Changes in the Amino Acid Composition of Dehydrated Orange Juice during Accelerated Nonenzymatic Browning

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Maillard reaction in dehydrated orange juice stored at 30 or 50 °C and $a_w = 0.44$ was studied. The decreases of the total amino acids were 30 and 65% of initial concentration after 14 days of storage at 30 and 50 °C, respectively. Storage at 50 °C for 14 days caused a decrease of 11.8 g/L of carbohydrates, and glucose was more reactive than fructose. Loss of sucrose due to hydrolysis was also observed. Presence of 1-(*N*-substituted)amino-1-deoxy-D-fructose compounds in stored dehydrated orange juice was detected by thin-layer chromatography.

Keywords: *Maillard reaction; storage; dehydrated orange juice*

INTRODUCTION

Nonenzymatic browning during processing or storage of citrus juice may affect the flavor, color, or other quality factors of the product. It is the most complex reaction in food chemistry due to the large number of food components able to participate in the reaction through different pathways giving rise to a complex mixture of products. Nonenzymatic browning may be originated by the condensation of a carbonyl group with amino acids (Maillard reaction); however, sugars and ascorbic acid also undergo browning reactions in the absence of free amino acids (caramelization), and many of the products formed are similar to those resulting from the Maillard reaction (Olano and Martínez-Castro, 1996). Although correlations of increased browning with increased storage time and temperature of citrus juices have been reported (Kennedy et al., 1990), the mechanism responsible for the deterioration with aging is not well understood and there is still a lack of understanding concerning the fundamental factors involved (Kacem et al., 1987).

This work was undertaken to study the compositional changes that occur in the amino acid fraction of dehydrated orange juice during storage under adverse conditions as a contribution to the knowledge of the role of individual amino acids in nonenzymatic browning of orange juice.

MATERIALS AND METHODS

Orange Juice Samples. Aliquots (5 mL) of single-strength fresh juice 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 the desired temperature (30 or 50 °C) for 14 days. Samples were taken at 4, 7, 11, and 14 days of storage. Before each analysis, samples were reconstituted to initial volume.

Model Systems. Mixtures of D-glucose and L-amino acids (alanine, γ-aminobutyric acid, arginine, asparagine, aspartic acid, glutamic acid, proline, and serine) in molar ratios of 6:1, in water (5 mL), were lyophilized and stored at 50 °C and $a_w = 0.44$. Storage was stopped when a brown color appeared.

Synthesis of *N*-(1-Deoxy-D-fructosyl)-γ-aminobutyric Acid. *N*-(1-Deoxy-D-fructosyl)-γ-aminobutyric acid was obtained according to the procedure of Reuter 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 washed with water (3 × 80 mL) in vacuo and lyophilized. The reaction mixture was subjected to ion-exchange chromatography (Finot and Mauron, 1969) on Dowex 50 WX4 in pyridinium form. The excess of glucose was eluted with water. The Amadori compound was eluted with 0.2 M pyridine/formic acid buffer, pH 3.25, followed by unreacted amino acid. The presence of the Amadori compound in the collected fractions was evidenced by TLC. Fractions containing the product were combined, lyophilized, and then dissolved in a minimum amount of methanol. Diethyl ether was added dropwise to turbidity with vigorous stirring. After 24 h, the precipitate formed was filtered, washed with diethyl ether, and dried with heat. The product was then characterized by ^{13}C NMR spectrum recorded using a Bruker AM-200 spectrometer operating at 50 MHz using D_2O as solvent under standard conditions.

Browning. Formation of browning pigments was determined by measuring the absorbance at 420 nm using a Shimadzau spectrophotometer UV (120-01) following the method of Ting and Rouseff (1986).

Chromatographic Methods. *Thin-Layer Chromatography (TLC).* Detection of Amadori compounds in reaction mixtures (carbohydrates and amino acids) and stored dehydrated orange juice was carried out by TLC on cellulose aluminum plates. The solvent system was 1-butanol/pyridine/water, 2:3:1 (v/v). Plates were sprayed with 0.5% 2,3,5-triphenyl-2*H*-tetrazolium chloride in 0.5 mol/L sodium hydroxide (Reuter and Eichner, 1989). The identification of Amadori compounds in stored dehydrated orange juice and model systems was achieved by comparison with the R_f values of synthesized standard.

High-Performance Liquid Chromatography (HPLC). Determination of Amino Acids. Reconstituted orange juice samples were centrifuged at 6940*g* for 20 min at 20 °C, and free amino acids were determined in supernatant previously diluted with 0.4 M borate buffer, pH 10. Analysis was carried out by HPLC using a Waters liquid chromatograph controlled by a Maxima 820 chromatography workstation. Samples were submitted to an automatic precolumn double derivatization with *o*-phthaldialdehyde (OPA) (González de Llano et al., 1991) to

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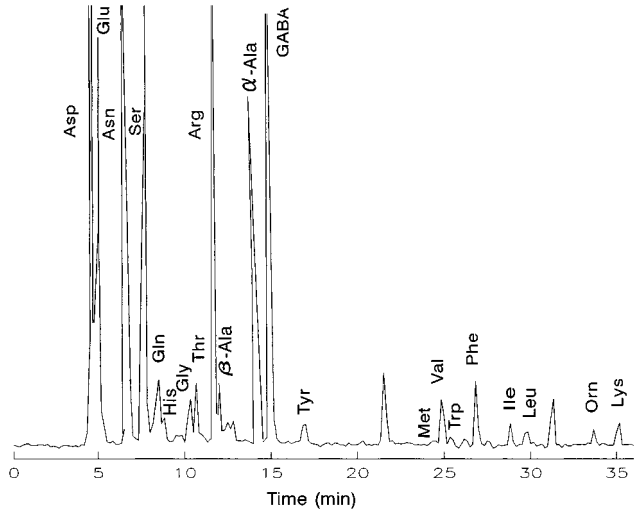

Figure 1. HPLC chromatogram of OPA amino acids of fresh dehydrated orange juice: aspartic acid (Asp), glutamic acid (Glu), asparagine (Asn), serine (Ser), glutamine (Gln), histidine (His), glycine (Gly), threonine (Thr), arginine (Arg), β-alanine (β-Ala), α-alanine (α-ala), γ-aminobutyric acid (GABA), tyrosine (Tyr), methionine (Met), valine (Val), tryptophan (Trp), phenylalanine (Phe), isoleucine (Ile), leucine (Leu), ornithine (Orn), and lysine (Lys).

determine primary amino acids and with 9-fluorenylmethylchloroformate (FMOC) to detect secondary amino acids (Einarsson, 1985). The separation of amino acids was performed in a Novapak C_{18} 60 Å 4 μm column (3.9 cm × 150 mm). Detection was by fluorescence using wavelengths of excitation and emission at 340 and 425 nm, respectively, for OPA derivatives. For FMOC derivatives the excitation and emission wavelengths were 250 and 335 nm, respectively. Samples were filtered through a 0.22 μm membrane filter before analysis.

Determination of Carbohydrates. Fructose, glucose, and sucrose were determined by HPLC in a Perkin-Elmer liquid chromatograph using a carbohydrate analysis column (Waters) (3.9 × 300 mm) and a Waters 410 refractive index detector. The elution was isocratic using acetonitrile/water 80:20 (v/v) as mobile phase (flow rate = 2 mL/min) following the method of Hurst et al. (1979). Calibration was made using an internal standard method. Samples were prepared by mixing 1 mL of orange juice, 1 mL of threitol solution (internal standard, 10 mg/mL), and 8 mL of mobile phase, followed by centrifugation at 6940*g* for 20 min at 20 °C. An aliquot was filtered through a 0.22 μm membrane filter and analyzed by HPLC.

RESULTS AND DISCUSSION

Figure 1 shows a chromatogram of the amino acids found in fresh dehydrated orange juice. The chromatographic profile of the amino acids was similar to that corresponding to fresh orange juice (Cavazos et al., 1996).

The total amino acid content in fresh dehydrated orange juice was 4.65 g/L. The loss of total amino acids during storage at 50 °C accounted for 41.5, 51.0, 59.0, and 65.1% of initial content at 4, 7, 11, and 14 days of storage, respectively (Figures 2−4). The decrease of the major amino acids proline, arginine, asparagine, and γ-aminobutyric acid represented ~78.1% of the total loss. Except for lysine, glycine, and ornithine, the main loss took place during the first 4 days.

Wolfrom et al. (1974) studied the effect of amino acid type on the extent of browning in a 1:1 molar ratio of a mixture of D-glucose/amino acid heated at 65 °C. They found that the most rapid color development was produced by L-arginine and γ-aminobutyric acid followed

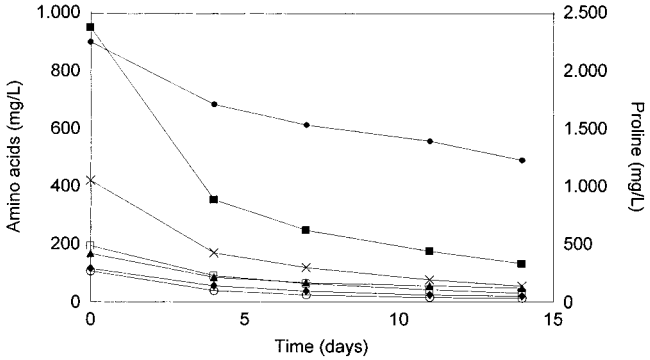

Figure 2. Loss of amino acids during storage of dehydrated orange juice at 50 °C and a_w = 0.44: γ-aminobutyric acid (□), arginine (■), asparagine (×), aspartic acid (▲), glutamic acid (○), proline (●), and serine (◆).

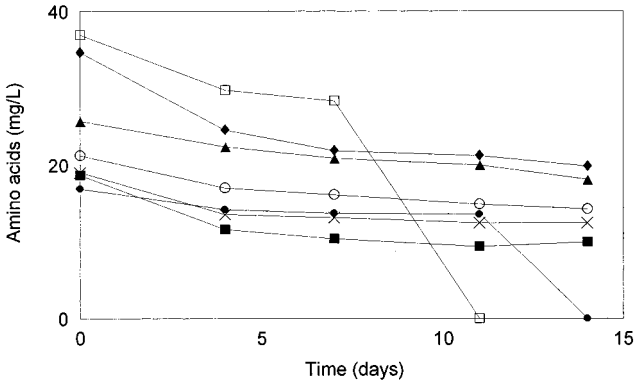

Figure 3. Loss of amino acids during storage of dehydrated orange juice at 50 °C and a_w = 0.44: glycine (○), isoleucine (●), leucine (▲), ornithine (□), phenylalanine (◆), threonine (■), and valine (×).

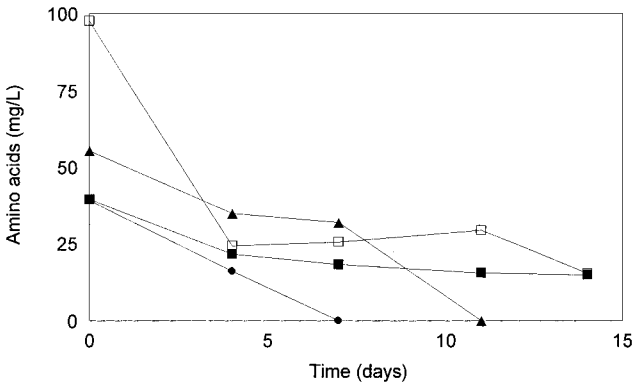

Figure 4. Loss of amino acids during storage of dehydrated orange juice at 50 °C and a_w = 0.44: α-alanine (■), β-alanine (□), glutamine (●), and lysine (▲).

by glycine, alanine, serine, and L-proline, which is consistent with present results.

Storage at 30 °C resulted in a slower loss of amino acids. The decrease of the total amino acid content was 29.7% of initial content at 14 days of storage. This loss was lower than that found after 4 days of storage at 50 °C.

In Figure 5 can be observed a chromatogram of carbohydrates present in dehydrated orange juice. The amounts of glucose, fructose, and sucrose found in control sample (Table 1) were those expected for this type of juice (Aristoy et al., 1989). During storage at 50 °C, the sucrose content decreased progressively from 41.4 to 30.4 g/L. Since nonreducing carbohydrates do

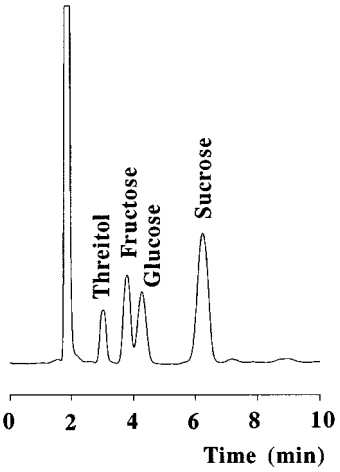

Figure 5. HPLC chromatogram of carbohydrates in fresh dehydrated orange juice: column, "carbohydrate analysis", 3.9 × 300 mm (Waters); mobile phase, acetonitrile/water 80:20 (v/v); dectector, refractive index; calibration, internal standard (threitol).

Table 1. Variation of Carbohydrate Fraction of Dehydrated Orange Juice during Storage at Different Temperatures[a]

storage		carbohydrates (g/L)		
temp (°C)	time (days)	fructose	glucose	sucrose
	control	18.7 (1.24)[b]	18.8 (3.26)	41.4 (0.80)
50	7	19.9 (0.22)	17.2 (0.7)	37.1 (0.28)
	14	19.8 (1.92)	16.9 (3.76)	30.4 (2.24)
30	14	18.0 (3.92)	18.2 (3.3)	37.2 (4.4)

[a] $n = 3$. [b] Numbers in parentheses are the relative standard deviations (in %).

not undergo Maillard reactions, the loss of sucrose is due to hydrolysis under the acidic conditions of the sample. Despite the considerable release of glucose and fructose due to sucrose hydrolysis, the total amount of reducing monosaccharides decreased along the storage period. Glucose was found to be the more reactive hexose, decreasing from 18.8 to 16.9 g/L, while fructose increased from 18.7 to 19.8 g/L. This indicates that degradation of fructose was lower than the amount released during sucrose hydrolysis. A higher loss of glucose was also observed by Ural (1978) during storage of orange juice at 50 °C from 22 days. The total loss of carbohydrates was 11.8 g/L, equivalent to 65 mM of hexoses, which is considerably higher than the corresponding loss of amino acids (3.03 g/L; 23 mM). This gives an indication of the extent of caramelization and Maillard reaction under the assayed conditions.

The color change during storage at 50 °C is shown in Figure 6. Despite the considerable loss of amino acids at the fourth day of storage, color development was relatively modest since the 1-(*N*-substituted)amino-1-deoxy-D-fructose compounds formed during the initial stages of storage are colorless. Development of color rose sharply at the end of the studied period when these compounds are degraded to brown pigments. After 14 days of storage at 30 °C, only a slight development of color was observed (Figure 6).

During storage of lyophilized model systems a considerable development of color was observed for alanine, arginine, and γ-aminobutyric acid, giving rise to absorbance values up to 9.36 after 7 days of storage. For proline and asparagine absorbance values of 1.25 were achieved after 14 days of storage. In model systems

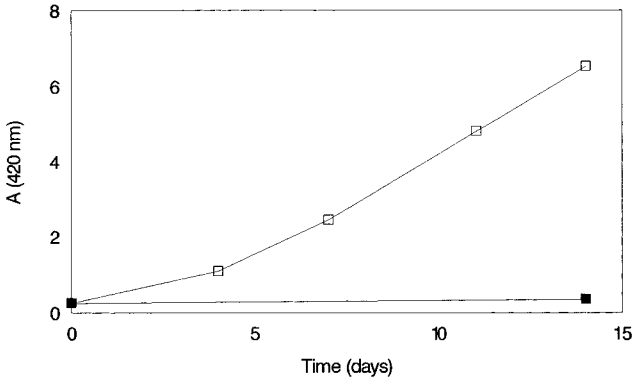

Figure 6. Development of color during storage at 50 (□) and 30 °C (■) at $a_w = 0.44$ of dehydrated orange juice.

Table 2. ^{13}C NMR Chemical Shifts for Fructosyl-γ-aminobutyric Acid in D_2O

carbon atom		
C-1	β-pyranose	54.12
	α-pyranose	50.19
	β-furanose	53.51
	α-furanose	52.29
C-2	β-pyranose	96.73
	α-pyranose	97.25
	β-furanose	100.09
	α-furanose	103.18
C-3	β-pyranose	70.85
	α-pyranose	72.91
	β-furanose	79.09
	α-furanose	83.79
C-4	β-pyranose	70.60
	α-pyranose	71.60
	β-furanose	75.44
	α-furanose	77.25
C-5	β-pyranose	70.18
	α-pyranose	66.83
	β-furanose	82.15
	α-furanose	83.55
C-6	β-pyranose	65.20
	α-pyranose	63.75
	β-furanose	63.09
	α-furanose	62.04
COO^-		182.46
C^α		35.68
C^β		23.06
C^γ		49.72

containing serine or glutamic or aspartic acids, development of color was not observed.

The ^{13}C NMR spectrum of fructosyl-γ-aminobutyric acid allowed the assignment of the characteristic signals for this compound (Table 2), showing a predominance of β-pyranose form as well as a constant distribution between four tautomeric forms. The results agree with those of Mossine et al. (1994).

TLC analysis of model systems allowed the detection of Amadori compounds formed during storage. The R_f values of the spots ranged from 0.06 (model system containing arginine) to 0.23 (model system containing proline), and the R_f of the isolated fructosyl-γ-aminobutyric acid was 0.13. In the case of model systems containing serine or aspartic or glutamic acids, only traces of Amadori compounds were detected, in agreement with the negligible development of color observed.

TLC of stored dehydrated orange juices showed the presence of spots with R_f values similar to those

observed in stored model systems. These spots were absent in fresh dehydrated orange juice.

These results seem to indicate that changes in amino acid composition and formation of Amadori compounds may be suitable indicators to characterize the storage conditions in orange juice before color change became significant. Further investigation is to be carried out on changes in single-strength and concentrated orange juice during storage.

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