JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Characterization of Color Fade during Frozen Storage of Red Grapefruit Juice Concentrates

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Color changes in red grapefruit juice concentrates during storage at −23 °C for 12 months were studied. Concentrate (38° Brix) was packed in both plastic (16 oz) and metal (6 oz) cans. Decrease in red intensity (CIE a*) in juice color and slight increases in CIE L*, b*, and hue values from analysis of reconstituted juices were the characteristic color changes in concentrate during frozen storage. With respect to fresh concentrate, juice color in stored concentrate shifted toward the direction between negative ΔC* and positive ΔL*, indicating the color became slightly paler. A color difference seems to exist between the two containers, especially for the magnitude of ΔE*; color changes were more pronounced in concentrates packed in plastic. There are significant changes ($P < 0.05$) in major carotenoid pigments (β-carotene and lycopene) in the concentrates. More than 20% loss of lycopene and about 7% loss of β-carotene occurred with plastic containers after a 12-month period. Regression analysis showed that the rate of decline was about 0.291 ppm per month ($r = 0.990$) for lycopene compared to 0.045 ppm ($r = 0.817$) for β-carotene in concentrate stored in plastic. In the metal can, the same trends were observed but pigment losses were slightly smaller than those with plastic. An estimated shelf life for lycopene was 26.1 months in the metal can compared to 18 months in plastic. Shelf life for β-carotene was more than 39 months, more than twice that of lycopene in plastic container.

KEYWORDS: Red grapefruit juice concentrates; frozen; color; carotenoid; metal; plastic

INTRODUCTION

In the citrus industry, red pigmented grapefruit juice concentrate (62−65 °Brix) is stored as frozen in bulk at temperatures from −6 to −25 °C to avoid degradation of product quality during long-term storage. However, discoloration of red grapefruit juice concentrate during frozen storage has often been noticed, but there have been no detailed reports regarding this phenomenon.

Color changes during frozen storage have often been reported in some high-carotenoid foods including tomato products (*1*, *2*), and fruit slices of pineapple (*3*), kiwi (*4*), and papaya (*5*). The typical color change during frozen storage of carotenoid foods was characterized as the diminishing of the red character, increasing of the yellow character, and simultaneous lightening of the color (*6*).

Furthermore, the quantities of carotenoids were diminished as a function of frozen storage time (*1*, *4*−*6*). Because carotenoids are highly unsaturated compounds, they are susceptible to oxidation, isomerization, and other chemical changes during processing and storage periods (*7*). Freezing and thawing could lead to cell disintegration, pigment degradation, and isomerization of carotenoids (*3*). However, the pigment stability during frozen storage appears to depend on the carotenoids, as described by Sheehan et al. (*8*).

Changes in carotenoids in red grapefruit juice can cause development of a muddy, brown, unappetizing color (*9*), thus, evaluating color changes during storage to provide for maximum color retention is of great importance when the concentrate is submitted to relatively long-term storage. More detailed knowledge of pigment behavior during frozen storage and its effect on visual color can be a benefit in maintaining the color of the concentrate.

This study aimed to characterize the changes in color and pigment composition of red grapefruit juice concentrate during frozen storage. We were further interested in comparing the color changes between the concentrates packed in two different containers: metal and polyethylene plastic cans.

MATERIALS AND METHODS

Samples. Flame grapefruits (*Citrus paradisi Macfad*) from the Indian River growing region in Florida were used in this study. The juices were prepared using commercial FMC extractors, finished in an FMC juice finisher, and concentrated using a TASTE evaporator at the pilot plant at the Citrus Research and Education Center, University of Florida (Lake Alfred, FL). Concentrate samples were adjusted to 38 °Brix and were packed in 16-oz polyethylene (HDPE) plastic and 6-oz metal cans. Samples were placed in a −23 °C walk-in freezer for 12 months. Stored concentrates were reconstituted with water (3:1), adjusted to 10 °Brix (pulp content, 8 vol %), and analyzed for color and pigment contents on the same day as reconstituted. Duplicate samples were evaluated for analysis.

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10.1021/jf020159q CCC: $22.00
Published on Web 06/07/2002

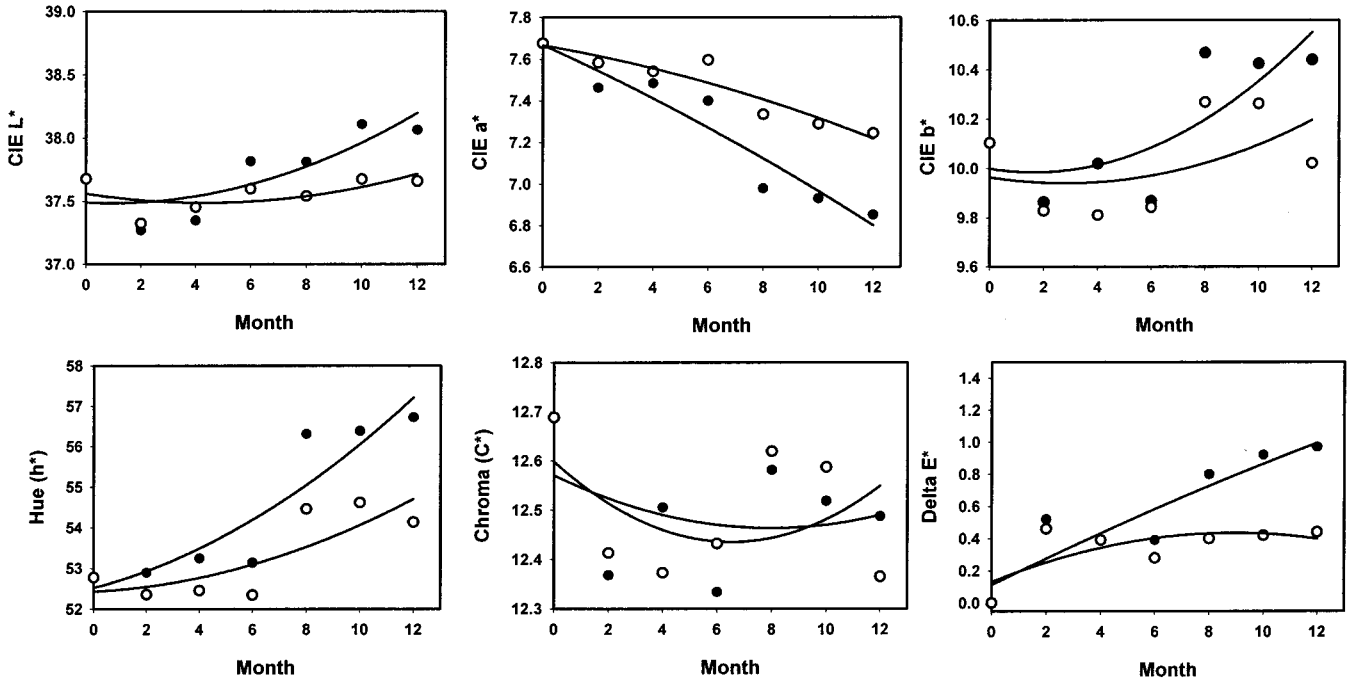

Figure 1. Changes in color as a function of storage time: metal can (○) and plastic (●).

Color Analysis. Color was measured on duplicate 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 (Kollmorgen Instruments Corp., Newburgh, NY) with 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. Total color differences, ΔE* of before and after storage samples, were also calculated using the (L*, a*, and b*) color coordinates, and were defined by the equation $(\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})^{1/2}$. Differences in CIE L*, a*, and b* values between replicates were less than 1%. The color data were plotted by fitting the data to polynomial equations using Sigma Plot software from SPSS, Inc. (Chicago, IL).

Pigment Analysis by HPLC. Pigment analysis was conducted using a previously described HPLC method (*10*). Grapefruit juice samples of 2-mL each were mixed with 5 mL of hexanes−ethanol−acetone (50:25:25), and the mixtures were agitated, and centrifuged for 5 min at 6 500 rpm at 5 °C. The solution separated into distinct polar and nonpolar layers. The upper hexane layer was used for pigment analysis. Reproducibility of analysis was less than 1% CV for the 6 runs of extracts prepared from the same juice.

The HPLC system consisted of a Waters 600E gradient pump and a 717 plus autosampler equipped with chiller (Waters Associates, Milford, MA). The analyses were carried out using a YMC (Wilmington, NC) C_{30} column (4.6 mm × 15 cm, 3 μm), and an 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 (linear step) was 75% A/25% B initial, to 65% A/35% B in 10 min, to 45% A/55% B in 10 min, isocratically run for 10 min, and then returned to the initial condition. The flow rate was 1.5 mL/min and the injection volume was 10 μL. For detection, a Spectra-Physics (Riviera Beach, FL) UV−Visible detector and a Waters 996 photodiode-array detector were used. β-carotene and lycopene were identified by their retention times and by comparison of the visible spectra with those of pure compounds. Standards of carotenoids were obtained from Sigma Chemical Co. (St. Louis, MO). All data acquisition and processing were done using Millennium Chromatography software from Waters. All the data were duplicate analyses and mean values were reported.

Statistical Analysis. Statistical analysis was conducted using the SigmaStat PC software from SPSS, Inc. (Chicago, IL). Trends were considered significant when means of compared sets differed at $P < 0.05$. Results were submitted to analysis of variance (ANOVA), regression analysis, and Fisher LSD test.

RESULTS AND DISCUSSION

Effects on Color Coordinates. Figure 1 illustrates the overall color changes in Flame grapefruit juice concentrates during storage at −23 °C. Among color parameters, the slight increase in CIE L*, b*, and hue values, and decrease in red intensity in juice color (CIE a* value) were the characteristic changes in concentrate during frozen storage. After 12 months frozen storage, color shifted toward positive b* ($P < 0.05$) and negative a* ($P < 0.05$) directions (**Figure 1**) indicating more yellow and less redness in concentrates. Also, the majority of samples showed slight increases in L* value ($P > 0.05$) after storage, which indicates a lightening of juice surface color. A subjective term, color fading (*11*−*13*), was often used to describe the lightening of the surface color. A similar observation of slight increases in L* values was also reported with frozen storage of pineapple (*3*) and tomato (*6*).

Total color differences (ΔE*), which indicate the magnitude of the color difference between fresh and stored concentrates, are also included in **Figure 1**. Changes in ΔE* are significant ($P < 0.05$) in juices after storage. During 12 months storage, the ΔE* values ranged from 0 to 1.1 for concentrates in plastic cans. A difference seems to exist between the two containers, especially for the magnitude of ΔE*; color changes are more pronounced in concentrates packed in plastic (**Figure 1**). For metal cans, the ΔE* values ranged from 0 to less than 0.5 (**Figure 1**), which could be hardly noticeable by the naked eye (*6*). However, in all of the tested samples stored in either metal or plastic, the total color difference between before and after storage was less than ΔE* of 2 (**Figure 1**), which is probably commercially acceptable. Earlier, Francis and Clydesdale (*14*) indicated that a ΔE* of 2 would be a noticeable difference in the visual perception of many products.

In most cases, a good correlation was found between color parameters and storage time. Among color parameters, changes in CIE a* ($r = -0.964$) value, h* ($r = 0.964$) angle, and ΔE*

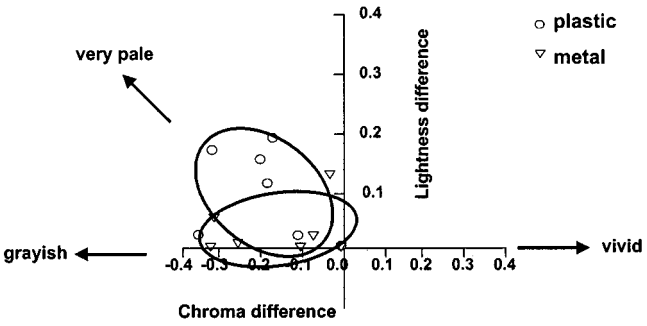

Figure 2. Differences in lightness (ΔL*) and chroma (ΔC*) in metal can and plastic.

($r = 0.857$) are the three color parameters showing significant ($p < 0.05$) correlation with storage time. Thus, the change in color of grapefruit concentrate is dependent on storage time as well.

Effects on Lightness and Chroma. Color differences in stored concentrates packed in both plastic and metal are also plotted on a chroma diagram (ΔL* versus ΔC*) as shown in **Figure 2**. This plot clearly illustrates changes of CIE L* and C* parameters after storage. Especially, a large change was found in the concentrates stored in plastic. The confidence ellipse ($p = 0.05$) is centered on the sample means of the x (ΔC*) and y (ΔL*) variables. It is interesting to note the slight differences in the direction of color changes between the metal can and plastic as plotted in **Figure 2**. These data in **Figure 2** indicate the direction of color difference in concentrates after frozen storage but do not describe the degree of color difference. After a year of frozen storage, juice color shifted toward the direction between negative ΔC* and positive ΔL* in the plot, indicating the color of stored concentrate became slightly paler with respect to fresh concentrate as presented in **Figure 2**.

Effects on Pigment Contents. The two major carotenoid pigments, lycopene and β-carotene, were measured by RP-HPLC and are presented as a function of storage time (months) in **Figure 3**. Lycopene is the major (>78%) colored pigment in Flame grapefruit juice with less β-carotene. Total pigment content, which is the sum of these two major pigments, was 21.1 ppm and decreased ($p < 0.05$) to 17.3 ppm for plastic and 18.3 ppm for metal can. Degradation of pigments was considered as one of the main chemical changes to frozen foods during storage, and can be accelerated by the high concentration of solutes surrounding the ice crystals (*15*).

Under this storage condition, clear differences in the stability of β-carotene and lycopene were observed. There was more than 20% lycopene loss and about 7% β-carotene loss with plastic after 12-months storage. The lycopene was more labile than β-carotene under this condition. Carotenoid degradation depends on many factors (*15*), and oxidative degradation of carotenoid is often described as zero-order kinetics (*15*). The regression analysis provided a good fit of a simple linear model (**Figure 3**) under this condition. Regression analysis showed that the rate of decline was about 0.291 ppm per month ($r = 0.990$) for lycopene compared to 0.045 ppm per month ($r = 0.817$) for β-carotene in juice stored in plastic. Juice packed in metal cans showed similar trends, but with a slightly slower rate of carotenoid changes. The higher susceptibility of lycopene is probably owing to its 11 conjugated double bonds compared to 9 conjugated double bonds in β-carotene. According to previous work of Henry et al. (*17*), carotenoid stability toward oxidation is a function of both the number of conjugated double bonds and the presence of functional groups.

To estimate the shelf life of carotenoids under this condition, a $^2/_3$ life of pigments was determined. A $^2/_3$ life is often used in the color industry, and is the time it takes for $^1/_3$ of the color of a carotenoid sample to fade under the conditions of the experiment (*13*). It has been considered as a highly reproducible measurement to evaluate the relative effectiveness of colorants. The $^2/_3$ life for lycopene was about 18 months in concentrate packed in plastic compared to 26.1 months for that in a metal can. The fate of lycopene in concentrate packed in plastic would be expected to be short, about 1.4 times, compared to concentrate in the can. The $^2/_3$ life for β-carotene was 39.5 months for concentrate in plastic containers, more than twice that of lycopene. Considering changes in lycopene contents had a good correlation with changes in CIE a* ($r = 0793$) and ΔE* ($r = -0.793$) values for the concentrates in both plastic and metal cans during storage suggests the important role of lycopene loss in the color fade in stored concentrate. Earlier, Lovric et al. (*12*) described that the main cause of color fading in dried tomato products was the oxidation of lycopene.

With regard to headspace, the metal can originally had a larger (12.2%) headspace than the plastic container (9.3%) in this study, but once the oxygen was consumed in the headspace, further oxidation appeared to be much slower in the hermetically sealed metal cans than in the plastic. Headspace volume of each container is based on space remaining after addition of 16 oz of concentrate to the plastic can and 6 oz of concentrate to the metal can. In a study of a model system with β-carotene (*18*), it was determined that the presence of oxygen in the headspace is crucial in carotenoid degradation. In contrast to metal containers that are hermetically sealed, plastic bottles are

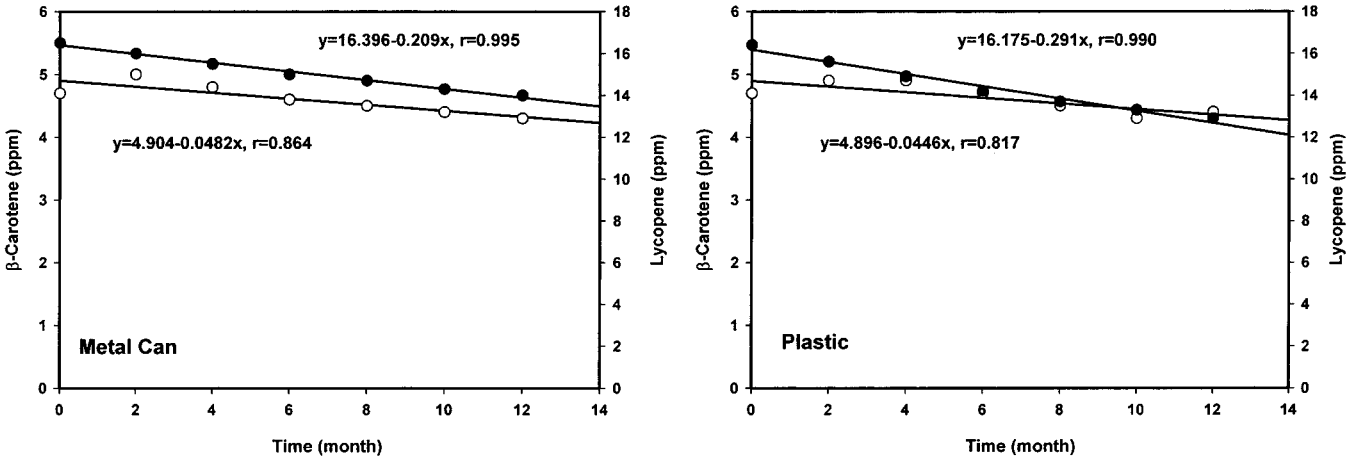

Figure 3. Changes in carotenoids (ppm) as a function of storage time: β-carotene (○) and lycopene (●).

permeable to oxygen, thus, this suggests the oxygen permeability of the container can also be one of the critical factors for pigment stability during frozen storage.

Loss of carotenoid pigments during frozen storage is probably due to autoxidation reaction as discussed with tomato products (*1*, *2*). At this frozen storage temperature (−23 °C), cell disintegration was speculated and could release the pigment, which would then become susceptible to oxidative changes as described by Fellows (*15*). And further degradation of lycopene, causing color loss, could involve a number of reactions as proposed by Boskovic (*7*).

In conclusion, this study showed that the color of grapefruit juice concentrate faded slightly during frozen storage at −23 °C, and it became apparent in the diminishing of red character and increase of the yellow character, while the sample became lighter. Color parameters indicated that color fade of concentrate in the plastic was more intense that the color fade of concentrate in the metal cans.

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Received for review February 6, 2002. Revised manuscript received April 23, 2002. Accepted April 29, 2002.

JF020159Q