Non-enzymic Browning in Orange Juice using a Model System

Application Note

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Introduction

Oxidative changes in foods during storage due to non-enzymic browning reactions lead to off-flavors and color deterioration. An important food constituent that has focussed attention on this type of browning is L-ascorbic acid (vitamin C). This acid is present in many foods, occurring naturally or added as an anti-oxidant.

L-ascorbic acid has a role in the non-enzymic browning of foods although the mechanism remains obscure. 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 the influences on L-ascorbic acid that lead to browning in fresh orange juice using a model system. In addition, some suspected browning precursors were investigated.

PLRP-S columns were used in this investigation. PLRP-S is a rigid macroporous styrene/divinylbenzene HPLC phase which is excellent for the determination of L-ascorbic acid in fruit juice without the need for sample preparation.

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Materials and Reagents

The juice of fresh Navel oranges was centrifuged at 10000 g at 20 ºC and the supernatants were pooled and divided into 5 mL lots. 5 µL of aqueous solutions of lysine, glucose, sorbitol, L-ascorbic acid or water (control) were added and vials were then stored at 5, 38 and 50 ºC.

Conditions

Columns: 2 x PLRP-S 100Å 5 µm, 150 x 4.6 mm (p/n PL1111-3500)
Eluent: 0.2 M NaH_2PO_4, pH 2.14
Flow rate: 0.5 mL/min
Detector: UV, 220 nm

Browning was detected visually, but optical density ranges of 420 nm were assigned for visually grading the amount of browning.

Results and Discussion

Ion suppression-reversed phase HPLC produced the chromatograms shown in Figure 1a, revealing the presence of L-ascorbic acid and citric acid. The addition of glucose, lysine or sorbitol did not in general affect the level of L-ascorbic acid. A number of new peaks indicated the presence of L-dehydroascorbic acid, oxalic acid and threonic acid. Storage at higher temperatures (Figure 1b) markedly decreased L-ascorbic acid values in control and varied samples. Model solutions maintained at 5 ºC did not show any signs of browning, with or without additives. However, an increase in temperature with any of the additives increased the amount of browning, whether the pH was 1.15, 4.15 or 7. Sugar concentrations remained almost constant, except for the ratio of the individual sugars due to sucrose inversion at pH <4 and high temperature.

There was no sign of browning in the solutions kept at 5 ºC with or without additives, even at very low levels of L-ascorbic acid. However, at 38 and 50 ºC browning was seen, and an increase in temperature combined with any of the additives revealed even greater browning. In addition, samples at pH 1.15, 4.15 and 7, maintained at elevated temperatures, showed a faster onset of browning.

The complete data set and analysis is available in Kennedy *et al.* (1990).

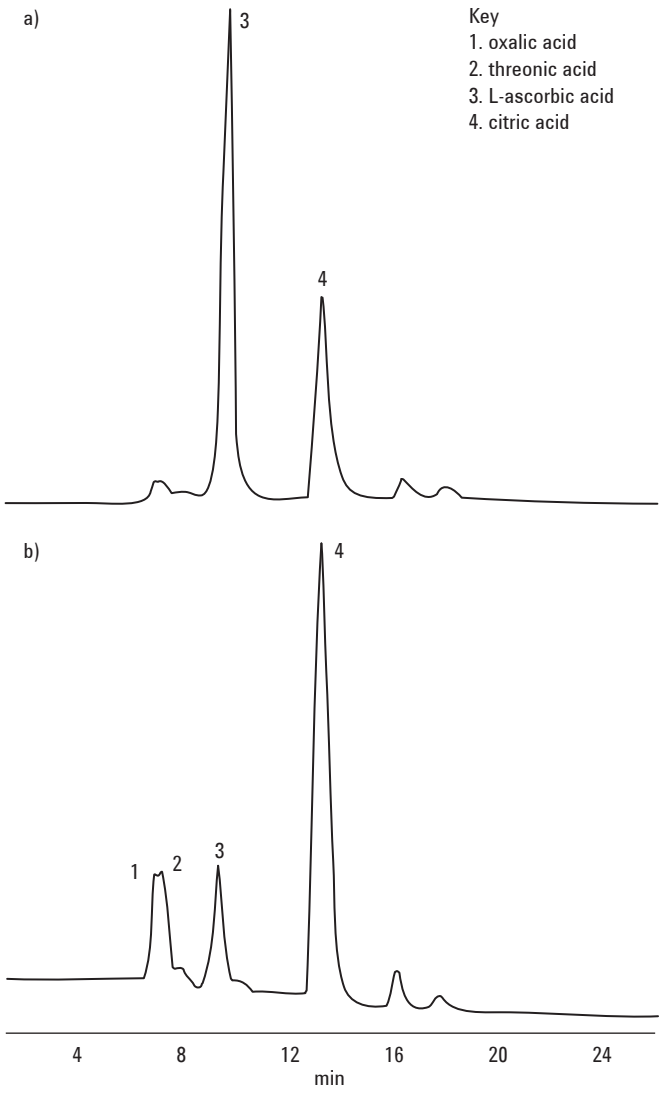

Figure 1. Separation of orange juice a) fresh squeezed (0.2 AUFS) and b) after storage at 50 ºC for five days (0.05 AUFS).

Conclusion

Ion suppression HPLC using PLRP-S columns successfully elucidated some of the effects of additives and temperature variations on L-acorbic acid in fresh orange juice. Using these polymeric reversed phase materials, an optimized system suggested that L-ascorbic acid is a precursor in the nonenzymic browning of orange juice because of the formation of reactive carbonyl compounds produced by its degradation.

Reference

Kennedy, JF, Rivera, ZS, Lloyd, LL, Warner, FP, Jumel, K (1990) Studies on non-enzymic browning in orange juice using a model system based on freshly squeezed orange juice. *J. Sci. Food Agric.,* 52, 85-95.

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