PACKAGING TECHNOLOGY AND SCIENCE
Packag. Technol. Sci. **12**, 111–118 (1999)

Comparative Effects of De-aeration and Package Permeability on Ascorbic Acid Loss in Refrigerated Orange Juice

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Several factors including pH, cultivar, extraction method, metal ion content and storage conditions affect the rate of ascorbic acid loss in refrigerated fruit juices. While oxygen permeation rate and product de-aeration also influence ascorbic acid loss, little comparative data on these two variables exist despite the potential usefulness of such data in optimizing the packaging of juice. De-aerated and non-de-aerated single-strength orange juices were packaged and stored at 7°C in experimental glass containers constructed with oxygen permeability rates of 0·35, 0·39, 0·43, 0·79, 1·18 and 1·60 ml/day/container at 7°C. The rate of ascorbic acid degradation inversely correlated with permeation rate for both de-aerated and non-de-aerated juices regardless of initial dissolved oxygen content. Degradation was best described by zero-order and first-order kinetics for de-aerated and non-de-aerated juices, respectively. Headspace volume had no effect on ascorbic acid loss in both de-aerated and non-de-aerated juices when nitrogen flushed. Juice in high oxygen permeability containers showed a faster decrease in ascorbic acid content, independent of initial dissolved oxygen content. These results indicate that both package barrier properties and de-aeration are major factors in maintaining ascorbic acid in refrigerated orange juice. Copyright © 1999 John Wiley & Sons, Ltd.

Received 7 December 1998; Accepted 17 March 1999

KEY WORDS: ascorbic acid stability; orange juice; oxygen permeability; package permeability; citrus juice

INTRODUCTION

Quality deterioration in the form of ascorbic acid loss, colour change and development of off-flavours occurs in refrigerated juices during storage. The factors affecting ascorbic acid loss in juices are complex and depend on several variables including cultivar, metal ion content, processing parameters, storage environment, pH, microbial load and protection provided by the container and cannot be readily predicted. Thus, the specific kinetics of ascorbic acid loss appears to be dependent on the system studied. Early work by Joslyn and Miller[1] concluded that the kinetics of auto-oxidation of ascorbic acid in the presence of dissolved oxygen was first-order with respect to the ascorbic acid. Singh, Heldman and Kirk[2] concluded that, at saturated dissolved oxygen levels (8·71 mg/l), oxidative breakdown of ascorbic acid followed first-order kinetics. However, at

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 CCC 0894–3214/99/030111–08 $17.50

low oxygen levels the reaction followed second-order kinetics. Recent work supports the conclusion that rate order depends on oxygen content.[3,4]

In both glass bottled grapefruit juice and orange juice stored at ambient temperatures ascorbic acid degradation was directly related to initial oxygen content.[5,6] Conversely, Robertson and Samaniego[7] working with lemon juice stored at 36°C found no significant relationship between initial dissolved oxygen concentrations (0·41, 1·44 and 3·74 mg/l) and ascorbic acid degradation.

It has been suggested that juice de-aeration and anaerobic storage results in improved ascorbic acid retention.[8] However, Passy and Mannheim[9] compared the effects of vacuum de-aeration, hot filling and nitrogen sparging on concentrated grapefruit juice quality when stored at ambient temperature. They concluded that there was no difference in quality, including ascorbic acid retention and shelf life, due to the different treatments. In hermetically sealed canned juice, it has been reported that the aerobic degradation of ascorbic acid occurs initially while oxygen is present.[10] After oxygen has been consumed, degradation occurs anaerobically at a substantially lower rate.

Ohta *et al.*[11] investigated the influences of headspace volume, pasteurization temperature, pasteurization time and storage temperature on the quality of Satsuma mandarin orange juice. Headspace volume and storage temperature were found to have greater influence on juice quality than did the other parameters studied.

Despite the large volume of work on ascorbic acid loss in citrus juices, few workers have directly investigated the relationship between package oxygen permeability and the rate of ascorbic acid degradation for refrigerated juices. The objective of the study reported here was to compare the effects of dissolved oxygen content, package oxygen permeability rate, headspace volume and storage time on ascorbic acid degradation in single-strength orange juice stored at 7°C. These data could be useful in designing processing and packaging parameters for refrigerated juices.

MATERIALS AND METHODS

Two batches of single-strength orange juice were obtained by diluting concentrated orange juice (60–65°Brix) to 12°Brix, followed by pasteurization at 72°C for 15 s. Potassium sorbate was added at 0·25% to inhibit microbial growth. The first batch of juice was de-aerated under a vacuum of 27 mm Hg for 1 hour. After de-aeration, the dissolved oxygen content was 2·7 mg/l and the ascorbic acid content 530 mg/l. 12 × 227 ml glass containers (approximately 5·7 cm inside diameter × 8·9 cm deep) were filled with 100 ml each and another 12 were filled with 200 ml of juice. All were flushed with N_2 prior to closure. A second batch of juice was packaged in the same manner and in the same size containers except the juice was not de-aerated (dissolved O_2 and ascorbic acid concentrations were 6·7 mg/l and 680 mg/l, respectively). Jars were filled with 100 or 200 ml of juice to determine if the ratio of volume of juice to headspace would affect the rate of ascorbic acid loss.

Adjustment in container permeability was achieved by drilling 0, 0·28, 0·56, 2·83, 4·90 and 8·04 cm^2 holes in the metal lids over which PVC film (thickness = 13 μm, GTO_2 = 0·16 ml/day/cm^2 at 7°C) was adhered with epoxyresin. This resulted in package O_2 permeability rates of 0·35, 0·39, 0·43, 0·79, 1·18 and 1·60 ml/day/container at 7°C. These rates were determined by measuring the ingress rate of O_2 after flushing empty containers with N_2 and sampling the headspace through a rubber septum which had been added to the lid (as described below).

Samples were taken at specific time intervals after storage in the dark at 7°C and analysed for ascorbic acid, dissolved oxygen and headspace oxygen. Duplicate containers were analysed for each treatment.

Ascorbic acid was analysed using the HPLC method of Carnevale.[12] An Alltech CN μBondapack column (25 cm × 4·6 mm ID, Alltech Associates, Deerfield, IL) was used with 2% acetic acid/methanol (19:1) as the mobile phase at a flow of 1·0 ml/min; detection was at 254 nm (Beckman Instruments, San Ramon, CA; Model 160). Single-strength juices were diluted 10× with mobile phase and filtered using a 0·45 μm filter (Acetate Plus, Micron Separators Inc.) and 10 μl aliquots of the final filtrate assayed.

A YSI Model 53 (Yellow Springs, OH) oxygen sensor calibrated with air-saturated water was utilized to measure the level of dissolved oxygen

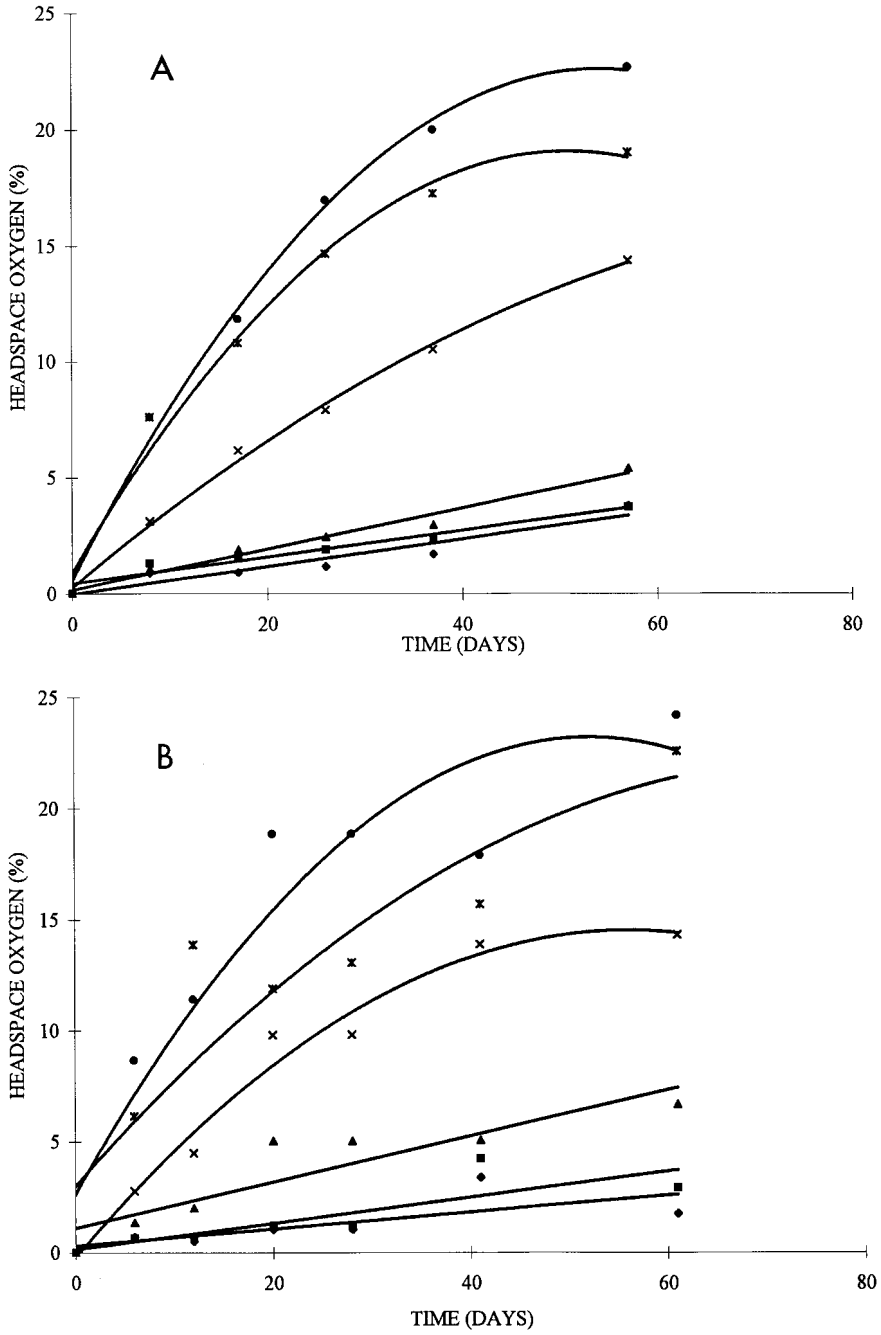

Figure 1. Headspace oxygen in de-aerated (A) and non-de-aerated (B) orange juice in containers with different oxygen permeability as affected by storage time at 7°C. Lines represent the best fit regression of the data for each permeation rate. (◆ 0.35, ■ 0.39, ▲ 0.43, × 0.79, ✻ 1.18, ● 1.60 ml O_2/day/container).

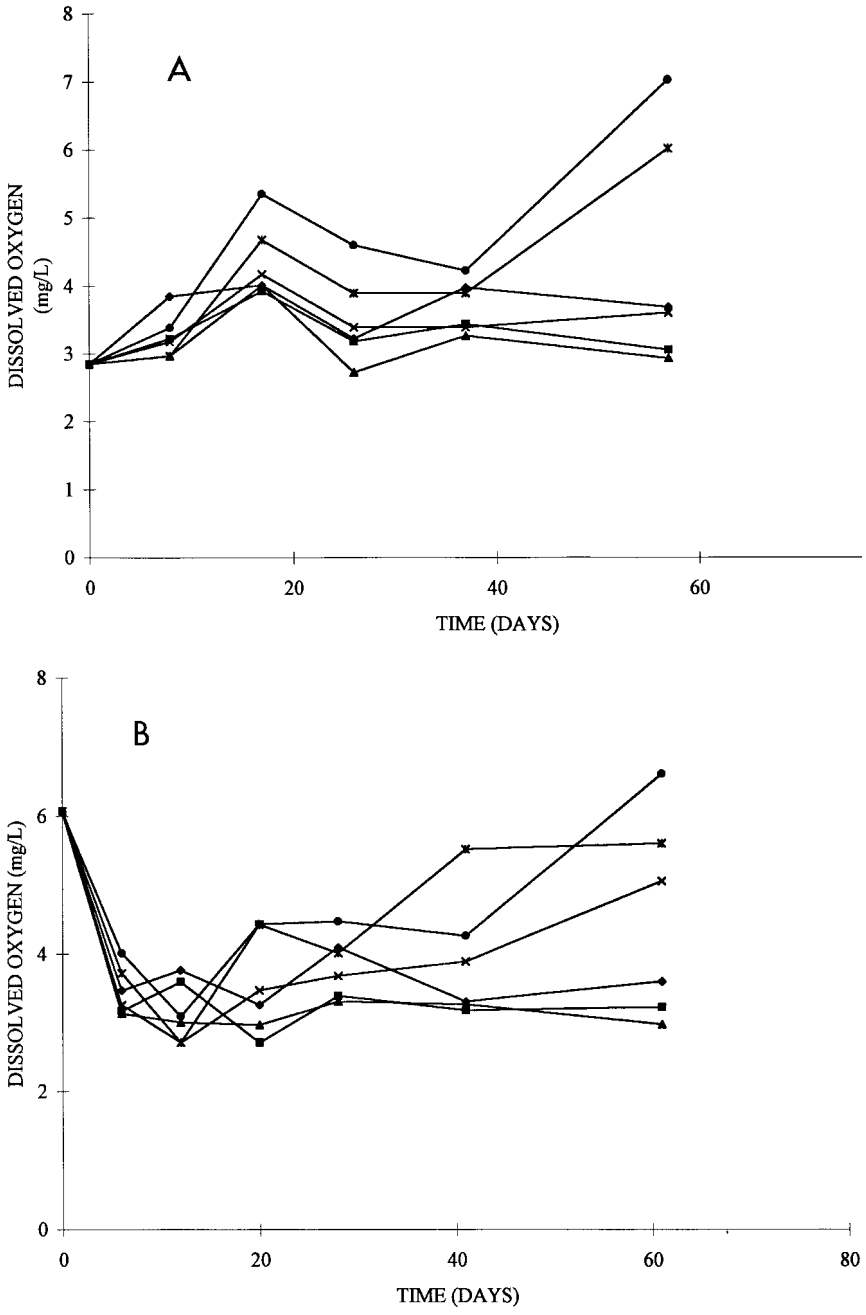

Figure 2. Dissolved oxygen content of de-aerated (A) and non-de-aerated (B) orange juice in containers with different oxygen permeability as affected by storage time at 7°C. (◆0.35, ■ 0.39, ▲ 0.43, × 0.79, ✻ 1.18, ● 1.60 ml O_2/day/container)

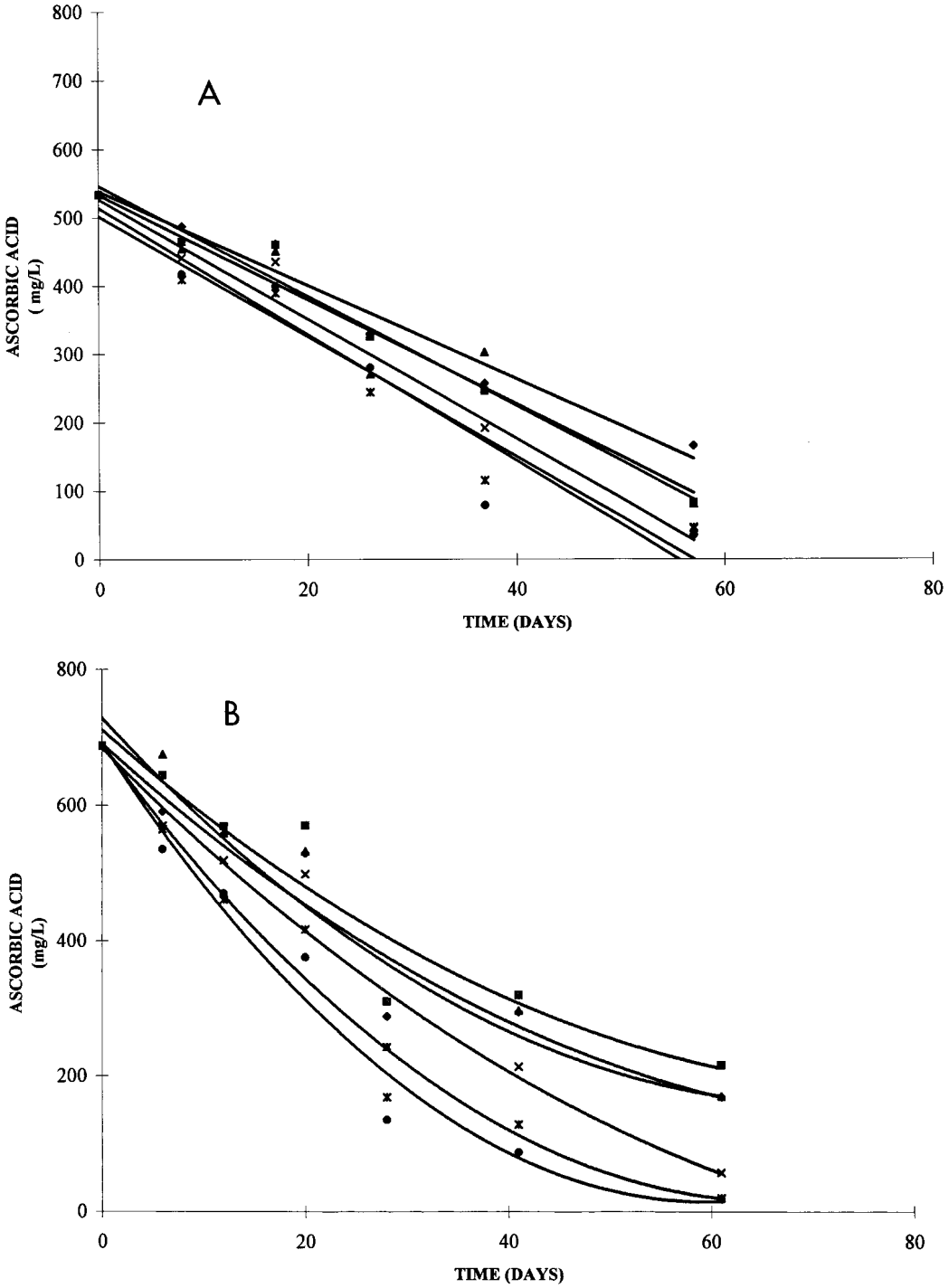

Figure 3. Loss of L-ascorbic acid in de-aerated (A) and non-de-aerated (B) orange juice stored in containers with different oxygen permeability as affected by storage time at 7°C. Lines represent the best fit regression of the data for each permeation rate. (◆ 0.35, ■ 0.39, ▲ 0.43, × 0.79, ✻ 1.18, ● 1.60 ml O_2/day/container).

in juice samples. 3 ml samples were withdrawn from the containers and kept at room temperature until they reached 23°C. The reading was taken when a steady value was registered (approximately 2 minutes).

The oxygen content of the headspace was assayed by gas chromatography (GC; Varian 2700; Sugarland, TX) using a 5 Å molecular sieve column under the following GC conditions:oven temperature 65°C; detector 150°C; injector 135°C. Aliquots of 100 µl were withdrawn using a gas-tight syringe from the headspace via a rubber septum fitted to the metal lid. Air was used as the standard.

RESULTS AND DISCUSSION

The headspace oxygen concentration in the jars containing 200 ml of juice and approximately 27 ml of headspace increased as expected based on the oxygen permeability rates (Figure 1(A,B)). The area of juice in contact with the headspace was approximately 26 cm^2. Both de-aerated and non-de-aerated juices showed similar trends in the lowest barrier containers and reached the same O_2 level as air in approximately 30 days in the non-de-aerated containers and 40 days in the de-aerated containers. In high barrier containers, the headspace oxygen content never reached more than 3% over the 60 day test period.

The dissolved oxygen content of the de-aerated juice in containers with oxygen permeability rates of 0·35–0·79 ml O_2/day/container increased only slightly during storage (Figure 2A). However, juices in containers with oxygen permeability rates between 1·18 and 1·60 ml O_2/day/container doubled in dissolved oxygen during the storage period (Figure 2A). The dissolved oxygen content of the non-de-aerated juice dropped during the first three days of storage (Figure 2B), probably as a result of N_2-flushing of the headspace and equilibration between the dissolved O_2 in the juice and the headspace. The juice in low oxygen permeability (i.e., high barrier) containers reached equilibrium at a dissolved oxygen content of 3–4 ml O_2/l (Figure 2(A,B)). Juices in high oxygen permeability (i.e., low barrier) containers approached oxygen saturation. Kennedy *et al.*[3] reported an equilibrium dissolved oxygen value of approximately 2 mg/l for single-strength orange juice in a Tetra Brix carton stored at 4°C and initial dissolved oxygen 4·45 mg/l.

The ascorbic acid content for both de-aerated and non-de-aerated juices declined over time (Figure 3(A,B)). As expected, the rate of ascorbic acid degradation was fastest in the juices packaged in containers with high oxygen permeability rates. The data were regressed by best-fit analyses, and correlation coefficients between percentage ascorbic acid remaining over time and dissolved oxygen level or oxygen content in the headspace for each oxygen permeability level were calculated. Generally, the ascorbic acid content correlated inversely with both dissolved and headspace oxygen, with the latter having higher r^2 values.

Degradation of ascorbic acid in de-aerated and non-de-aerated juices followed different kinetics. The best-fit equations were first order for non-de-aerated (r^2 = 87·8 to 97·2) and zero order for de-aerated samples (r^2 = 93·0 to 98·1). Second-order models did not have significant correlation coefficients ($p < 0{\cdot}05$). Kennedy *et al.*[3] reported similar r^2 values for zero, first and second-order equations in single-strength orange juice stored at 4°C with 4·45 mg dissolved oxygen/l. They concluded that it was not possible to determine whether ascorbic acid degradation was zero, first or second order. Other workers[6] reported that the loss of ascorbic acid in concentrated orange juice (58°Brix) followed first-order reaction kinetics at temperatures of 25°C and below. The water activity of concentrated juice would be significantly lower than single-strength juice. A study carried out using canned single-strength grapefruit juice,[4] however, found that the degradation of ascorbic acid was explained by a zero-order reaction over the temperature range of 10–50°C. The zero-order kinetics at low O_2 concentration suggests that anaerobic degradation is a complex reaction.

In both experiments oxygen permeability rate and the length of storage were important parameters for the degradation of ascorbic acid. Whether the containers were filled with 100 or 200 ml of juice did not significantly affect the rate of ascorbic acid loss ($p < 0{\cdot}01$) for both de-aerated and non-de-aerated juice. This was likely due to removal of oxygen from the headspace by nitrogen flushing. The following quadratic equations were found best fitted the de-aerated and non-de-aerated juices:

Packaging Technology and Science

De-aerated

$$\%AA \text{ remaining} = 119 - 1{\cdot}54 \times TIME - 35{\cdot}4 \times PERM + 12{\cdot}5 \times PERM^2, \quad (1)$$

$$r^2 = 93$$

Non-de-aerated

$$\%AA \text{ remaining} = 123 - 2{\cdot}42 \times TIME + 0{\cdot}0168 \times TIME^2 - 40{\cdot}4 \times PERM + 12{\cdot}3 \times PERM^2, \quad (2)$$

$$r^2 = 81$$

where: *TIME* = time (days); *PERM* = oxygen permeation rate (ml/day/container).

These equations had significant coefficients ($p < 0{\cdot}05$) for time and oxygen permeability variables. Both equations gave high r^2 values suggesting that they accurately described the results obtained under the conditions of these tests. These results showed that in addition to de-aeration processing, the oxygen permeability rate of the package is an important factor affecting ascorbic acid retention. For example, an increase in oxygen permeability rate from 0·5 to 1·0 ml/day/container reduces the ascorbic acid remaining after 30 days from 58 to 50% for de-aerated juice (equation 1) and from 49 to 38% for non-de-aerated juice (equation 2). Using an average of the oxygen permeability rates tested (0·65 ml/day/container) and one-month storage, the difference in ascorbic acid remaining between de-aerated and non-de-aerated juices was approximately 10%. This difference increased as the oxygen permeability rate increased within the range studied. This difference is similar to the effect of de-aeration on ascorbic acid retention in juices packaged in high barrier containers. This suggests that container permeability rate is as, or more, important than de-aeration in ascorbic acid retention.

CONCLUSION

These data suggest that ascorbic acid degradation for de-aerated (2·7 mg dissolved oxygen/ml) and non-de-aerated (6·8 mg dissolved oxygen/ml) single-strength orange juices stored at 7°C followed zero and first-order kinetics, respectively. The high correlation coefficients between rate of ascorbic acid degradation and oxygen permeability rate for de-aerated ($r^2 = 0{\cdot}93$) and non-de-aerated ($r^2 = 0{\cdot}81$) juices suggest that the ascorbic acid degradation was predominantly aerobic. Two equations were fitted to the empirical data for describing the resulting ascorbic acid retention according to the storage time and container oxygen permeability. Using an average of the oxygen permeability rate tested (0·65 ml/day/container) the difference between ascorbic acid remaining in de-aerated and non-de-aerated juices after one month of storage was 10 %. Juice in high oxygen permeability containers showed a faster decrease in ascorbic acid content, independent of initial dissolved oxygen. The rate of ascorbic acid loss was greater in non-de-aerated juice. For instance, an increase in oxygen permeability rate from 0·5 to 1·0 ml/day/container showed a difference in ascorbic acid remaining of approximately 12% for non-de-aerated juice, after one month of storage.

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