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Technical paper

Color Changes and Carotenoid Pigment Loss in Retentate from *Star Ruby* Grapefruit Juice under Refrigerated Conditions

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Retentate, highly pigmented pulp, from red colored *Star Ruby* grapefruit juice was placed in glass test tubes and stored in a refrigerated locker at 4.5°C for 8 weeks. The effect of light exposure (Cool White Fluorescence, 4150 K) on visual color changes using CIE color parameters (*L, *a**, *b**, hue angle, and chroma), and predominant carotenoid pigment (lycopene, β-carotene) contents by HPLC were investigated during the storage period. The changes in color parameters in the retentate were small, causing a slight color shift. Gradual decline of CIE *a** value indicated the apparent loss of red character in the samples. Total carotenoid concentration gradually declined by more than 25% for both samples after 8 weeks of storage, but no significant effect of light-exposure on pigment loss was observed. Analysis of lycopene and β-carotene by HPLC indicated slight differences in loss but were not statistically significant under this condition.**

Keywords: *Star Ruby* grapefruit, retentate, visual color changes, lycopene, β-carotene, HPLC

The *Star Ruby* grapefruit originated from an irradiated seed of the Hudson grapefruit, a minor seedy red grapefruit, and has a pleasing visual appearance to attract consumers (Cruse *et al*., 1979; Huffman *et al*., 1953; Rousseff *et al*., 1992). Since major carotenoids are responsible for the red color of the grapefruit, color is used as an index of quality for grapefruit products. But it often yields a juice product with a color which is neither distinctive nor pleasing. Carotenoid oxidation is considered as one of the major causes for color loss.

It has been widely reported that carotenoids are susceptible to light, oxygen, heat, and acid degradation due to the presence of highly unsaturated molecules comprising many conjugated double bonds (Nguyen & Schwartz, 1999). Significant effects of light-exposure on carotenoid stability and different degradation reaction kinetics between major carotenoids in aqueous model systems has been reported (Pesek & Warthesen, 1987; 1988). Some authors had suggested that the photostability of carotenoids remains important with the continued use of glass-packaging for juices (Pesek & Warthesen, 1988) and reported that carotenoids may be degraded due to light-permeable packaging (Pesek & Warthesen, 1990).

In a recent study with red grapefruit juices packed in transparent plastic bottles, the effects of dissolved oxygen and vitamin C contents on carotenoid stability and their effect on visual color were presented (Lee, 1998). A greater understanding of the carotenoid pigment stability and its effect on visual color can be a benefit to the improvement of juice quality of red grapefruit products. Moreover, since some red pigmented grapefruit products are packed in transparent packages and marketed under light exposure, the evaluation of effects of light on carotenoid stability is necessary.

The objectives of this study were to evaluate the stability of carotenoid pigments from *Star Ruby* grapefruit under refrigerated condition (4.5°C) and their potential effects on juice color changes. The effects of light exposure on color change were also studied.

Materials and Methods

Sample preparation Retentate (highly pigmented pulp, 9.3 °Brix) was separated from red colored *Star Ruby* grapefruit juice using an ultra-filtration membrane (pilot plant scale, Citrus Research & Education Center, Univ. of Florida, Lake Alfred, FL). Samples were homogenized using an Omni Mixer Homogenizer (Omni International, Marietta, GA) for 1 min at speed 4 and then were equally divided (14 ml) into 15 ml glass test tubes and pasteurized in a thermostatic water bath operating at 90°C for 90 s before storage to prevent fermentation. Samples were placed in a light chamber with Cool White Fluorescent at 4.5°C (Gretag Macbeth, New Windsor, NY). A second set of samples was covered with aluminum foil to prevent light exposure and stored in the same light chamber. The custom built light chamber was maintained at a constant light intensity of 4150 K, at 4.5°C to simulate market conditions. All procedures for sample preparation were done excluding light.

Color analysis Samples were diluted (17.85 times) with water before color measurement. 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. Chroma $[(a^{*2}+b^{*2})^{1/2}]$, hue angle ($\tan^{-1}$ b^*/a^*), and total color difference (ΔE^*) were calculated from tristimulus values CIE *L** (lightness), *a** (redness), and *b** (yellowness) (Francis & Clydesdale, 1975; McGuire, 1992).

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Total color difference (ΔE^*) expressed the magnitude of difference between initial non-aged retentate and storage-aged retentate.

Determination of total carotenoid pigment content Total carotenoid determination was carried out on an aliquot of hexane by spectrophotometric method. Extraction of carotenoid pigment in retentate followed the method of Sadler *et al*. (1990). Samples were filled to 250 ml with distilled water and homogenized for 60 s at speed 4. Two milliliters of sample was mixed with 5 ml of extracting solvent (hexane : acetone : ethanol, 500 : 250 : 250, v/v/v), agitated, and centrifuged (International Equipment Company, Needham Heights, MA) for 5 min at 6500 rpm at 5°C. Absorbance of the top layer of hexane containing the carotenoids was measured at 472 nm using a Spectronic Genesis 5 spectrophotometer (Milton Roy, Rochester, NY). Total carotenoid contents (ppm) were calculated from an extinction coefficient of 3450 in hexane (Arroyave *et al*., 1982).

Determination of carotenoid pigment by HPLC Analysis of lycopene and β-carotene was performed by an HPLC method (Lee & Coates, 1999). Two model 510 pumps (Waters Associates, Milford, MA) with a variable UV/vis detector (Scientific Systems, Inc., State College, PA) at 450 nm, YMC carotenoid TM reversed-phased column (C30, 4.6 mm×250 mm), and a gradient mobile phase of methanol (eluent A) and methyl-*t*-butyl ether (eluent B) comprised the HPLC system. A 10 μl injection volume of sample was analyzed at a flow rate of 1.0 ml/min. Duplicate injections were conducted.

Statistical analysis All test results were the average of duplicate samples and all statistical analyses including means, standard deviations of the means, and analysis of variance (ANOVA) were performed using Sigma Stat 2.0 PC software with significant level, α=0.05. Differences between means were evaluated using T-test. Plots from statistical evaluation of the data were done by using Microsoft Excel computer software.

Results and Discussion

Visual color changes Table 1 presents the changes in CIE color parameters in retentate (highly pigmented pulp) during the storage period at 4.5°C. The color changes as a function of storage time under refrigerated condition were small, and color changes are probably due to gradual decline in CIE a^* value, which has a red hue. However, changes in other color parameters (CIE b^*, and L^*) were relatively small, and appeared to increase as a function of storage time. Hue values are presented in Table 1 to describe the general trend of sample hue over time. Hue angles increased slightly and then decreased over time, indicating a shift towards redness especially for light-exposed samples. This is probably due to the fact that dominant red lycopene loss is accompanied by loss of yellow β-carotene.

The effect of light-exposure on color was not significant (p>0.05). Reduction of b^*-value and chroma (color intensity) in the light exposed sample were faster than the non-light exposed (control) sample during storage. But there were no significant differences (p>0.05) between the control and light exposed sample. The retentate can be classified as a yellow-orange color based on the initial hue angle, and a significant difference (p <0.05) was observed in change of this angle between dark and light exposed sample. These light-exposure periods contributed significantly to changes in the "Hue" angle of retentate.

Total color difference (ΔE^*), which is calculated based on color differences compared to the initial retentate, was investigated to better understand the overall visual color change. The ΔE^* value was gradually increased during refrigerated storage in both samples as a function of time (Table 1). The ΔE^* value was less than 1.0 unit after 8 weeks of storage, which could be considered as no noticeable difference after refrigerated storage. Thus, effect of storage temperature on visual color change appears minimal under this storage condition. Furthermore, there are no signficant differences in ΔE^* values between the control (0.6) and light-exposed samples (0.7) after 8 weeks storage.

Changes in carotenoid pigment Total carotenoid concentration, which was estimated by absorbance at 472 nm, also declined gradually for both samples (Table 1). Initially, the total carotenoid content in retentate wass 98.9 ppm and gradually declined by 25.5% for the control and 28.6 % for light-exposed

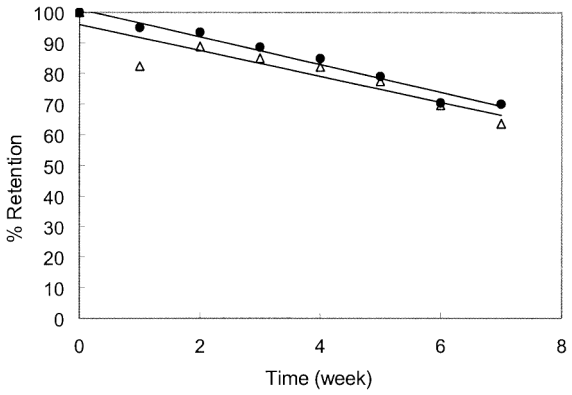

Fig. 1. Changes in β-carotene retention as a function of storage time (control, ●; light-exposed, △).

Table 1. Changes in CIE color parameters of retentate from *Star Ruby* grapefruit juice during storage at 4.5°C.

Week	Control							Light-exposed						
	L^*	a^*	b^*	Hue	Chroma	ΔE^*	Total Carotenoid (ppm)	L^*	a^*	b^*	Hue	Chroma	ΔE^*	Total Carotenoid (ppm)
0	33.5	3.2	4.3	53.4	5.3	—	98.8	33.5	3.2	4.3	53.4	5.3	—	98.8
1	33.4	3.2	4.6	54.9	5.6	0.3	91.8	33.7	3.3	4.6	54.2	5.7	0.4	93.7
2	33.6	3.0	4.5	56.4	5.3	0.3	90.5	33.3	3.1	4.2	54.0	5.2	0.2	85.2
3	33.4	3.3	4.9	56.4	5.9	0.6	90.2	33.2	3.1	4.5	55.0	5.5	0.3	86.9
4	33.1	2.9	4.6	57.3	5.4	0.6	88.8	33.0	3.0	4.0	52.8	5.0	0.6	86.3
5	33.5	3.1	5.0	58.1	5.9	0.7	86.4	33.3	3.2	4.3	53.1	5.3	0.2	84.1
6	33.6	3.0	3.9	52.3	4.9	0.5	83.7	33.5	2.9	4.8	58.6	5.6	0.5	78.7
7	33.4	3.0	4.9	58.9	5.7	0.6	75.3	33.7	3.0	3.7	51.1	4.8	0.6	74.9
8	33.4	2.9	4.9	59.1	5.7	0.6	73.9	33.6	3.0	3.7	51.4	4.7	0.7	70.5

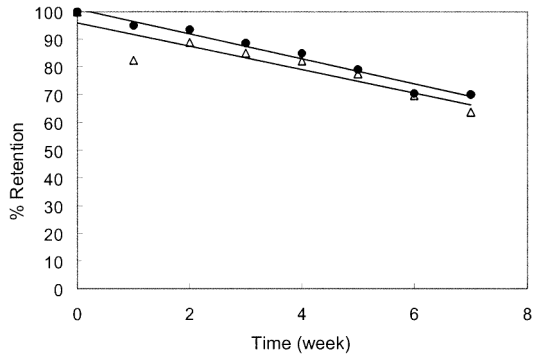

Fig. 2. Changes in lycopene retention as a function of storage time (control, ●; light-exposed, △).

samples after 8 weeks of storage.

Figure 1 and Figure 2 illustrate the percent retention of β-carotene and lycopene content by HPLC in retentate as a function of storage time. Initially, the retentate contained 7.61 ppm β-carotene and 30.28 ppm lycopene. Regression analysis shows that about 75% β-carotene and 70% lycopene in the control sample, and 63% β-carotene and 64% lycopene in light exposed sample still remained after 8 weeks storage at 4.5˚C.

Quantitative losses of the β-carotene and lycopene in both samples appeared to be significant as a function of storage time. This result also implied that the degradation rate of lycopene was greater under lighted condition. Lycopene in the light exposed sample showed slightly less retention compared to the control sample, but differences were not significant ($p>0.05$) under this condition.

A previous study (Pesek & Warthesen, 1987) reported that lycopene degraded at a rate approximately one-fifth that of β-carotene in a vegetable juice. Slight differences in structure between the two hydrocarbons could account for differences in the degradation rate. However, in this experiment, no discernable difference ($p>0.05$) in retention between β-carotene and lycopene was observed.

The decrease of red color in retentate is probably due to degradation of lycopene, the major carotenoid responsible for red color in grapefruits (Ting & Deszyck, 1958). In a previous study, a decrease in Gardner *b*-value showed a significant correlation with photodegradation of yellow pigments, α- and β-carotenes in vegetable juice system (Pesek & Warthesen, 1987).

In conclusion, there was a quantitative pigment loss during refrigerated storage, but effects of light-catalyzed color degradation appear to be limited under this condition which is probably due to the fact that grapefruit pigments are embedded in the pulp.

References

Arroyave, G., Chichester, C.O., Flores, H.G., Glover, J., Mejia, L.A., Aolson, J.A., Simpson, K.L. and Underwood, B.A. (1982). Biochemical methodology for the assessment of vitamin A status (A report of the international vitamin A consultative group, IVACG). Nutrition Foundation Publication, Washington D.C, 529–531.

Cruse, R., Lime, B. and Hensz, R. (1979). Pigmentation and color comparison of Ruby and Star Ruby grapefruit juice. *J. Agric. Food Chem.*, **27**, 641–647.

Francis, F.J. and Clydesdale, F.M. (1975). Food colorimetery: Theory and Applications. AVI Publishing Company Inc., Westport, CT.

Huffman, W.A.H., Lime, B.J., and Scott, W.C. (1953). Processed juices from Texas red and pink grapefruit. A progress report. *Proc. Rio Grande Valley Hort. Inst.*, **7**, 102–105.

Lee, H.S. (1998). Color and vitamin C of red grapefruit juices in refrigerated storage. Citrus Processing and Technology Conference. Lake Alfred, FL.

Lee, H.S. and Coates, G.A. (1999). Thermal pasteurization effects on color of red grapefruit juice. *J. Food Sci.,* **64**, 663–666.

McGuire R.G. (1992). Reporting of objective color measurements. *Hortscience*, **27**, 1254–1259.

Nguyen, M.L. and Schwartz, S.J. (1999). Lycopene: Chemical and biological properties. *Food Tech.*, **53**, 38–45.

Pesek, C.A. and Warthesen, J.J. (1987). Photodegradation of carotenoids in a vegetables juice system. *J. Food Sci.*, **52**, 744–746.

Pesek, C.A. and Warthesen, J.J. (1988). Characterization of the photodegradation of β-carotene in aqueous model systems. *J. Food Sci.*, **53**, 1517–1520.

Pesek, C.A. and Warthesen, J.J. (1990). Kinetic model for photoisomerization and concomitant photodegradation of β-carotenes. *J. Agric. Food Chem.*, **38**, 1313–1315.

Rousseff, R., Sadler, G.D., Putnam, T.J. and Davis, J.E. (1992). Determination of β-carotene and other hydrocarbon carotenoids in Red Grapefruit cultivars. *J. Agric. Food Chem.*, **40**, 47–51.

Sadler, G., Davis, J. and Dezman, D. (1990). Rapid extraction of lycopene and β-carotene from reconstituted tomato paste and pink grapefruit homogenates. *J. Food Sci.*, **55**, 1460–1461.

Ting, S.V. and Deszyck, E.J. (1958). The internal color and carotenoid pigments of Florida red and pink grapefruit. *Pro. Am. Soc. Hort. Sci.*, **71**, 271–277.