Introduction

L-ascorbic acid (vitamin C) has a role in the non-enzymic browning of foods although the mechanism remains obscure; browning might occur from direct participation of ascorbic acid or from reactions between degradation products of the vitamin and free amino acids or other compounds. However, some factors are known to influence the browning process, including temperature, time, pH, oxygen content, amino acids, sugars and trace metals. This note examines some of these influences using model systems and PLRP-S columns. PLRP-S is a rigid macroporous styrene/divinylbenzene HPLC phase with outstanding chemical stability with acidic eluents.
Materials and Reagents

Two model systems containing L-ascorbic acid, amino acids and carbohydrates were established and stored at 5 ºC and 25 ºC to simulate storage of fruit juices in retail premises, and at 5 ºC and 40 ºC to accelerate degradation and browning.

Model 1
L-ascorbic acid 400 mg/L + amino acid 1 g/L + monosaccharides 120 g/L, pH 3

Model 2
L-ascorbic acid 402 mg/L + L-lysine 0.843 g/L + glucose 250 g/L, pH not adjusted

These concentrations are typical of orange juice.

Conditions

Analysis of vitamin C
Columns: 2 x PLRP-S 100Å 5 µm, 150 x 4.6 mm (p/n PL1111-3500)
Eluent: 0.2 M NaH2PO4, pH 2.14
Flow rate: 0.5 mL/min
Detector: UV, 268 and 220 nm

The system was calibrated with fresh solutions of L-ascorbic acid and oxalic acid in eluting buffer.

Analysis of carbohydrates
Column: irregular silica 5 µm, 200 x 8 mm
Eluent: ACN:water 70:30 (v/v) + 0.01% 1.4 diaminobutane as amine modifier
Detector: RI

This system was calibrated with aqueous solutions of glucose and fructose.

Browning was measured at 280 and 480 nm.

Results and Discussion

The amounts of the constituent compounds were assessed after storage times of one day to four weeks. In model system 1, L-ascorbic acid loss was in the range of 51 to 91.7% at 5 ºC storage and 59.7 to 98% at 25 ºC storage, both for four weeks. In model system 2, L-ascorbic acid loss values ranged from 41 to 64.2% at 5 ºC and from 99.2 to 99.9% at 25 ºC, both for four days.

Degradation products in the model 2 system after storage at 40 ºC are shown in the figures, at two wavelengths. Figure 1 is L-ascorbic acid without sugars or amino acids after three days, and Figure 2 shows L-ascorbic acid with glucose, also after three days. Figure 3 is L-ascorbic acid containing glucose and aspartic acid after four days. The chromatograms reveal the presence of degradation products, possibly, in elution order, oxalic acid, threonic acid, 2,3-diketogulonic acid (DKGA), dehydroascorbic acid (DHAA), furfural and acetic acid. Dual wavelength detection was useful in verifying the absence of interferences and revealing the presence of new compounds. This is particularly noteworthy in Figure 3, which reveals at 268 nm the presence of a peak at the back of the ascorbic acid peak, this peak not being apparent at 220 nm.

With regard to browning, there were no absorbance readings at 280 or 480 nm in samples containing glucose and amino acids without L-ascorbic acid, irrespective of temperature. This suggests that a Maillard type reaction is not the predominant cause of non-enzymic browning. Reactions between sugars and amino acids, leading to increased amounts of reactive carbonyl compounds, appear unlikely to be the main contributors to the formation of brown pigments, even though their presence enhances browning.

The complete data set and analysis is available in Kennedy et al. (1989)1.

Figure 1. L-Ascorbic acid solution without sugars or amino acids after three days.
Conclusion

PLRP-S columns successfully revealed the presence of breakdown products of vitamin C after extended storage periods. Degradation was accelerated at increasing storage temperature, and degradation pathways other than DHAA to DKGA to oxalic and threonic acids seemed to operate. Glucose and/or fructose increased L-ascorbic acid retention but amino acids had no clear cut effect. In addition, the presence of amino acids did not appear to have any effect on glucose and fructose levels at 5 °C or 25 °C storage.

The macroreticular structure of PLRP-S is well suited to this type of application because it provides good retention of vitamin C molecules.

Reference