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The vitamin C content of orange juice packed in an oxygen scavenger material

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Abstract

A storage study of orange juice packed in oxygen scavenging (OS) film and oxygen barrier film was conducted to determine the extent of ascorbic acid loss due to oxygen as a function of time and temperature. The initial concentration of ascorbic acid in the orange juice was 374 mg/l and this was found to decrease by 74 and 104 mg/l after 3 days of storage at 25 °C in the OS and oxygen barrier film, respectively. This rapid loss in ascorbic acid correlated well with the amount of oxygen initially present in the headspace and that dissolved in the juice. The loss of ascorbic acid also correlated with an increase in the browning of the juice, where the extent of browning was found to be lower for the juice packed in the OS film than that packed in the oxygen barrier material. The rapid removal of oxygen was found to be an important factor in sustaining a higher concentration of ascorbic acid over long storage times.

Keywords: Oxygen scavenging; Packaging; Oxygen; Orange juice; Ascorbic acid

1. Introduction

The nutritional quality of orange juice is related primarily to its content of L-ascorbic acid and dehydroascorbic acid, and the decomposition of ascorbic acid, together with non-enzymatic browning, are the main deteriorative reactions that occur during storage. Ascorbic acid is highly sensitive to various modes of deterioration. The main factors that can affect ascorbic acid loss in orange juice include temperature, salt and sugar concentration, pH, oxygen, enzymes, light, metal catalysts, initial concentration of ascorbic acid, the ratio of ascorbic acid to dehydroascorbic acid, microbial load and protection provided by the container (Tannebaum, Archer, & Young, 1985).

Ascorbic acid consists of an enediol structure which is conjugated with the carbonyl group in a lactone ring (Tannenbaum et al., 1985). In the presence of oxygen, ascorbic acid is degraded primarily to dehydroascorbic acid via its monoanion. The rate at which dehydroascorbic acid is formed is approximately first order with respect to the concentrations of ascorbic acid, oxygen, and metal catalysts, whereas the rate of the uncatalysed reaction is independent of the oxygen concentration at low partial pressures of oxygen up to 0.4 atm (Khan & Martell, 1967). At high concentrations of sugars and solutes in orange juice, a salting-out effect on dissolved oxygen is caused, and this also has an effect on the stability of ascorbic acid (Tannenbaum et al., 1985).

The adverse effects of dissolved oxygen on the quality attributes of fruit juices have been reported by many researchers, and these include increased degradation of ascorbic acid (Kennedy, Rivera, Lloyd, Warner, & Jumel, 1992; Solomon, Svanberg, & Sahlstrom, 1995), increased browning (Meydav, Saguy, & Kopelman, 1977; Solomon et al., 1995) and growth of aerobic bacteria and moulds (Eiroa, Junqueira, & Schmidt, 1999).

Ascorbic acid is readily converted to dehydroascorbic acid by mild oxidation, but the loss of vitamin activity only arises after hydrolysis of the lactone to form 2, 3-diketogulonic acid (DKG) (Tannenbaum et al., 1985). For this reason, both the ascorbic acid and dehydroascorbic acid were analysed in the present study. Kurata

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and Saurai (1967) suggest that, under anaerobic conditions, ascorbic acid reacts via its keto tautomer which is in equilibrium with its anion, which undergoes delactonisation to form DKG.

Decarboxylation of DKG may result in the formation of xylosone (X) and 3-deoxypentaone (DP), which is formed by β-elimination at the C4 of DKG, followed by decarboxylation. It is at this point that the pathways begin to assume the features of non-enzymic browning reactions. Xylosone is further degraded to form reductones and ethylglyoxal, whereas DP degrades to form furfural and 2-furancarboxylic acid. These compounds may combine with amino acids to form brown pigments (Tannenbaum et al., 1985). Solomon et al. (1995) have correlated the browning of orange juice with the degradation of ascorbic acid. Other investigators have also suggested that browning follows ascorbic acid loss (Clegg, 1966; Clegg & Morton, 1965; Trammell, Dalsis, & Malone, 1986).

Studies by Joslyn and Miller (1949) concluded that the kinetics of oxidation of ascorbic acid in the presence of dissolved oxygen was first-order with respect to ascorbic acid. Likewise, Singh, Heldman, and Kirk (1976) concluded that, at saturated dissolved oxygen levels, oxidation of ascorbic acid is a first-order reaction, although at low oxygen levels the reaction follows second-order kinetics. Wilson, Beezer, and Mitchell (1995), found that the rate of oxidation of ascorbic acid in a closed system without any headspace was first order with respect to the concentration of oxygen. Ascorbic acid will degrade, even in the absence of oxygen, although the rate of degradation under anaerobic conditions is even slower than that of the uncatalysed aerobic reaction.

Methods of packaging orange juice have traditionally been aimed at reducing the exposure of the juice to oxygen through the use of high barrier materials, such as foil laminates in brickpacks, with or without nitrogen flushing. Recent developments in oxygen scavenging packaging now provide an opportunity to further reduce the exposure of packaged juice products to oxygen (Brody, 2001; Gontard, 2000; Rooney, 1995).

The use of oxygen scavenging packaging materials means that oxygen dissolved in the juice, or present initially in the headspace, can potentially be reduced to levels much lower than those achievable by modified atmosphere packaging. However, the rate of oxygen scavenging packaging for this oxygen is likely to be important because the depletion of oxygen present at the time of packaging can also occur through reaction with the ascorbic acid.

This study was undertaken to investigate the effects of removal of dissolved oxygen, by an experimental oxygen scavenging packaging film, on the quality and shelf life of commercial orange juice, and the potential for oxygen scavenging materials to provide extended periods of high oxygen barrier.

2. Materials and methods

2.1. Packaging and storage of orange juice

Combiblock cartons (1 l) containing '100% Just Juice Orange Juice®' were provided by National Foods – Sunburst Foods Ltd. The juice had a "best before date" that was 1 year after the date of packaging, and was stored at −18 °C until re-packing.

The juice was thawed just prior to re-packing which took place 6 days after the original packaging date. The initial dissolved oxygen concentration of the juice was measured just prior to re-packing.

The juice was re-packed into pouches made using EVOH[1] (18 μm)/CPP[2] (22 μm) laminate (reference), and EVOH (18 μm)/OS[3] film (21 μm)/CPP (22 μm) laminate (OS) with total contact surface areas of 172 cm^2 for each type of pouch. The pouches had a surface-to-volume ratio of 6:1 to maximise any impact of oxygen permeability of the laminate on ascorbic acid retention. The orange juice (30 ml) was transferred aseptically via a syringe into the vacuum-sealed pouches. The vacuum-sealed OS pouches were activated just prior to filling. Dimethyl dicarbonate (BASF), a sterilising agent, was added to the orange juice in each pouch at a concentration of 250 mg/l of juice.

The sugar content, pH, browning index, dissolved oxygen content and vitamin C (L-ascorbic acid and dehydroascorbic acid, each individually) concentrations of the juice were determined immediately after re-packing.

Samples were stored at 4 °C and at 25 °C in the dark. Packs of orange juice from each treatment (in duplicate packs) were then analysed in duplicate, over time, to monitor the concentrations of dissolved oxygen, browning, and vitamin-C (different packs were used at each sampling time). This provided information on the rate of degradation of the orange juice which enables a shelf life estimate to be made based on the time at which the L-ascorbic acid content falls below 400 mg/l—Australian Food Standards Code (ANZFA, 1991), 200 mg/l—Leitsätze für Fruchtsäfte (Anon, 1982), or 250 mg/l—UK Food Law Notes (CCFRA, 1998). A microbiological investigation of the juice packed under the above-mentioned conditions, was carried out at day 0 and after 2 weeks, to ensure that microorganisms did not interfere with the oxidation of vitamin-C by competitively consuming the available oxygen.

2.2. Oxygen scavenging film performance

Air saturated distilled water (10 ml) was packed into pouches made from experimental oxygen scavenging

[1] EVOH, ethylene-vinyl alcohol copolymer.
[2] CPP, cast polypropylene.
[3] OS, oxygen scavenger.

film, EVOH (18 μm)/OS film (25 μm)/ EVA[4] (22 μm), with total contact surface areas of 100 cm^2 each. Five pouches of each material were made and vacuum-sealed prior to filling. They were then immediately UV-activated, re-opened, filled and re-sealed.

Samples were stored at 25 °C; the water from each treatment was then analysed over time to monitor dissolved oxygen concentration.

2.3. Microbiological testing

Juice samples were checked for microbial growth after re-packing into the pouches. Four of the pouches containing orange juice were sampled at random, two of which were inspected for microbial spoilage after two weeks of storage at 30 °C, and the other two were analyzed on the day of re-packing. The microbiological tests were performed by plating on orange serum agar for a total plate count (incubated at 30 °C), and on malt extract agar for yeast and moulds (incubated at 25 °C).

2.4. Dissolved oxygen

The dissolved oxygen content of the juice was measured using a calibrated MI-730 Micro-Oxygen Electrode (Microelectrode Inc, USA) and OM-4 Oxygen Meter (Microelectrodes Inc, USA). The lid was opened on the Combiblock orange juice carton and the oxygen probe was immediately inserted into the juice at approximately half the depth of the container. The oxygen content of the juice in the test pouches was measured by cutting them open at the top and immediately inserting the probe into the juice. The latter was then stirred (with a magnetic stirrer) to expose the sensor to the maximum amount of juice. A value was taken once a steady reading was displayed, typically at 5 min.

2.5. L-ascorbic acid and dehydroascorbic acid analysis

The ascorbic acid content was determined by a HPLC analytical procedure using an Iso Chrom VS pump at a flow rate of 2.5 ml/min, an injection valve with a 10 μl loop, a 250 × 4.6 mm NH_2 5 micron column (Altima), and an Etpkortec UV-detector set at 254 nm. The mobile phase was 75:25 (v/v) acetonitrile/0.05 M KH_2PO_4, pH 6.0. Orange juice (1 ml) was transferred to a 10 ml volumetric flask and made to volume with distilled water. The diluted orange juice was kept on ice and then filtered through two filters, 0.45 and 0.2 μm, before being injected into the HPLC. The dehydroascorbic acid was quantified indirectly after reduction to ascorbic acid by adding L(+)-cysteine $C_3H_7NO_2S$ (1% w/v) to the diluted orange juice sample. The pH of the sample was adjusted to between 7.0 and 7.5 by addition of ammonia solution and was maintained at that level for 2 min. The pH was then lowered to the initial value with concentrated HCl, after which the sample was analysed for total ascorbic acid content. (Solomon et al., 1995).

2.6. Browning analysis

Single strength orange juice (10 ml) was centrifuged at 2000 rpm for 20 min to remove pulp and coarse cloud particles. The supernatant was diluted 1:1 with 100% ethanol and filtered through a 0.45-μm filter, to obtain a fully clarified extract. Transmittance spectra and browning indices (absorbance at 420 nm) of the clear extracts were then determined (Meydev et al., 1977).

2.7. Statistical analysis

Conventional linear regression analysis of the data obtained in this study was performed using MINITAB software.

3. Results and discussion

3.1. Oxygen scavenging film performance

The initial concentration of oxygen in the aerated water in OS pouches was 8.2 ppm. This concentration decreased within 2–3 h to below 0.5 ppm (Fig. 1). This is the concentration observed in the reference among juice packs after 3 days at 25 °C (Fig. 2).

The OS film used in this study scavenged 7.2 ppm (6.75×10^{-3} millimoles) within an hour of storage, at 25 °C. This removal of oxygen from the water occurs at a rate which is rapid compared with that at which ascorbic acid reacts with oxygen in orange juice (Fig. 1; Table 1 a).

The pouches had a surface-to-volume-ratio of 10:1. At this surface-to-volume-ratio, 96% of the oxygen was essentially removed within 3 h. The surface-to-volume-ratio of the pouches used for the orange juice was 6:1, so it would be expected that the oxygen would be removed by the OS film somewhat more slowly.

3.2. Dissolved oxygen in orange juice

The dissolved oxygen concentration in the orange juice in the Combiblocks as received was found to be 0.04 ppm. The juice was intentionally aerated on transfer to the pouches and its initial concentration in both the OS and reference pouches was 2.7 ppm. This concentration decreased during storage to less than 0.04 ppm in both treatments, although this occurred substantially faster in the OS treatment than in the control at both temperatures (Table 1a and b).

[4] EVA, ethylene vinyl acetate.

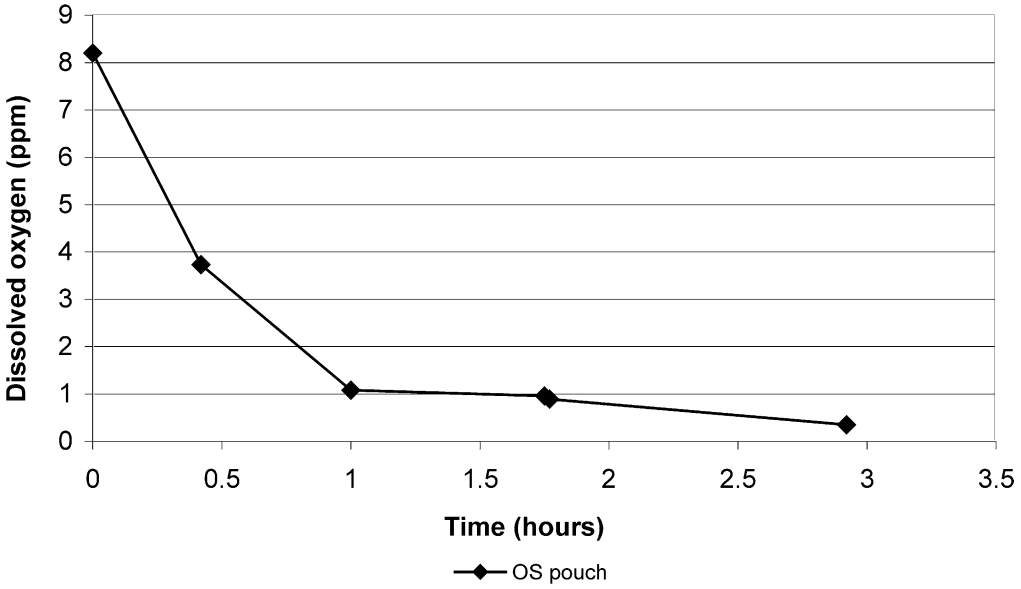

Fig. 1. Scavenging of dissolved oxygen from aerated water by use of an OS film, at 25 °C.

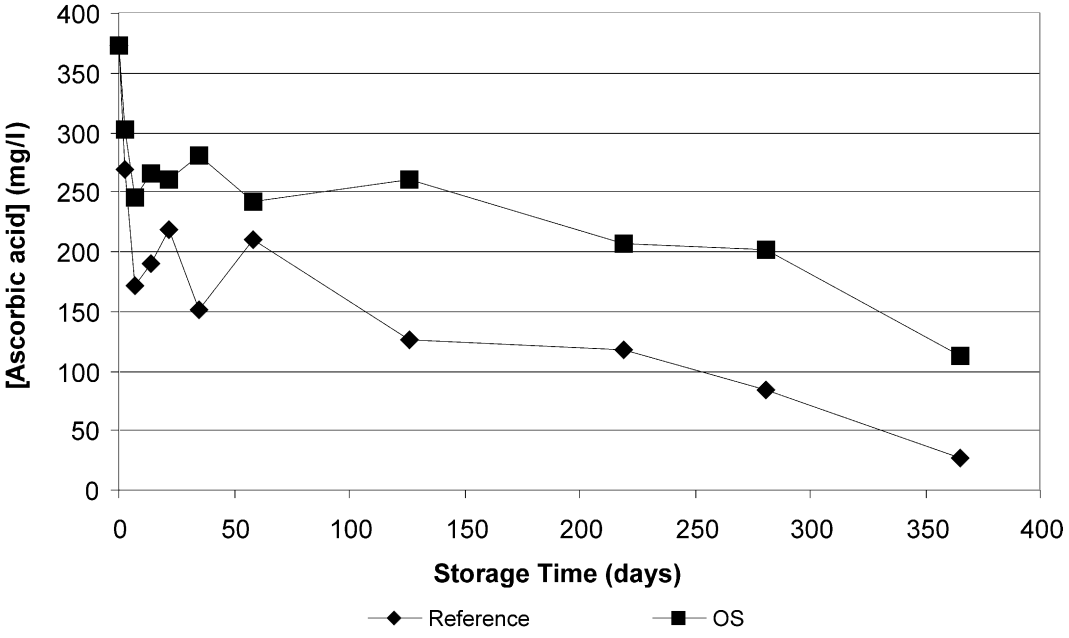

Fig. 2. Concentration of ascorbic acid in orange juice packed in OS and reference pouches stored at 25 °C.

At 25 and 4 °C the dissolved oxygen concentration in the orange juice samples packed in OS pouches reached less than 0.04 ppm within 3 and 7 days, respectively, whereas in the controls this occurred after at least 35 and 77 days at 25 and 4 °C, respectively. This difference reflects the impact of the competition for the oxygen between the OS film and the ascorbic acid. At 25 °C the 2.7 ppm (2.53×10^{-3} millimoles) of oxygen was consumed in 3 days or less for the OS packages, whereas in the reference packs the oxygen content was 0.5 ppm after this time. The OS film therefore consumed at least 0.5 ppm within those 3 days (refer to Table 1a). At 4 °C, at least 1.33 ppm was consumed by the OS film in 7 days, or less (refer to Table 1b). These values are lower limits, and to determine the maximum oxygen scavenging by the OS film, analysis would be required in the first few days of storage time.

The impact of oxygen permeation is not likely to be significant over the shelf life time at either temperature, with the expected quantity of oxygen permeating the reference packs being 3.14 ml after 365 days assuming an oxygen transmission rate of 2.5 ml/m^2/day/atm at 25 °C for the laminate. This amount appears to have been intercepted by the OS packs since the oxygen concentration remained at zero, whereas that in the reference packs varied between zero and 1 ppm after the initial oxygen had been scavenged, after 22 days.

Table 1
Dissolved oxygen content in orange juice

Storage time (days)	Reference		OS	
	O_2 (ppm)	S.D[a]	O_2 (ppm)	S.D[a]
Stored at 25 °C				
0	2.75	0.0	2.7	0.0
3	0.5	0.06	0.0	0.0
7	0.3	0.1	0.0	0.0
14	0.3	0.1	0.0	0.0
22	0.2	0.14	0.0	0.0
35	0.0	0.0	0.0	0.0
58	0.2	0.08	0.0	0.0
126	0.8	0.02	0.02	0.02
219	0.0	0.0	0.0	0.0
281	0.4	0.04	0.06	0.06
365	0.2	0.1	0.06	0.06
Stored at 4 °C				
0	2.75	0.0	2.75	0.0
7	1.33	0.12	0.0	0.0
14	0.67	0.67	0.0	0.0
23	0.43	0.43	0.0	0.0
36	0.86	0.86	0.0	0.0
72	0.20	0.04	0.0	0.0
77	0.0	0.0	0.0	0.0
156	0.04	0.04	0.02	0.02
288	0.04	0.04	0.0	0.0
373	0.20	0.04	0.10	0.02

[a] Standard deviation.

The oxygen scavenged by the OS film in the first 3 days resulted in the reduced L-ascorbic acid loss seen in Figs. 2 and 3 compared with the loss in the reference pouches. Once the oxygen was depleted, by either the combined effects of the OS film and oxidation with ascorbic acid (OS curve), or solely by oxidation with ascorbic acid (Reference curve), the ascorbic acid contents decreased to 300 and 270 mg/l, respectively. Thereafter, the ascorbic acid continued to be depleted substantially in the reference, and to a lesser extent in the OS treatment. By 25 days, the rate of depletion of the ascorbic acid was the same rate in both types of pouches. Hence, the initial rate of oxygen removal is important to sustain a higher content of ascorbic acid over the storage time.

3.3. L-ascorbic acid and dehydroascorbic acid contents in orange juice

The ascorbic acid content of the orange juice was 374 mg/l at the time of re-packaging from the Combiblocks into the pouches. This was 6 days after processing by the manufacturer.

The large decrease in ascorbic acid concentration, evident from day 0 to day 3 at 25 °C, or day 7 at 4 °C (Figs. 2 and 3), coincides with the consumption of dissolved oxygen as seen in Table 1a and b. In general, the results show that there was a rapid degradation of vitamin C in the early stage of storage, followed by a gradual loss. This is consistent with evidence from other investigators (Kennedy et al., 1992; Roig, Bello, Rivera, & Kennedy, 1999; Soares & Hotchkiss, 1999) and can be attributed largely to the oxygen that is dissolved in the juice and in the headspace in the early stages of storage.

The rate of oxidation of ascorbic acid is highly dependent on the dissolved oxygen concentration. Solomon et al. (1995) and Wilson et al. (1995) have reported results showing that the rate of oxidation of ascorbic acid is significantly correlated ($P<0.01$, $r^2=0.78$) with the level of dissolved oxygen and with the length of storage. Oxygen permeation through the

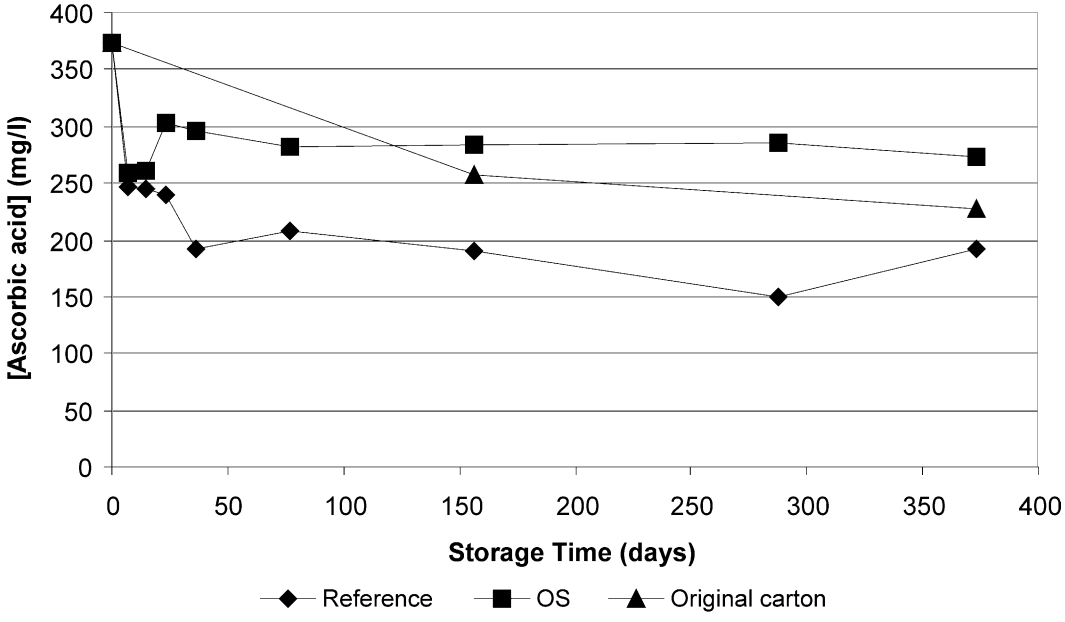

Fig. 3. Concentration of ascorbic acid in orange juice packed in OS and reference pouches stored at 4 °C.

packaging during storage is another factor contributing to the continuation of the aerobic mechanism of ascorbic acid oxidation in the reference packs in the present work.

The decrease in vitamin C (ascorbic acid plus dehydroascorbic acid) concentration over storage time followed the same trend as that of the ascorbic acid (Figs. 2–4).

The decrease in ascorbic acid concentration in one of the original Combiblocks stored at 4 °C was found to be 31% after 156 days, compared with 24 and 49% in the OS and reference pouches, respectively. The rate of loss of ascorbic acid in the orange juice was significantly lower ($P<0.05$) when it was packed in OS pouches. The rate of loss of ascorbic acid in the orange juice samples held at 25 °C was significantly higher ($P<0.05$) than in those held at 4 °C. All treatments were significantly different ($P<0.05$) from each other but, of all the conditions studied in this work, the ascorbic acid was best retained when it was stored in OS pouches and at 4 °C. It has been reported previously that the kinetics of ascorbic acid degradation is dependent on temperature (Kennedy et al., 1992).

Dehydroascorbic acid (DHA) is the oxidised form of ascorbic acid. The orange juice stored in the OS pouches at 25 °C had a DHA concentration similar to, or less than, the reference pouches for the first 120 days (Fig. 5). Thereafter, the reference pouches contained less DHA. Pouches stored at 4 °C showed a similar initial trend (up to 150 days storage) and the results at 365 days showed the reference pouches to contain less ascorbic acid than the OS pouches. These results might be interpreted in terms of oxygen being necessary for some of the degradation of the DHA, as the reference pouches had a measurable oxygen concentration throughout most of the storage period. This is consistent with oxygen slowly permeating the reference pouch material but being stopped in the OS pouches. The increase, followed by a decrease in the DHA concentration, observed in this work, is consistent with the results of Solomon et al. (1995) who showed that the DHA concentration increased over the storage time, while ascorbic acid underwent oxidation to DHA through the aerobic pathway. The DHA concentration then decreased and reached a relatively constant concentration as the supply of oxygen diminished and/or the DHA was converted to DKG. At 4 °C, this process was slower than at 25 °C.

3.4. Browning of orange juice

Browning of the juice samples occurred at a significantly ($P<0.05$) faster rate in the reference pouches than in the OS pouches at both temperatures, especially at 25 °C (Fig. 6), demonstrating that the rate of browning is in part dependent on the presence of oxygen. Roig et al. (1999) found that a relationship existed between the browning index and the oxidative loss of L-ascorbic acid in citrus juice. The rate of browning is also temperature dependent, in this study browning occurred at a significantly ($P<0.05$) faster rate at 25 °C than that at 4 °C (Fig. 6). Roig et al. (1999) also found that the rate of browning of citrus juice samples was directly related to temperature. The mean browning index of freshly squeezed orange juice, as reported by Johnson, Htoon, and Shaw (1995) is 0.15 ± 0.07 at ambient temperature; the OS juice stored at 4 °C had a browning index below

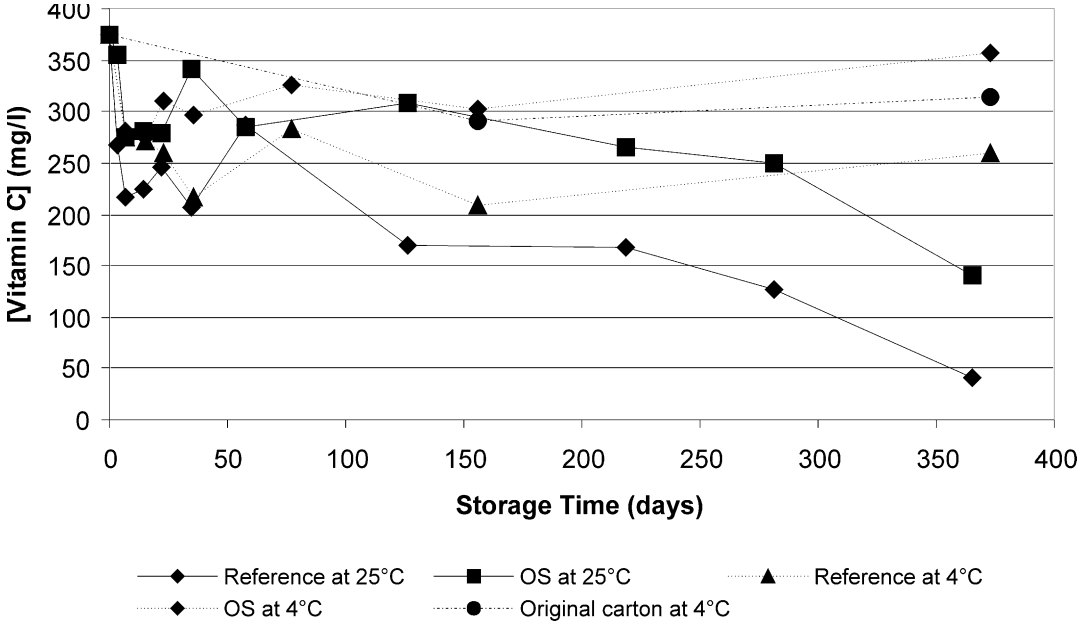

Fig. 4. Concentration of vitamin C (L-ascorbic acid and dehydroascorbic acid) in orange juice packed in OS and reference pouches stored at 25 and 4 °C.

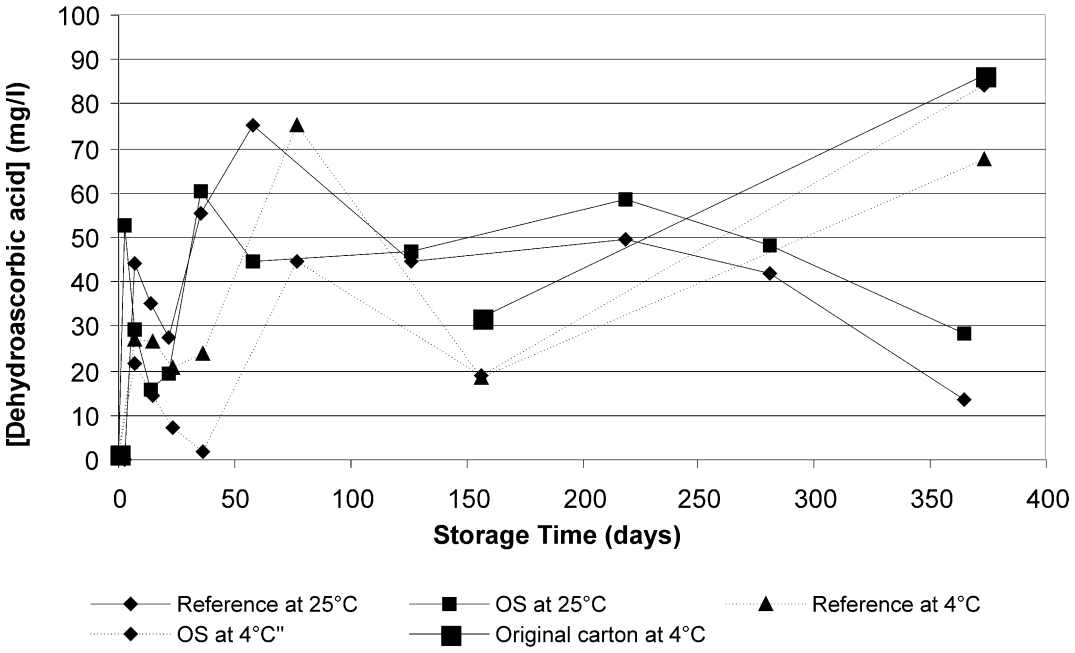

Fig. 5. Concentration of dehydroascorbic acid in orange juice packed in OS and reference pouches stored at 25 and 4 °C.

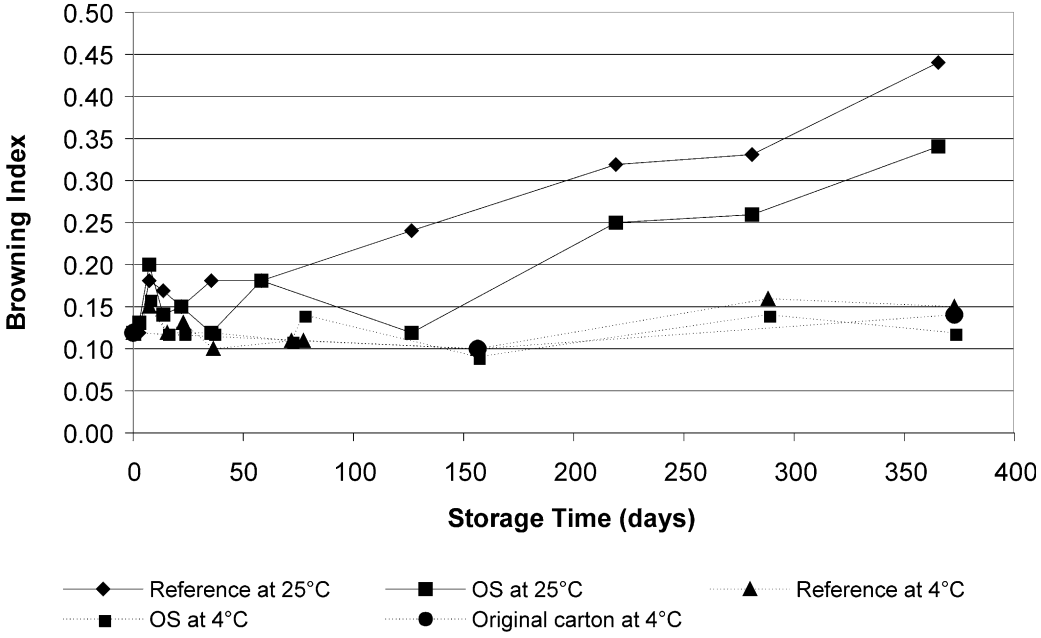

Fig. 6. Browning of orange juice packed in OS and reference pouches stored at 25 and 4 °C.

0.15 during the entire storage period in this study. DHA is the major degradation product of ascorbic acid and is converted to DKG, forming xylosone through the aerobic pathway, which is further degraded to form reductones or ethylglyoxal, which further react with amino acids and contribute to the browning of the orange juice. DHA also degrades through the anaerobic pathway, to form 3-deoxypentosone; this degrades to furfural, which also reacts with amino acids, causing the undesirable brown pigments in the orange juice.

3.5. Soluble solids

The soluble solids content of the orange juice, as supplied, was determined to be 10.3°Brix at 25 °C, using a refractometer.

3.6. Microbiological test

After packing, no viable organisms were detected in the duplicate samples of the orange juice. The duplicate

Table 2
Ascorbic acid (aa) retention (%) in orange juice packed in OS and reference pouches stored at 25 and 4 °C

Storage time (days)	% aa retained in Reference pouches at 25 °C	% aa retained in OS pouches at 25 °C	% aa retained in Reference pouches at 4 °C	% aa retained in OS pouches at 4 °C	% aa retained in original carton at 4 °C
0	100	100	100	100	100
3	72.1	81.1			
7	45.9	65.6	66.2	69.2	
14	50.7	70.9			
15			65.5	70.0	
22	58.4	69.6			
23			64.0	81.3	
35	40.4	75.2			
36			51.5	79.1	
58	56.4	64.6			
77			55.8	75.3	
126	33.5	69.8			
156			50.7	76.0	69.0
219	31.1	55.2			
281	22.6	54.1			
288			39.9	76.6	
365	7.29	30.0			
373			51.3	73.2	60.84

samples, stored at 30 °C for 2 weeks, were inspected for microbial spoilage. No spoilage, in either pouch, was found, indicating a good quality orange juice, and/or the microbiocidal effects of dimethyl dicarbonate.

4. Conclusion

The rate of oxidation of ascorbic acid in orange juice was found to be lower when packed in OS pouches than when packed in reference pouches or in the original carton. The rate of oxidation of ascorbic acid in the orange juice samples stored at 25 °C was greater than those stored at 4 °C. The rate of oxidation of ascorbic acid and browning of the orange juice were found to be significantly ($P<0.05$) dependent on the dissolved oxygen concentration, temperature, and storage time.

A direct estimate of the shelf life cannot be determined since the initial concentration of the ascorbic acid was below the legal concentration in Australia. However, the rate of degradation of the ascorbic acid can be used to extrapolate results. According to the UK Food Law Notes (CCFRA, 1998), 250 mg/l of ascorbic acid is the legal minimum concentration in orange juice; therefore the OS packed orange juice stored at 25 °C is still acceptable after 288 days, but would have a shelf life of less than 126 days with just the oxygen barrier packaging.

These results demonstrate that an oxygen scavenging package can remove oxygen from the juice and headspace at a rate sufficient to extend the shelf life. Furthermore, the residual oxygen scavenging capacity in the OS film is able to provide an ongoing barrier to oxygen permeation, thereby providing extended protection of the juice from oxidative degradation. This protection of the juice (during the 1 year storage trial), by the use of the OS film, is potentially similar to that achieved using an aluminium foil laminate in commercial brick-packs.

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