Early Stages of Maillard Reaction in Dehydrated Orange Juice

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The formation of furoylmethyl derivatives of amino acids as indicators of the early stages of Maillard reaction in dehydrated orange juices and model systems was studied. In stored dehydrated orange juices, the presence of furoylmethyl derivatives of arginine, asparagine, proline, alanine, glutamic acid, and GABA was detected. Their formation increased with temperature of storage. After 2 weeks at 30 °C and $a_w = 0.44$, the reconstituted orange juice contained 94 mg/L furoylmethyl derivatives, whereas up to 1215 mg/L was detected in samples stored at 50 °C.

Keywords: *Maillard reaction; storage; orange juice*

INTRODUCTION

The Maillard reaction is one of the most complex reactions that occur during the processing and storage of foods. The reaction is initiated by a condensation between the free amino group of an amino acid, peptide, or protein and the carbonyl group of a reducing sugar, leading to the formation of Amadori compounds. The activity of water, pH, time, and temperature govern the extent of the reaction. In the case of foods containing proteins and glucose, Amadori compounds are largely represented by protein-bound ϵ-fructosyl-lysine (Olano and Martínez-Castro, 1996).

The determination of the level of Amadori compounds present in foods allows one to detect the onset of the reaction before detrimental changes occur (del Castillo et al., 1998) as well as to retrospectively assess the heat treatment or storage conditions to which a product has been submitted.

In the case of foods containing free amino acids, free Amadori compounds can be present. Although Amadori compounds can be analyzed by HPLC and detected by differential refractometry (Möll and Gross 1981), the sensitivity of the method does not allow the detection of the low amounts present in foods. To improve sensitivity, HPLC methods involving derivatization have been proposed (Walton and McPherson 1987; Reutter and Eichner 1989).

A sensitive method for evaluating Amadori compounds involves an acid hydrolysis that releases protein-bound ϵ-fructosyl-lysine and generates furosine (ϵ-*N*-2-furoylmethyl-lysine) (Erbersdobler et al., 1995), which can be directly measured by HPLC (Resmini et al., 1990). In foods containing free Amadori compounds, acid hydrolysis would give rise to the formation of the corresponding 2-furoylmethyl derivatives, which could be easily determined by UV detection.

The purpose of this paper was to study the formation of furoylmethyl derivatives from the Amadori compounds of major amino acids present in orange juice and further study their presence in the hydrolysates of processed orange juices.

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MATERIALS AND METHODS

Model Systems. Mixtures of D-glucose and the corresponding L-amino acid [alanine, γ-aminobutyric acid (GABA), arginine, asparagine, aspartic acid, glutamic acid, proline, and serine] in molar ratios of 6:1, in water (5 mL), were lyophilized and equilibrated to $a_w = 0.44$ in a desiccator over saturated K_2CO_3 solution using the method of Labuza and Saltmarch (1981) and then stored at 50 °C.

Dehydrated Orange Juice Samples. Freshly squeezed orange juice was prepared in the laboratory from 2 kg of oranges (navel variety) using a domestic juicer Braun Citromatic MPZ2 (Braun Española, S.A., Spain). The extracted juice was filtered through a double layer of gauze to remove seeds and albedo fragments and to reduce pulp content. pH was measured (pH-meter MicropH2001 Crison Instruments, S.A., Barcelona, Spain) in aliquots (5 mL), which were then lyophilized, equilibrated to $a_w = 0.44$, and stored at the desired temperature (30 or 50 °C) for 14 days. Samples were taken at 4, 7, and 14 days of storage. Before analysis, samples were reconstituted to initial volume.

Synthesis of *N*-(1-Deoxy-D-frutosyl)-γ-aminobutyric Acid. *N*-(1-Deoxy-D-frutosyl)-γ-aminobutyric acid was obtained according to the procedure of Reutter and Eichner (1989). A mixture of glucose (3.25 g), γ-aminobutyric acid (0.5 g), microcrystalline cellulose (16.41 g), and water (100 mL) was lyophilized and stored for 14 days at 40 °C and $a_w = 0.35$ in a desiccator over saturated $CaCl_2$ solution. Following the storage, the mixture was treated with water (3 × 80 mL) at vacuum and the aqueous solution lyophilized. The reaction mixture was subjected to a purification by ion-exchange chromatography (Finot and Mauron, 1969) on Dowex 50 WX4 in pyridinium form. The characterization of *N*-(1-deoxy-D-frutosyl)-γ-aminobutyric acid was made by ^{13}C NMR spectroscopy (del Castillo et al., 1998).

HPLC Analysis of Furoylmethyl Derivatives. An ion-pair RP-HPLC method (Resmini et al., 1990) was used to analyze furoylmethyl derivatives using a C_8 column (250 mm × 4.6 mm i.d.) (Alltech Furosine-dedicated) with a linear binary gradient. A Dionex chromatograph (DX-300) and variable wavelength detector (LDC Analytical, SM 4000) were used. Acquisition and processing of data were achieved in an HPChem Station (Hewlett-Packard).

Before HPLC analysis, 1.3 mL of sample was hydrolyzed with 3 mL of 11.4 M HCl at 110 °C for 24 h in a Pyrex screw-cap vial with a PTFE-faced septum. High-purity helium gas was bubbled through the solution for 2 min. The hydrolysate was filtered with a medium-grade paper filter. A 0.5 mL portion of the filtrate was applied to a Sep-Pak C_{18} cartridge (Millipore, Madrid, Spain) prewetted with 5 mL of methanol

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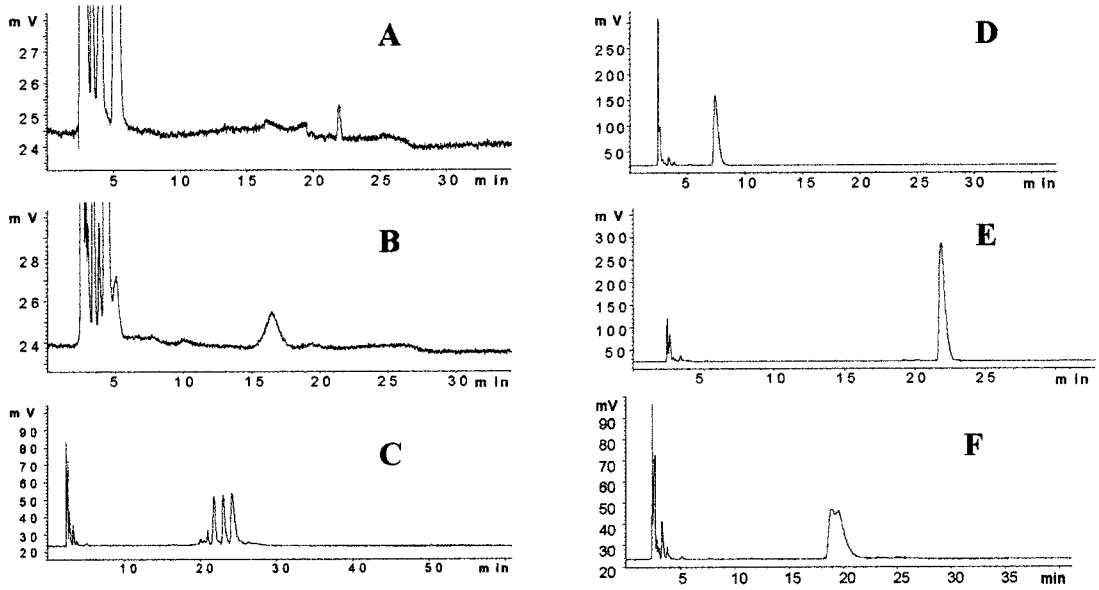

Figure 1. HPLC chromatograms of acid hydrolysates of different model systems containing glucose and amino acid (A, glutamic acid; B, asparagine; C, arginine; D, alanine; E, GABA; F, proline) stored at 50 °C and $a_w = 0.44$.

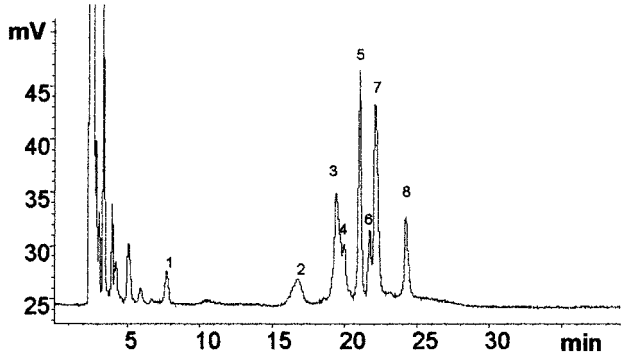

Figure 2. HPLC chromatogram of acid hydrolysate of dehydrated orange juice stored at 50 °C and $a_w = 0.44$. Peaks 1−8: furoylmethyl derivatives of (1) alanine, (2) asparagine, (3−4) proline, (5) GABA, (6) glutamic acid or arginine, (7) arginine, and (8) unknown.

and 10 mL of water, and furoylmethyl derivatives were eluted with 3 mL of 3 M HCl; 50 μL was injected in the chromatograph.

Calibration was performed by the external standard method using a commercial standard of pure furosine (Neosystem Laboratories, Strasbourg, France) that exhibits the same response factor as the furoylmethyl derivatives of GABA and arginine (del Castillo, 1999).

RESULTS AND DISCUSSION

Model Systems. Figure 1 shows the HPLC chromatograms of the acid hydrolysates of the stored model systems of glutamic acid, asparagine, arginine, alanine, GABA, and proline (parts A−F, respectively). No formation of furoylmethyl derivatives of aspartic acid and serine was observed. A simple peak was observed in model systems of glutamic acid, asparagine, alanine, and GABA, whereas multiple peaks were observed in model systems of proline and arginine. This could be attributed to the presence of different anomers and the formation of different Amadori compounds with the α-amino group and guanidino group of arginine. The participation of the guanidino group of arginine in the Maillard reaction has been described (Ledl and Schleider, 1990; Sopio and Lederer, 1995; Friedman, 1996).

Table 1. Formation of Furoylmethyl Derivatives during Storage of Dehydrated Orange Juice at Different Temperatures and $a_w = 0.44$

storage conditions		furoylmethyl derivatives (mg/L)[a]
temp (°C)	time (days)	
30	4	25.64
	7	58.49
	14	94.15
50	4	376.90
	7	663.88
	14	1215.38

[a] mg/L of reconstituted orange juice.

Previous studies on the formation of Amadori compounds in dehydrated model systems showed that only traces of these compounds were formed from serine, aspartic acid, and glutamic acid, whereas considerable amounts of Amadori compounds were detected in model systems containing alanine, arginine, asparagine, GABA, and proline (del Castillo et al., 1998).

The HPLC chromatogram of the hydrolysate of purified *N*-(1-deoxy-D-fructosyl)-γ-aminobutyric acid was identical to that of the model system containing glucose and γ-aminobutyric acid; thus, the peaks observed in the hydrolysates of model systems were tentatively assigned to the furoylmethyl derivatives of the corresponding amino acid.

Dehydrated Orange Juice. Freshly squeezed orange juice showed a pH value of 3.9, and no variation was observed in the reconstituted samples during the storage period.

Formation of furoylmethyl derivatives in dehydrated orange juice was detected during storage at 30 and 50 °C. No formation of these compounds was observed in freshly prepared dehydrated orange juice (control). Figure 2 shows the chromatogram of the acid hydrolysate of dehydrated orange juice stored for 14 days at 50 °C and $a_w = 0.44$; the presence of eight peaks can be observed. According to the retention times, seven of them were assigned as the furoylmethyl derivatives of alanine, asparagine, proline, GABA, glutamic acid, and arginine. The last peak in the chromatogram was not identified.

Table 1 shows the furoylmethyl derivative content in stored dehydrated orange juice. During storage considerable formation was observed at the two temperatures studied, at levels up to 94.1 and 1215.4 mg/L at 30 and 50 °C, respectively.

Because the formation of these compounds in the hydrolysates of orange juices is related to the extent of early Maillard reaction, they can be used to evaluate quality changes either during processing or during subsequent storage and could probably characterize them better.

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