during the 1996−1997 and 1997−1998 seasons. Thus, a seasonal difference in color was not significant in the juice samples processed for this study.

**Conclusion.** In summary, the CIELAB parameters were effective for measurement of color differences in red grapefruit juices of different cultivars, maturities, and processing seasons. Among cultivars, the highest juice color score (CIE $a^*$) was found for Star Ruby (mean = 6.54) followed by Rio Red (mean = 6.02) and Flame (mean = 5.47). The color score of Ruby Red juice was low; the highest color score found with Ruby Red juice was 1.18 during two processing seasons. The unique color quality in highly pigmented cultivars such as Star Ruby probably provide marketing advantages over the Ruby Red, a standard red cultivar in Florida, for both fresh fruit and processed juice. The color of red grapefruit juices was dominated by lycopene and declined as the season progresses. A highly significant correlation between CIE $a^*$ value and lycopene content indicated the practical importance of tristimulus measurement of red grapefruit juice color. CIE $a^*$ values could be a good criterion of the acceptability of color of red grapefruit juices because it characterizes redness, and this red color is dominant in juice.

## ABBREVIATIONS USED

CIE, Commission International de l'Eclairage (International Commission on Illumination); CIELAB, CIE 1976 ($L^*a^*b^*$) color space; USDA, U.S. Department of Agriculture; CR, citrus red; CY, citrus yellow, CN, color number; HPLC, high-performance liquid chromatography; CV, coefficient of variation; ANOVA, analysis of variance; $t_R$, retention time; BHT, butylated hydroxytoluene; MTBE, methyl *tert*-butyl ether; THF, tetrahydrofuran.

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