Rates of Vitamin C Loss and Discoloration in Clear Orange Juice Concentrate during Storage at Temperatures of 4−24 °C†

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The rate of vitamin C degradation in clear orange juice concentrate (80.2 °Brix) produced by the combined membrane−evaporation technique was studied at storage temperatures of 4, 14, and 24 °C for 19 weeks. Absorption at 420 nm and CIE *L**, *a**, *b** color parameters were determined to evaluate color changes due to nonenzymic browning during storage. Calculated values of reaction rate constants indicated that vitamin C degradation occurred slowly in clear orange juice concentrate at 4 °C. Rates of vitamin C degradation ranged from 4.79×10^{-4} to 3.13×10^{-2} per week. The activation energy for vitamin C degradation was on the order of 34.3 kcal/g·mol. Main color changes in stored clear orange juice concentrate in amber glass vials were due to increases in *b** value and chroma.

Keywords: *Clear orange juice concentrate; vitamin C; color; browning, membrane*

INTRODUCTION

Recently, a combined membrane−evaporation process was applied to develop an improved method for concentrating orange juice (Johnson, 1993). A semipermeable membrane was used to separate the juice into pulpy suspended solids (retentate) and clarified juice (permeate). The membrane-clarified clear juice was then concentrated by a conventional evaporative method using a TASTE (thermally accelerated short time evaporator) evaporator. The pulpy retentate may be pasteurized by a heat exchanger. The clear juice concentrate from the combined membrane−evaporation technique can reach a higher degree Brix than traditional whole juice concentrate processed in a TASTE evaporator under similar conditions (Hernandez et al., 1995). Higher degree Brix orange juice concentrates offer significant energy savings during storage and distribution due to reduced volume and increased microbial stability at higher temperatures (Crandall and Graumlich, 1982). The resulting clear juice concentrated can also be further utilized to formulate new fruit beverage juice blends.

Previously, storage stability values of whole orange juice concentrate (Crandall and Graumlich, 1982; Johnson and Toledo, 1975; Kanner et al., 1982; Marcy et al., 1984) as well as clarified citrus syrups prepared by centrifuge techniques (Bruemmer and Bowers, 1977; Onayemi and Bruemmer, 1984) were reported. Juice concentrates containing >65% total solids are normally stable at any temperature from the standpoint of fermentation (Sand, 1973), but when stored at relatively high temperatures, nonenzymic browning reactions are expected (Toribio and Lozano, 1984). Previously, Johnson and Toledo (1975) indicated that short-term storage at ambient temperature for whole orange juice concentrate is feasible if the package is impermeable to oxygen and oxygen is eliminated from the headspace. Our preliminary study (Lee et al., 1994) with high-density clear orange juice concentrate of 80 °Brix also indicated that major changes in clear orange juice concentrate during storage were due to chemical reactions rather than microbial deterioration. Recently, first-order rate constants for nonenzymic browning and ascorbic acid loss of membrane-clarified clear juice during conventional evaporative processes at 70.3−97.6 °C have been presented (Johnson et al., 1995).

Because clear orange juice concentrate produced by the combined membrane−evaporation technique is a new type of product and this highly concentrated (>80°Brix) clear orange juice resembles honey or sugar syrups, which are usually stored and marketed at ambient temperature, we desired to evaluate the storage stability of membrane-clarified clear orange juice concentrate at temperatures above freezing. This could feasibly replace conventional frozen storage of orange juice concentrates. This paper presents experimental kinetic constants of estimating vitamin C retention and visual color changes in membrane-clarified orange juice concentrate during storage at 4, 14, and 24 °C.

MATERIALS AND METHODS

Clear Orange Juice Concentrates. Valencia oranges were used to prepare membrane-clarified juice concentrate in the pilot plant at the Citrus Research and Education Center, University of Florida, Lake Alfred, FL. Briefly, oranges were extracted and the juice finished according to standard operating procedures (Redd et al., 1986).

For membrane-clarified clear juice concentrate, single-strength orange juice was processed through hollow-fiber polysulfone ultrafiltration membrane cartridges (Koch Membrane System, Inc., Wilmington, MA) to remove the pulp, and then the clarified juice (permeate) was concentrated using a pilot plant scale TASTE evaporator (Gulf Machinery Co.,

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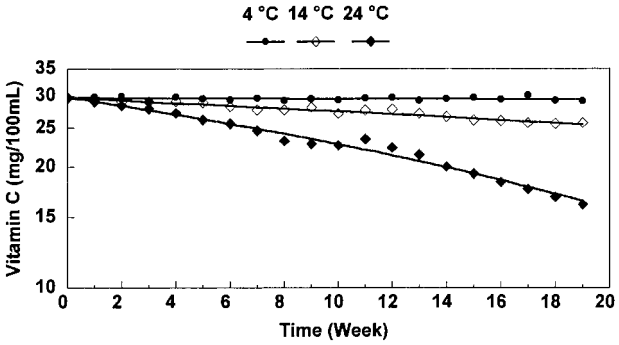

Figure 1. Kinetics of vitamin C loss in clear orange juice concentrate during storage.

Clearwater, FL). The membrane system consisted of three polysulfone hollow-fiber membrane cartridges arranged in parallel. The molecular weight cutoff was 5×10^5. The TASTE evaporator was a five-effect, five-stage pilot plant evaporator. The °Brix was 80.2 for clear juice concentrates, which were stored in a −23 °C freezer before testing.

Storage Test. The clear juice concentrates were each weighed (4 ± 0.02 g) into small amber glass vials (15 × 45 mm) and capped with septa, PTFE/silicone caps, and stored in 24, 14, and 4 °C storage lockers for 19 weeks. Each stored clear juice concentrate was reconstituted to 11.8 °Brix using deionized water and filtered through a 0.45 μm Acrodisc filter cartridge from Gelman Co. (Ann Arbor, MI) before analysis of vitamin C, browning index, and color.

Vitamin C Analysis. Analysis of vitamin C was carried out according to the HPLC procedure of Lee and Coates (1987). The chromatographic system consisted of a Waters model 600 E system controller/pump, a Waters model 717 plus autoinjector with cooler (Waters Corp., Milford, MA), a Spectra-Physics model 200 programmable wavelength UV detector, and a Zorbax ODS column with 5 μm packing (250 × 4.6 mm). Integration and data storage were performed using Millennium chromatography software from Waters Corp. The ascorbic acid was eluted isocratically with a mobile phase of 2% KH_2PO_4 (pH 2.4) at a flow rate of 0.5 mL/min elution. The eluate was monitored at 245 nm.

Browning and Visual Color Measurements. Browning was measured by absorbance at 420 nm with a Bausch and Lomb Spectronic 88 using a 13 mm cuvette. Color (CIE L^*, a^*, b^*) values were measured by a Macbeth color-eye 3000 spectrophotometer (Macbeth Division of Kollmergen Instruments Co., Newburgh, NY). Chroma $[(a^{*2} + b^{*2})^{1/2}]$ and hue angle ($\tan^{-1} b^*/a^*$) were calculated from tristimulus values CIE L^*, a^*, and b^*.

Data Analysis. All test results are the average of duplicate samples and presented on the basis of single strength (11.8 °Brix). All statistical analyses were performed using SigmaStat 2.0 PC software from SPSS, Inc. (Chicago, IL) with significance at $p < 0.05$. Plots from statistical evaluation of the data were done by using TableCurve 2D computer software from SPSS Science (Chicago, IL).

Kinetics of Vitamin C Loss. Vitamin C degradation data were assumed to fit first-order kinetics (Johnson et al., 1995). The integral form of a first-order reaction can be written as the following expression: $\ln([D]_0/[D]_t) = kt$, where $[D]_0$ is the vitamin C content at time 0, $[D]_t$ is the value after reaction time t, and k is the reaction rate constant. The Arrhenius relationship was assumed for the temperature dependence for the reaction rate constant k as follows: $k = A_0 \exp(-E_a/RT)$, where E_a is the activation energy of the reaction, R is the gas constant, T is the absolute temperature, and A_0 is a pre-exponential constant.

RESULTS AND DISCUSSION

Vitamin C Loss. In Figure 1, the kinetics of vitamin C degradation in membrane-clarified clear orange juice concentrate as a function of storage time and temperature are presented in a semilogarithmic plot. As noted, retention of vitamin C decreased as storage temperature and time increased. During 19 weeks of storage, vitamin C content decreased 45.8% at a storage temperature of 24 °C. In the samples stored at 14 °C, vitamin C loss was ~13.2%, and at 4 °C, the loss was limited to 2.7%. Since membrane-clarified orange juice concentrate is a relatively new citrus product, there are no previous data available in the literature on the kinetics of vitamin C degradation for comparison. Loss of vitamin C in clear orange juice concentrate could be due to aerobic and anaerobic reactions as reported in whole orange juice concentrate by Crandall et al. (1981), Johnson and Toledo (1975), and Kanner et al. (1982). Vitamin C destruction beyond pasteurization treatment in whole orange concentrate is known to be affected significantly by the storage time and temperatures (Marcy et al., 1984) and by the degree of juice concentration (Kanner et al., 1982).

Most quality-related reaction rates are either zero- or first-order reactions, and the statistical differences between both types may be small (Labuza and Roboh, 1982). There were no statistically significant differences between zero-order and first-order models at 4 and 14 °C, but they were significantly different at 24 °C. Vitamin C degradation in this stored clear orange juice concentrate was assumed to follow first-order reaction kinetics as suggested by previous work (Kanner et al., 1982), which showed that degradation of ascorbic acid in whole orange juice concentrate follows first-order reaction kinetics at 25 °C and below. However, at higher storage temperatures, departures from pure first-order reactions have been observed (Kanner et al., 1982; Nagy and Smoot, 1977). Acceleration effects from many breakdown products of vitamin C degradation at higher storage temperatures were responsible for departures from pure first-order reactions (Clegg, 1964).

The rate constants, calculated by computer programs, of vitamin C degradation (mg/100 mL) for 19 weeks were 4.79×10^{-4} per week at 4 °C, 7.95×10^{-3} per week at 14 °C, and 3.13×10^{-2} per week at 24 °C. As expected, the rate of ascorbic acid degradation in juice concentrate increased with temperature. The rate at 24 °C is ~65.2-fold higher than the rate at 4 °C.

The activation energy for the degradation of vitamin C in clear orange juice concentrate stored between 4 and 24 °C was on the order of 34.3 kcal/g·mol. Note that the E_a for vitamin C loss determined from this study was slightly higher than the reported value (29.7 kcal/g·mol) from whole orange juice concentrate at high-temperature, short-time processing condition (Johnson et al., 1995). A higher E_a implies that orange serum concentrate could be more susceptible to thermal degradation than whole orange juice concentrate.

Because vitamin C is one of the principal nutrients in citrus products, and degrades rapidly, quantitative analysis of vitamin C content was considered as one of the simple approaches to predict the shelf life of citrus juices. The estimated vitamin C retention values for storing this clear juice concentrate for up to one year at 4, 14, and 24 °C are calculated and presented in Table 1. Information on percent of daily value (DV) of vitamin C based on a 240 mL serving size of single-strength juice is also included in Table 1. The percent DVs are computed using the Reference Daily Intake (RDI) for vitamin C, 60 mg, as established by the U.S. Food and

Table 1. Estimated Vitamin C Retention in Clarified Orange Juice during Storage Based on 240 mL Serving

time (weeks)	vitamin C content (mg/240 mL) at storage temp of		
	4 °C	14 °C	24 °C
0	71.4 (119%)[a]	71.4 (119%)	71.4 (119%)
10	70.8 (118%)	65.9 (110%)	53.7 (90%)
20	70.5 (117%)	60.9 (101%)	39.3 (66%)
30	70.2 (117%)	56.2 (94%)	28.8 (48%)
40	69.8 (116%)	51.9 (87%)	21.0 (35%)
50	69.5 (116%)	48.0 (80%)	15.4 (26%)
52	69.4 (116%)	47.2 (79%)	14.5 (24%)

[a] Values in parentheses are the % DV (daily value).

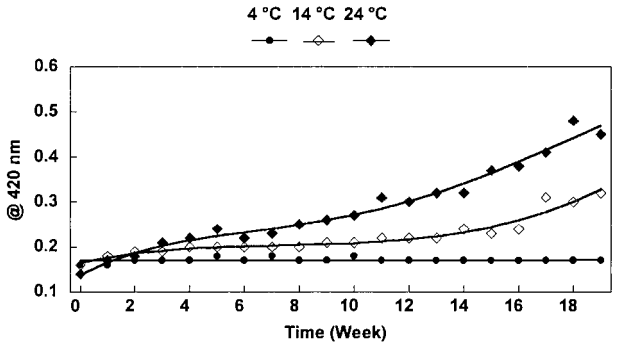

Figure 2. Changes in browning index (at 420 nm) in clear orange juice concentrate during storage.

Drug Administration (FDA, 1993). As high as 116% of the DV of vitamin C can be retained on the basis of a 240 mL serving size at 4 °C for 1 year.

Nonenzymic Browning. The extent of discoloration due to browning of clear juice concentrate during storage periods was determined by measurement of absorbance at 420 nm. Figure 2 presents regression plots from the extent of browning in clear orange juice concentrates at the three storage temperatures. Polynomial regression equations were applied to describe the absorbance changes over storage times. At 24 °C, absorbance at 420 nm sharply increased ~1.5 times within 3 weeks compared to the initial sample and then gradually increased to definite browning at the end of storage. After 19 weeks storage, the absorbance at 420 nm in clear juice concentrates increased to ~3.2 times (24 °C) and ~2.0 times (14 °C) compared to the initial samples. For clear juice concentrate stored at 4 °C, browning did not show a definite pattern, with only slight fluctuation in the value. Thus, for clear juice concentrate stored at 4 °C, no significant change in absorbance versus time over a 19 week period was observed as evidenced by a virtually zero slope in Figure 2. Previous work with whole orange juice concentrate (72 °Brix) also reported no significant browning during 12 months of storage at 4.4 °C or lower (Crandall and Graumlich, 1982).

Previously, absorbance changes related to browning in stored citrus juices were best described by complex equations with higher polynomial functions (Nagy et al., 1990). Polynomial regression equations for best curve fittings for browning of serum concentrate stored at 14 °C yielded curved linear profiles [$Y = 0.165 + 0.013x - 0.002x^2 + (7.024 \times 10^{-5})x^3$, $r^2 = 0.9449$]. The equation that described 24 °C stored serum concentrate also contained higher polynomial functions [$Y = 0.149 + 0.019x - 0.001x^2 + (6.428 \times 10^{-5})x^3$] with correlation coefficients >0.97.

Because previous work with browning pigment formation in stored citrus juices indicated the presence of numerous brown pigments with multiple reaction kinetics (Rouseff et al., 1989), no simplistic model is suggested to define the brown discoloration of citrus juices based on relative changes in absorbance at 420 nm, but complex polynomial models were applied to statistically define the nonenzymic browning profiles (Nagy et al., 1990). Johnson et al. (1995) indicated that the nonenzymic browning in orange serum concentrate during the evaporative process is complex and true order may be between 0 and 1. The development of browning pigment formation in orange serum concentrate as plotted in Figure 2 suggests faster and more extensive browning changes compared to the browning in single-strength citrus juices (Nagy et al., 1990), in which minimal browning occurred during storage at 10−20 °C for an 18 week period. Nonenzymic browning in citrus juice occurs much more quickly with concentrated juice than with single-strength juice and was found to be positively correlated to the level of juice concentration (Kanner et al., 1982).

The results of the browning measurements are in accordance with vitamin C destruction, and it is generally agreed that vitamin C degradation provides reactive carbonyl groups which can be precursors that play a major role in brown pigment formation and darkening in citrus juices during storage (Clegg, 1964). Because clear juice concentrate is high in vitamin C and its destruction appears to mainly depend on storage time and temperature after processing, low temperature is necessary to retain the vitamin C and retard the consequent browning during storage. This is especially true for juice concentrate for which susceptibility to nonenzymic browning increases as the level of juice concentrate increases.

Color changes. CIE L^*, a^*, b^* color values were also measured to evaluate the visual color changes associated with nonenzymic browning during storage. The clear juice concentrate had a light orange-yellow color similar in appearance to sugar syrups such as honey. The L^* value, which can be an indicator of lightness of color, decreased slightly as a function of storage time and temperature. At 24 °C, even though the color of the concentrate was definitely manifested by nonenzymic browning, its L^* value decreased to only ~2.1% of its initial value after 19 weeks of storage. At 14 °C, the L^* value decreased to ~0.2% of its initial value, and at 4 °C, there were virtually no changes in L^* value during the 19 week storage period. Changes in lightness of clear juice concentrate color were not significant ($p > 0.05$) during storage. Even though the absorption at one fixed wavelength may not be adequate for representing the entire visual color changes in clear orange juice concentrate, the absorbance measurement at 420 nm appeared to be sensitive to the degree of discoloration in clear juice concentrate rather than the L^* value measurement (lightness), especially at relatively low temperatures. In a previous storage test with citrus juices, Lee and Nagy (1988) also reported no significant ($p > 0.05$) differences in the L^* values from the storage test of grapefruit juice at 20 °C for 21 weeks.

CIE a^* and b^* values are used to represent serum colors and calculate color differences between initial and stored clear juice concentrates. Color difference measurements of the a^* and b^* values are plotted in a chroma diagram (Δa^* versus Δb^*) in Figure 3. This plot clearly illustrates that changes of a^* and b^* parameters became progressively more pronounced with each stor-

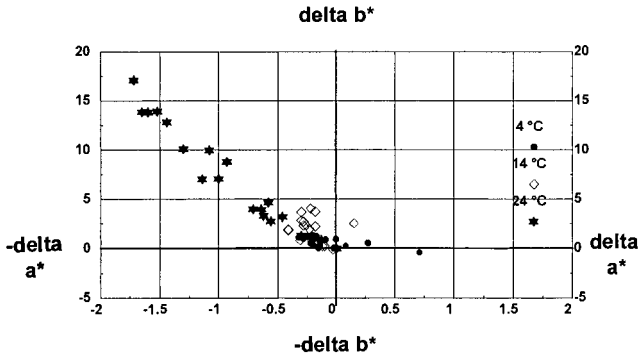

Figure 3. Changes in Δa^* and Δb^* values in clear orange juice concentrate during storage.

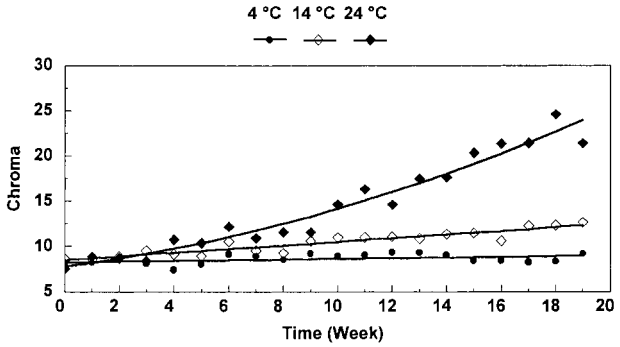

Figure 4. Changes in chroma in clear orange juice concentrate during storage.

age period as the storage temperature increased (Figure 3). Especially large changes were found in the b^* value. During storage, b^* values gradually increased in all samples, indicating a general change to yellower hues, and negative a^* (greenness) values declined slightly during storage. The decline of negative a^* values corresponded to a greener hue upon storage. Thus, Δa^* increased to more negative direction and Δb^* shifted toward more positive direction as shown in Figure 3. A positive Δb^* indicated that there is more yellowness than blueness, and negative Δa^* indicated that there is more greenness than redness in the color of stored clear juice concentrate. It is interesting to note that these changes in a^* and b^* values in membrane-clarified orange juice concentrate are opposite of the color change observed with whole orange juice concentrate, in which values changed to more red hue (increase in a^* value) and less yellowness (decrease in b^* value) after 12 months of storage at 26.7 °C (Crandall and Graumlich, 1982). The discrepancy in color change between membrane-clarified orange juice and whole orange juice concentrates is probably due to the loss of pigmented pulp in clear juice during membrane filtration. Most of the suspended pigmented pulp in orange juice is not allowed to pass through to the filtrate side but is retained in the retentate during membrane filtration. No appreciable visual color changes in stored clear juice concentrate at 4 °C were found, which is in good agreement with previous work with whole orange juice concentrate (72 °Brix) at 4.4 °C for 1 year of storage (Crandall et al., 1981; Crandall and Graumlich, 1982).

Chroma (color intensity) increased gradually as the clear juice concentrate became more intense in color due to browning (Figure 4). Changes in chroma versus time over a 19 week period were analyzed by fitting the data to the equations from the curve fitting software, and correlation coefficients and parameters for best line fit were calculated. For clear juice concentrate stored at 4 °C, no significant change in chroma versus time over the 19 week period was observed. Equations that fit the 14 and 24 °C serums yielded curve lines (Figure 4) similar to browning but contained statistical quadratic functions (x^2). The regression equation for chroma is $8.586 + 0.172x + 0.002x^2$ for clear juice concentrate at 14 °C and is $7.785 + 0.398x + 0.024x^2$ for 24 °C, where x is a week. Color measurement of clear orange juice concentrate showed that the main color changes ($p < 0.05$) during storage were due to an increase in b^* value and chroma rather than changes in L^* and a^* values or hue angle. Also, among color parameters, b^* value ($r = 0.962$) and chroma ($r = 0.961$) were well correlated with the visual browning measurement based on absorbance at 420 nm. However, in a previous storage study with whole orange juice concentrate (Crandall and Graumlich, 1982), the L^* value appeared to be the better indication of color changes or browning than other color parameters.

In summary, the results of this storage test showed that the rate of quality changes in clear orange juice concentrate stored at 4 °C was relatively slow based on vitamin C retention and visual browning and might have economic feasibility for storage at refrigerated temperature. As high as 116% of the DV of vitamin C retention was estimated at 4 °C for up to 1 year with little color deterioration. According to our results, the main color change in stored clear orange juice concentrate was due to increases in chroma and b^* values, which were in high correlation to browning measurement.

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