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L-Ascorbic acid stability in aseptically processed orange juice in TetraBrik cartons and the effect of oxygen

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The rate of degradation of L-ascorbic acid in commercial single-strength orange juice aseptically processed in TetraBrik cartons was evaluated at different storage temperatures. In this type of sample, the level of dissolved oxygen present in the sample after packaging significantly affected the L-ascorbic acid content, the effect being directly related to temperature. Likewise, the rate of consumption of dissolved oxygen is directly dependent on the concentration of L-ascorbic acid. Both aerobic and anaerobic degradation of L-ascorbic acid occurs in the same system. The aerobic process predominates and the anaerobic process takes place when the level of dissolved oxygen has reached equilibrium.

INTRODUCTION

Due to the increasing health consciousness of the consuming public, the importance of L-ascorbic acid in human nutrition has gained recognition. This awareness has boosted the fruit juice industry due to the demand for healthy and convenient foods. One of the most popular of the juice drinks in the market today is orange juice in TetraBrik cartons. These have been processed and packaged aseptically to reduce the thermal load during processing.

The instability of L-ascorbic acid, however, has been very well documented and has been demonstrated to be affected by a number of factors. Temperature has been reported to be the most critical factor in preserving L-ascorbic acid (Nagy, 1980). In solutions, and even in the pure dry state, L-ascorbic acid has been demonstrated to degrade with time (Kennedy *et al.*, 1989*a*). Other factors and constituents of juice such as pH, heavy metals like copper and iron, oxygen, amino acids and sugars and type of packaging have been shown to affect L-ascorbic acid stability (Nagy, 1980; Trammell *et al.*, 1986; Kanner & Shapira, 1989; Kennedy *et al.*, 1989*b*).

In aseptic packages using semi-permeable cartons, oxygen remains a problem due to the accelerated oxidation of L-ascorbic acid (Sizer *et al.*, 1988). The loss in nutritional value of food and drinks containing L-ascorbic acid is accompanied by flavour and quality deterioration, thereby decreasing its shelf-life.

Studies have been conducted on L-ascorbic acid stability in different types of juices stored under various conditions. However, there is no available data on the stability of L-ascorbic acid in reconstituted single-strength orange juice in a TetraBrik carton stored at low, medium and high temperature. Thus, this work was undertaken to determine the rate of degradation of L-ascorbic acid in reconstituted single-strength orange juice aseptically processed and packaged in TetraBrik cartons and stored at various temperatures.

The effect of dissolved oxygen content on L-ascorbic acid degradation was also investigated.

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MATERIALS AND METHODS

Juice samples

Juice samples in TetraBrik cartons consisted of ready-to-drink commercial orange juice. These were obtained from orange juice concentrates (60–65° Brix) that were reconstituted with sterile water to give single-strength orange juice at 11·2° Brix. They were obtained from the manufacturer approximately 2 h after production and packaging.

Samples were taken at specific time intervals after storage at controlled temperatures of 4, 20, 37, 76 and 105°C, then analysed for L-ascorbic acid content and dissolved oxygen level. It should be noted that the samples do not contain artificial colours, flavours, preservatives or added sugars.

L-Ascorbic acid supplementation of degraded juice samples in TetraBrik cartons

Some juice samples were stored at 37°C for 1 week; then a representative sample was analysed as the rest of the samples. The remaining incubated samples were then supplemented with a freshly prepared aqueous solution of L-ascorbic acid (0·08 M, 1 ml) to bring their L-ascorbic acid content to the original level (approximately 300 mg litre^{-1}). L-Ascorbic acid solution was introduced by means of a sterile syringe and needle (23G × 1·25 mm 63/100) pushed through the laminated hole in the carton which was immediately sealed with wax. Care was taken not to introduce air into the samples. The samples were stored again at 37°C for known time intervals prior to analysis.

L-Ascorbic acid supplementation of newly manufactured juice samples in TetraBrik cartons

Parallel to the samples described above, some untreated samples which were frozen (−20°C) for 23 days, but without prior incubation, were supplemented with freshly prepared aqueous solutions of L-ascorbic acid (0·34 M, 1 ml). The samples were incubated at 37°C for known time intervals then analysed parallel to the other samples.

L-Ascorbic acid

Analysis of L-ascorbic acid was performed following a previously described HPLC method (Kennedy *et al.*, 1989*a*). Two macroreticular reverse phase PLRP-S columns, Polymer Laboratories, 5 μm, 150 mm × 4·6 mm ID were used in series. The eluent was an aqueous solution of sodium dihydrogenphosphate (0·2 M) with the pH adjusted to 2·14 with hydrochloric acid (12 M) and pumped at a flowrate of 0·5 ml min^{-1}. Detection was by a Knauer variable wavelength detector Model 287 set at 268 nm. Prior to analysis, samples were centrifuged at 10 000*g* RCF at 20°C for 45 min before filtration through a 0·45 μm cellulose nitrate membrane filter (Millipore).

Dissolved oxygen

A YSI Model 57 polarographic oxygen sensor calibrated with water vapour-saturated air (one-point calibration) was utilized to measure the level of dissolved oxygen in juice samples in TetraBrik cartons.

A small opening was made in the carton, just large enough to fit the oxygen probe which was immediately inserted until the membrane was immersed in the liquid. The sample was continuously shaken at a reproducible rate to ensure a constant oxygen supply to the sensor. The reading was taken when a steady value was registered (after approximately 5 min).

Statistical analysis

Regression analysis of data was performed using the Minitab program on an IBM 1040 Computer.

RESULTS AND DISCUSSION

L-Ascorbic acid retention

The expected instability of L-ascorbic acid as a function of time and temperature is well demonstrated in Fig. 1. The percentages of L-ascorbic acid retained after storage at various temperatures were: 60·4% after 64 days at 4°C, 48·6% after 64 days at 20°C, 11·9% after 64 days at 37°C, 2·0% after 6 days at 76°C, and 3·6% after 3 days at 105°C. These figures correlate with that of Moshonas & Shaw (1989) who showed a decrease of 27% of L-ascorbic acid for commercial orange juice samples

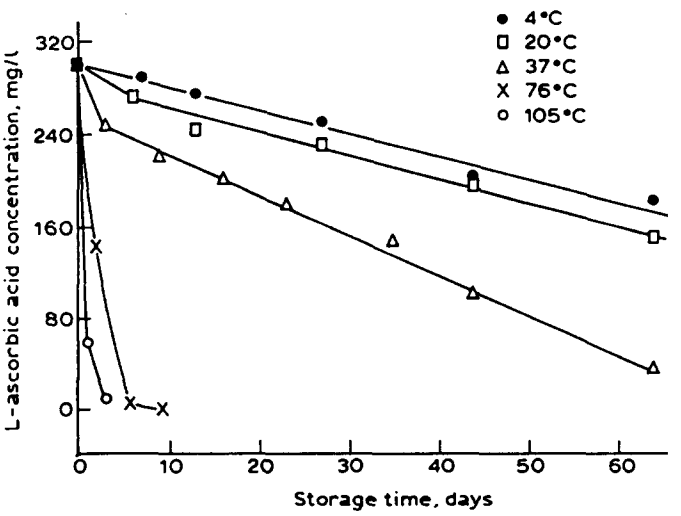

Fig. 1. Concentration of L-ascorbic acid as a function of time in aseptically processed reconstituted single-strength orange juice in TetraBrik cartons after storage at various temperatures.

Table 1. Rate of L-ascorbic acid degradation in single-strength orange juice in TetraBrik cartons

Storage temperature (°C)	Zero order[a]		First order[a]		Second order[a]	
	k (mg litre^{-1} day^{-1})	r^2 (%)	k (day^{-1})	r^2 (%)	k	r^2 (%)
4	1·98	98·5	0·008	99·0	—	—
20	2·23	97·2	0·010	97·6		97·4
37	3·75	96·7	0·029	93·0		97·2

[a] All results obtained were included.

Storage temperature (°C)	Zero order[b]		First order[b]		Second order[b]	
	k (mg litre^{-1} day^{-1})	r^2 (%)	k (day^{-1})	r^2 (%)	k	r^2 (%)
4	1·97	98·0	0·008 5	98·8		—
20	2·05	98·2	0·010 1	96·8		98·6
37	3·42	99·6	0·029 8	91·7		99·6

[b] Data for time 0 were excluded.

aseptically packaged and stored at 21°C for 35 days. The data are similar to that reported for an earlier study by Wilson & Shaw (1987).

The results of L-ascorbic acid retention were fitted into zero order (concentration against time), first order ($\log_e$ concentration against time) and second order (concentration against time^2) equations (see Table 1). Data obtained for samples stored at 76°C and 105°C were not enough to do an accurate regression. r^2 values (which are a measure of the closeness of fit) for each storage temperature of 4 and 20°C do not show great variations, i.e. 98·5 and 99·0% for 4°C and 97·2, 97·6 and 97·4% for 20°C for zero, first and second order equations, respectively. From these values, it is impossible to distinguish the order of the reaction at this level of degradation at these storage temperatures.

In contrast to the above observations, data obtained for samples stored at 37°C generated relatively lower r^2 values when fitted into any of the three equations. Other workers (Kanner *et al.*, 1982) reported that the loss of L-ascorbic acid in 58° Brix orange concentrates followed first order reaction kinetics at temperatures of 25°C and below. At 36°C the degradation did not follow a first order reaction. A study carried out using canned single strength grapefruit juice (Smoot & Nagy, 1980) however, demonstrated that the degradation of L-ascorbic acid was explained by a zero order reaction over the temperature range 10–50°C.

A dramatic fall in the initial L-ascorbic acid level for samples stored at 20, 37, 76 and 105°C was noted after the first few days of storage. This seems to coincide with the initial drop of the dissolved oxygen level (see Fig. 2).

Increasing the original level of L-ascorbic acid in the sample produced a greater initial loss of L-ascorbic acid than the control, i.e. 18·3 mg litre^{-1} day^{-1} for the control and 25·5 mg litre^{-1} day^{-1} for supplemented samples (see Table 2). Samples which have been previously incubated at 37°C (dissolved oxygen was greatly reduced) exhibited a marked decrease in the rate of loss of L-ascorbic acid even after supplementation to the original L-ascorbic acid level. This observation indicates a close correlation between the rate of L-ascorbic acid and the level of dissolved oxygen (discussed in detail in the next section). Due to the apparent participation of dissolved oxygen in L-ascorbic acid decomposition, additional data during the initial stage of degradation, wherein oxygen appears to be a limiting factor, should be generated. This is necessary to fully ascertain the order of reaction of L-ascorbic acid degradation in this particular study.

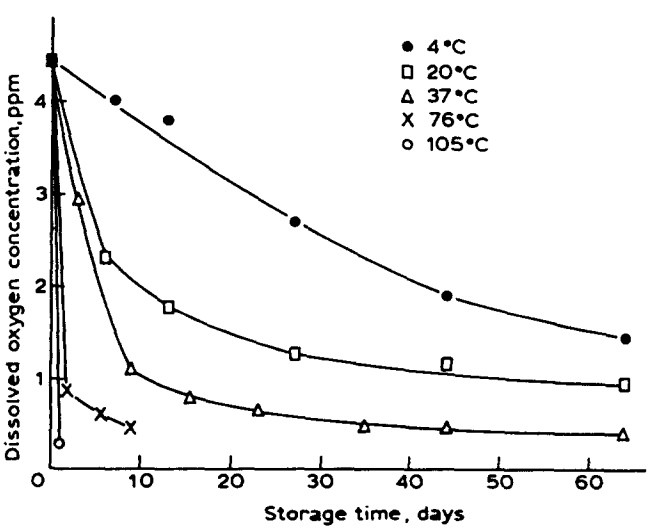

Fig. 2. Level of dissolved oxygen as a function of time in orange juice in TetraBrik cartons stored at various temperatures.

Table 2. Effect of initial L-ascorbic acid and dissolved oxygen concentrations on the rate of L-ascorbic acid decomposition after storage at 37°C

Sample	Initial L-ascorbic acid (mg litre^{-1})	Rate of loss of L-ascorbic acid[c] (mg litre^{-1} day^{-1})	Initial dissolved oxygen (ppm)	Final dissolved oxygen[d] (ppm)
Control	303	18·3	4·45	2·95
Supplemented sample[a]	565	25·5	4·30	2·20
Supplemented sample[b]	308	6·5	1·70	0·90

[a] Frozen for 23 days prior to addition of L-ascorbic acid (270 mg litre^{-1} added).
[b] Frozen for 16 days, then incubated at 37°C for 7 days before L-ascorbic acid supplementation (70 mg litre^{-1} added).
[c] Calculated after 3 days storage for the control and 4 days after supplementation and storage for the supplemented samples.
[d] Level of dissolved oxygen after 3 days storage for the control and 4 days after supplementation and storage for the supplemented samples.

Effect of dissolved oxygen levels on L-ascorbic acid stability

Initial analysis performed on juice samples was the determination of dissolved oxygen level. The consumption of dissolved oxygen in juice samples as a function of time is shown in Fig. 2.

An initial dissolved oxygen level of 4·45 ppm was recorded in the orange juice samples. This represents the amount of oxygen incorporated into the juice during reconstitution with water, mixing, etc., prior to thermal processing (Kacem *et al.*, 1987). No deaeration of the samples was performed. Headspace oxygen was negligible since the cartons were filled just below the seal line (Cabrera, 1989) and there was negligible variation in the net content of the samples.

It could be noted from Fig. 2 that after storage, there was an initial sudden drop in the dissolved oxygen content, which was intensified at the higher temperature. It then continued to decrease, then levelled off after prolonged storage.

A similar trend was observed by previous workers for the disappearance of oxygen in lemon juice stored in rubber stoppered flasks (Robertson & Samaniego, 1986) and from the headspace of canned grapefruit concentrate (Passy & Mannheim, 1979). Comparison with the latter however is inappropriate in relation to the objective of this investigation, since oxygen is involved in corrosion reactions in cans (Marshall *et al.*, 1986). It should be noted that these TetraBrik cartons are incompletely impermeable (theoretical rate of oxygen leakage into the 250 ml carton is 0·2713 ml oxygen per month at 18°C). Nonetheless, the results indicate a point wherein the level of dissolved oxygen reached an equilibrium.

The initial dramatic loss of dissolved oxygen seemed to correlate with the greater rate of decomposition of L-ascorbic acid during the initial stage of storage (see Fig. 3). Hence an aerobic degradation process was taking place. After the dissolved oxygen level reached equilibrium, further L-ascorbic acid decomposition occurred independently of oxygen. The anaerobic pathway is related to the inherent characteristics of the product and is mainly influenced by temperature.

These observations indicate that aerobic and anaerobic pathways of L-ascorbic acid degradation can operate simultaneously in the same system as previously reported (Sizer *et al.*, 1988). The aerobic pathway predominates and operates at a higher rate as noted from the initial drop of L-ascorbic acid level when oxygen was still present at a high concentration.

Samples stored at 37°C showed an initial rate of 18·3 mg litre^{-1} day^{-1} L-ascorbic acid loss for the first 3 days (see Table 2). Subsequent rate of loss was 3·42 mg litre^{-1} day^{-1} (see Table 1). Similar results were obtained by other workers for single-strength orange juice stored in glass bottles (Trammell *et al.*, 1986).

The excellent r^2 values obtained by eliminating the initial results of L-ascorbic acid levels prior to regression of L-ascorbic acid content against time (Table 1) exemplify this effect of oxygen on L-ascorbic acid degradation.

These observations suggest that the dissolved oxygen level is a limiting factor in the decomposition of L-ascorbic acid. Other workers investigating the degradation kinetics of L-ascorbic acid at high temperature and water

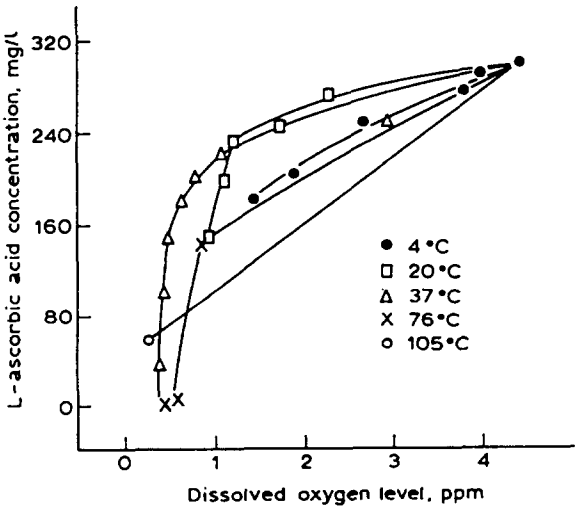

Fig. 3. Loss of L-ascorbic acid against dissolved oxygen concentration.

activity suggested that dissolved oxygen concentration was limiting above 92°C (Laing *et al.*, 1978). The initial dissolved oxygen concentration likewise seemed to affect the rate of degradation. Samples which were initially incubated at 37°C contained 1·70 ppm of dissolved oxygen upon supplementation of L-ascorbic acid to the original level whilst the control contained 4·45 ppm of dissolved oxygen. A comparison between the rate of loss of L-ascorbic acid against the dissolved oxygen level calculated for the first two values of L-ascorbic acid content against the dissolved oxygen level for these two samples showed the loss of L-ascorbic acid in the control to be greater than the supplemented sample by 64·5% (see Table 2).

This correlation between dissolved oxygen and L-ascorbic acid stability is further exhibited in frozen samples (stored at −20°C). Due to the high level of oxygen incorporated into the samples during processing (4·45 ppm), losses of 2·6% L-ascorbic acid and 0·15 ppm of dissolved oxygen were recorded after 23 day storage at −20°C. Although the stability of L-ascorbic acid generally increases as the temperature of the food is lowered, some investigators have reported L-ascorbic acid loss on freezing or frozen storage (Charoenrein & Reid, 1989).

The rate of oxidation of L-ascorbic acid, however, as affected by the dissolved oxygen level, is temperature dependent. At the lower storage temperatures of 4 and 20°C, r^2 and k values (rate constant) remained almost constant for a particular reaction order equation (Table 1).

In a separate study (Robertson & Samaniego, 1986) on the effect of different initial dissolved oxygen concentrations (0·41, 1·44 and 3·74 ppm), it was concluded that no significant effect on the rate of L-ascorbic acid degradation at 36°C could be attributed to the different oxygen levels used. It has been suggested that L-ascorbic acid degradation is predominantly anaerobic. Nevertheless, in spite of the differences in the initial rates of L-ascorbic acid decomposition, the shelf-lives of the products (in terms of L-ascorbic acid retention) did not vary significantly.

A dissolved oxygen level of 4·45 ppm is typical for normal plant operating conditions whilst a dissolved oxygen concentration of approximately 1·8 ppm is representative of commercially deaerated orange juice (Trammell *et al.*, 1986).

CONCLUSIONS

The level of dissolved oxygen normally present in the juice after packaging coupled with storage at temperatures of about 37°C are sufficient to cause a dramatic loss in the L-ascorbic acid content of the sample. Hence, deaeration during processing could possibly increase the retention of L-ascorbic acid since the dissolved oxygen level appears to be a limiting factor in the degradation process. However, attention should also be drawn to the fact that, although the rate of oxidation of L-ascorbic acid is reduced in the presence of low levels of dissolved oxygen, the shelf-life of the samples may not be significantly extended.

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