

# Use of 2-Furoylmethyl Derivatives of GABA and Arginine as Indicators of the Initial Steps of Maillard Reaction in Orange Juice

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The formation of 2-furoylmethyl derivatives of GABA (2-FM-GABA) and arginine (2-FM-Arg) as early indicators of nonenzymatic browning in different types of orange juice was studied. In dehydrated orange juice, the presence of 2-FM-GABA and 2-FM-Arg was detected from the first day of storage at 30 °C. In this type of juice, the content of these two compounds increased with temperature (30, 50 °C) and time (1–7 days) of storage. A noticeable increase in 5-hydroxymethylfurfural was only observed after 4 days of storage at 50 °C. No formation of 2-FM-GABA and 2-FM-Arg was detected in liquid orange juice heated under conditions similar to those used in the industry. These furoylmethyl derivatives were also found in commercial orange juice made from concentrates. A slight increase in their concentration was observed in the two samples stored during 8 months at room temperature. According to the results obtained, 2-FM-GABA and 2-FM-Arg contents could be suitable indicators to assess the main modifications due to Maillard reaction produced during the manufacture and/or storage of orange juice concentrates.

**Keywords:** 2-Furoylmethyl-GABA; 2-furoylmethyl-Arg; nonenzymatic browning; orange juice

## INTRODUCTION

Nonenzymatic browning is one of the most important chemical reactions responsible for quality and color changes during the heating or prolonged storage of citrus products (Rodríguez et al., 1991). Nonenzymatic browning is referred to as a Maillard reaction when it is initiated by the condensation of the carbonyl group of reducing sugars with free amino groups of amino acids and/or proteins.

5-Hydroxymethylfurfural (HMF) formed from Maillard reaction, hexoses, and/or vitamin C degradation (Hodge, 1953; Nagy 1980; Lee and Nagy, 1988) has been correlated with browning in orange juice (Meydav and Berk, 1978). HMF production is considered to be a useful indicator of the quality of stored fruit products (Pollard and Timberlake, 1971). Amadori compounds, the first stable compounds of the Maillard reaction, are very sensitive indicators to detect changes in food quality. These compounds can be measured after their transformation into 2-furoylmethyl amino acids (2-FM-AA) by acid hydrolysis. 2-Furoylmethyl derivatives of alanine (2-FM-Ala), asparagine (2-FM-Asn), proline (2-FM-Pro),  $\gamma$ -aminobutyric acid (2-FM-GABA), glutamic acid (2-FM-Glu), and arginine (2-FM-Arg) have been also reported as indicators of the Maillard reaction development in orange juice (del Castillo et al., 1999a).

In this work the determination of furoylmethyl derivatives 2-FM-GABA and 2-FM-Arg has been studied in different types of orange juice in order to use these compounds as indicators of nonenzymatic browning.

## MATERIALS AND METHODS

**Standard Substances.** *Synthesis, Isolation, and Characterization of 2-FM-Arg.* Amadori compound  $\alpha$ -N-(1-deoxy-D-

fructosyl)-Arg (600 mg), synthesized following the procedure described by Finot and Mauron (1969), was hydrolyzed with 7.95 M HCl (500 mL) for 24 h (Finot et al., 1968) under reflux, obtaining 2-FM-Arg. Then, HCl was evaporated in a vacuum at 40 °C, and the dried residue was dissolved in a minimum amount of water and placed on a 345 cm<sup>3</sup> column of Dowex 50Wx4 ion-exchange resin in acid form. The mixture of the reaction was eluted with 2 M HCl. The presence of 2-FM-Arg was detected by measurement of the optical absorbance at 280 nm and by thin-layer chromatography (TLC). Fractions 241–320 containing the product were combined and lyophilized.

The identification of the synthesized compounds was carried out by <sup>1</sup>H NMR and by fast-atom bombardment mass spectroscopy (FABMS) (del Castillo et al., 1999b).

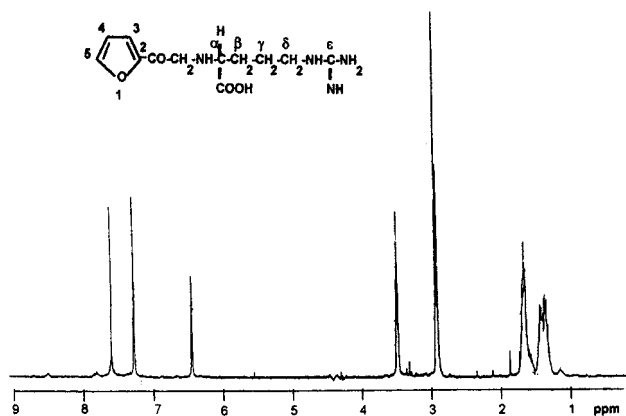
**Orange Juice Prepared in the Laboratory.** *Samples.* Freshly squeezed orange juice was prepared in the laboratory from 15 kg of oranges (Navel (10 kg) and Navel late (5 kg) varieties) using a domestic juicer Braun Citromatic MPZ2 (Braun Española, Barcelona, Spain). The extracted juice was filtered through a double layer of gauze to remove seeds and albedo fragments and to reduce pulp content. The pH of the samples was measured using a Crison MicropH2001 pH-meter (Crison Instruments S.A, Barcelona, Spain).

*Storage.* Aliquots of 5 mL of freshly squeezed orange juice (Navel late variety) were lyophilized and then equilibrated to  $a_w = 0.44$  in a desiccator over saturated K<sub>2</sub>CO<sub>3</sub> solution using the method of Labuza and Saltmarch (1981) and then stored at the desired temperature (30 or 50 °C) for 7 days. Samples were withdrawn at 0, 1, 2, 3, 4, and 7 days of storage and analyzed in duplicate. Before analysis, samples were reconstituted to initial volume.

*Heat Treatments.* Freshly squeezed orange juice (Navel variety) was heated in a continuous-flow system (Villamiel et al., 1998). Flow rates of 98 and 87.2 mL min<sup>-1</sup> were used to achieve outlet temperatures near 90 and 96 °C and residence times of 24 and 27s, respectively. The orange juice leaving the oven was then passed through a tube immersed in an ice/water-bath to cool the heated juice rapidly. Heat treatments were performed in duplicate.

**Commercial Orange Juices.** *Samples.* Six samples of straight-processed orange juices and 19 samples of single-

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**Figure 1.**  $^1\text{H}$  NMR spectrum ( $\text{D}_2\text{O}$ ) of 2-FM-Arg.

strength orange juices from concentrates were purchased at different local markets.

**Storage.** Two batches (I and II) of commercial single-strength orange juice from concentrates were stored at room temperature for 8 months. Two containers of each batch were opened after 0, 30, 60, 180, and 240 days of storage, and samples were analyzed in duplicate.

**Analytical Determinations.** *Colorimetric methods.* HMF was determined by measuring the absorbance at 550 nm in a Beckman spectrophotometer UV-Vis (DU-70) according to the method reported by IFFJP (1972).

Browning development was measured as the absorbance at 420 nm using a Shimadzu spectrophotometer UV (120-01) following the method of Meydav et al. (1977).

*Chromatographic Methods.* Thin-layer chromatography (TLC) analysis was performed on Silicagel G 60 F254 aluminum plates following the method of Finot and Mauron (1969).

2-FM-AA were analyzed by ion-pair RP-HPLC following the method of Resmini et al. (1990). Calibration was performed by the external-standard method using standards of pure 2-FM-GABA (del Castillo et al., 1999b) and 2-FM-Arg.

**Statistical Analysis.** The BMDP package (Dixon, 1988) was used for discriminant analysis (BMDP7M).

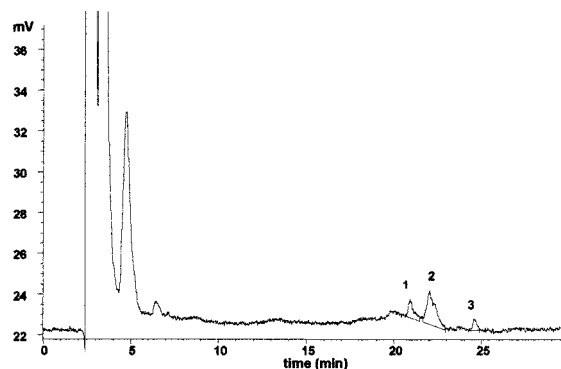
## RESULTS AND DISCUSSION

**Synthesis of 2-FM-Arg.** 2-FM-Arg, obtained by acid hydrolysis of Amadori compound, showed a low stability in dry state and high stability in acid conditions. The exact mass of the  $(\text{M}+\text{H})^+$  ion for  $\text{C}_{12}\text{H}_{19}\text{N}_4\text{O}_4$  was calculated to be 283.3084 (found, 283.1393).

The  $^1\text{H}$  NMR spectrum is shown in Figure 1. The low field multiple shifts (6.50, 7.32, and 7.62) are due to the 3 protons present in the 2-substituted furanic ring, results which are in agreement with those previously reported for 2-FM-lys (furosine) (Delgado et al., 1992) and 2-FM-GABA (del Castillo et al., 1999b). The other signals correspond to the deuterated arginine moiety of the molecule.

HPLC analysis showed a peak assigned as 2-FM-Arg with retention time near 23 min. Peak identities were checked by retention times and chromatographic patterns and confirmed by the spike of standard to the sample.

**Orange Juice Samples Prepared in the Laboratory.** *Storage Assays.* The presence of 2-FM-GABA and 2-FM-Arg was detected from the first day of storage at 30 °C, and they could be quantified from the second day (Figure 2). The formation of 2-FM-GABA and 2-FM-Arg during storage at 30 and 50 °C is shown in Figure 3 (A and B). Values up to 15.04 and 25.56 mg/L of 2-FM-GABA and 2-FM-Arg, respectively, were found at 30 °C,



**Figure 2.** HPLC chromatographic profile of acid hydrolyzate of dehydrated orange juice stored for 2 days at 30 °C and  $a_w = 0.44$ . 1, 2-FM-GABA; 2, 2-FM-Arg; 3, unknown.

whereas 125.5 and 201.55 mg/L were obtained, respectively, when storage was carried out at 50 °C.

Other established indicators of nonenzymatic browning in orange juices, such as HMF, were also determined. Figure 4 shows the formation of HMF in the dehydrated orange juice during storage at 30 and 50 °C and  $a_w = 0.44$ . No changes in HMF were detected in the samples stored at 30 °C. At 50 °C an increase in  $A_{550 \text{ nm}}$  was found during the first 4 days of storage, reaching values up to 18.4 mg/L at the end of the storage period.

The browning development ( $A_{420 \text{ nm}}$ ) in stored dehydrated orange juice was also measured. At 30 °C, in agreement with the data of HMF, no modifications were observed, whereas storage at 50 °C provoked a progressive browning (from 0.134 to 0.829) mainly after 4 days (0.453) of storage.

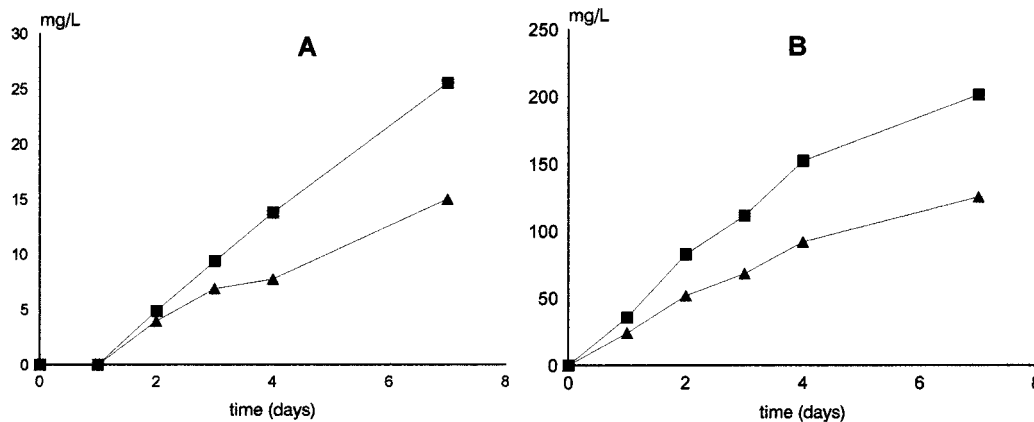
During the storage of dehydrated orange juice at 30 °C, the initial stage of Maillard reaction takes place giving rise to the formation of colorless compounds (Amadori compounds). At 50 °C, formation of HMF and color development were observed as a consequence of the progress of the Maillard reaction.

From these results it can be stated that 2-FM-GABA and 2-FM-Arg seem to be the most sensitive and useful indicators of Maillard reaction in stored dehydrated orange juice because they are the first compounds formed prior to browning development.

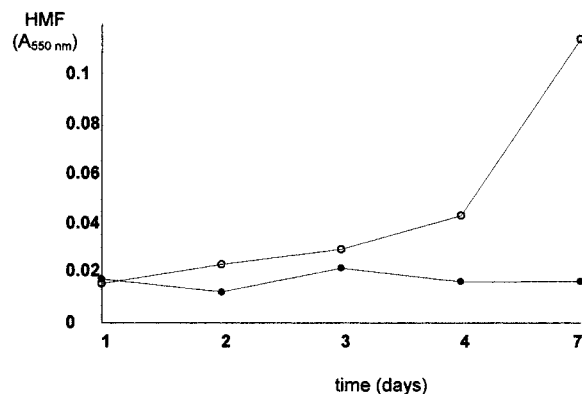
*Heat Treatment Assays.* To evaluate the possible formation of 2-FM-GABA and 2-FM-Arg from heat treatments applied during orange juice manufacture, fresh orange juice was heat treated under conditions similar to those used in the industry. No formation of 2-FM-AA was detected in any of the heated orange juices. This fact is in agreement with previous results showed by Villamiel et al. (1998) who did not observed changes in the carbohydrate and amino acid composition of orange juice heated under the same conditions.

**Commercial Orange Juices.** Two types of commercial orange juice were analyzed to study the presence of 2-FM-GABA and 2-FM-Arg. No formation of 2-FM-GABA and 2-FM-Arg was detected in any of the straight-processed orange juice samples. However, these 2-FM derivatives were found in 15 samples of single-strength orange juices made from concentrate (Table 1). Discriminant analysis permitted a 100% and a 76.5% of proper assignment for the straight-processed and single-strength made-from-concentrates orange juices, respectively.

Because single-strength made-from-concentrates orange juices showed presence of 2-FM-GABA and 2-FM-



**Figure 3.** Formation (mg/L) of 2-FM-GABA (▲) and 2-FM-Arg (■) during the storage of dehydrated orange juice at 30 °C (A) and 50 °C (B) under  $a_w = 0.44$ .



**Figure 4.** HMF formation during storage of dehydrated orange juice at 30 °C (●) and 50 °C (○) under  $a_w = 0.44$ .

**Table 1. 2-FM-GABA and 2-FM-Arg (mg/L) Content in Commercial Orange Juices Made from Concentrates**

juice	2-FM-GABA	2-FM-Arg
1	2.63	4.28
2	1.78	3.13
3	2.85	4.72
4	1.55	3.81
5	1.38	2.59
6	1.30	4.33
7	0.99	1.28
8	1.99	2.06
9	1.67	2.86
10	1.50	1.40
11	1.32	1.90
12	2.63	4.48
13	3.40	2.86
14 <sup>a</sup>	1.07	1.17
15 <sup>a</sup>	2.75	2.38
mean	1.92	2.88
rsd <sup>b</sup> (%)	38.80	42.05

<sup>a</sup> Samples used for storage assays. <sup>b</sup> rsd, relative standard deviation.

Arg, a study of their evolution during room temperature storage was carried out. After 8 months of storage a scarce increase in the content of these compounds was detected in the analyzed orange juices, this increase being slightly higher in the case of batch I. These data could point out that the main modifications due to Maillard reaction are produced during the manufacture and/or storage of concentrates used in this type of orange juice.

On the basis of the results here obtained, 2-FM-GABA and 2-FM-Arg contents may be suitable indicators for assessing processing and/or storage conditions of orange

juice and concentrates, and their determination may be useful for the control of material and processes in the orange juice industry.

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