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Study of 2-furoylmethyl amino acids in processed foods by HPLC–mass spectrometry

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Abstract

High-performance liquid chromatography (HPLC) in combination with electrospray ionisation mass spectrometric detection in positive ion mode, has been used in order to confirm the identity of 2-furoylmethyl amino acids in stored, dehydrated orange juice and tomato products. Three new compounds, identified as 2-furoylmethyl aspartic acid, 2-furoylmethyl pyrrolidone carboxylic acid and 2-furoylmethyl lysine (furosine), have been detected in stored orange juice. In stored dehydrated tomato product, two new compounds were identified as 2-furoylmethyl pyrrolidone carboxylic acid and 2-furoylmethyl arginine.

Keywords: HPLC–MS; 2-Furoylmethyl amino acids; Processed foods

1. Introduction

The determination of level of Amadori compounds, first stable products formed by Maillard reaction during processing and/or storage of foods, allows detection of the onset of this reaction before detrimental changes occur, as well as to retrospective assessment of the heat treatment or storage conditions to which a product has been submitted.

Amadori compounds can be measured after their transformation into 2-furoylmethyl- amino acids (2-FM-AA) and they could be used as indicators to evaluate quality changes of processed and/or stored foods. Thus, 2-furoylmethyl-lysine (2-FM-Lys, furosine) has been used in milk and dairy products to assess heat treatments and storage conditions (Pellegrino, Resmini, & Luf, 1995; Villamiel, Arias, Corzo, & Olano, 1999). We have previously reported the presence of the 2-FM-AA in orange juice (del Castillo, Corzo, & Olano, 1999; del Castillo, Villamiel, Corzo, & Olano, 2000) and in tomato products (Sanz, del Castillo, Corzo, & Olano, 2000). Because these products were tentatively assigned according to the retention times and quantified by HPLC–UV, the objective of this work was to confirm their identity by high performace–mass spectomety (HPL–MS).

2. Materials and methods

2.1. Standards

As standard substances, commercial furosine (Neosystem, S.A); and 2-furoylmethyl-γ-amino butyric acid (2-FM-GABA) and 2-furoylmethyl-arginine (2-FM-Arg) previously obtained in our laboratory (del Castillo et al., 1999, 2000) were used.

2.2. Synthesis of Amadori compounds

Mixtures of D-glucose and the corresponding L-amino acid [alanine (Ala), asparagine (Asn), aspartic acid (Asp), glutamic acid (Glu), proline (Pro), serine (Ser) and threonine (Thr)], in molar ratios of 6:1, in water (5

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Table 1
Retention times (rt; min) and efficiency obtained after HPLC–UV ($\lambda = 280$ nm) analysis of 2 furoylmethyl amino acids (2-FM-AA)

Compounds	Acetic acid					
	1%		2%			
	32 °C		32 °C		50 °C	
	Efficiency	rt (min)	Efficiency	rt (min)	Efficiency	rt (min)
2-FM-GABA	6670	24.75	7740	16.76	7307	15.17
2-FM-Arg + furosine	3927	29.55	4493	19.98	4318	18.90

ml) were lyophilised 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 for 14 days (del Castillo et al., 1999; Sanz et al., 2000). Before analysis, samples were reconstituted to initial volume.

2.3. Samples

Aliquots of 5 ml of single-strength fresh juice and 5 g of tomato pulp (7°Brix) were lyophilised and equilibrated to $a_w = 0.44$ in a desiccator over saturated K_2CO_3 solution, using the method of Labuza and Saltmarch (1981). Then, samples were stored at 50 °C for 14 and 11 days, respectively (del Castillo et al., 1999; Sanz et al., 2000). Before analysis, samples were reconstituted to initial volume.

2.4. 2-Furoylmethyl amino acids

2-Furoylmethyl amino acids were obtained by acid hydrolysis at 110 °C for 24 h in a Pyrex screw-cap vial with PTFE-faced septa. Tomato products samples (2 g) were hydrolysed with 6 ml of 10.6 N HCl; reconstituted orange juice samples (1.3 ml) and model systems samples (1.3 ml) were hydrolysed with 3 ml of 11.4 M HCl. 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), prewetted with 5 ml of methanol and 10 ml of water, and furoylmethyl derivatives were eluted with 3 ml of 3 M HCl.

2.5. HPLC–MS

HPLC–MS analysis was carried out on a Hewlett-Packard 1100 instrument. This instrument, which consists of an HPLC HP Series 1100, equipped with a diode array detector (DAD) coupled to a quadrupole HP-1100 mass detector, was used in the electrospray positive mode (API–ES). Samples (100 μl) were injected into a C_8 column (4.6 × 250 mm, 5 μm; Alltech), maintained at 32 °C. The mobile phase was acetic acid (2%) in water and elution was in isocratic conditions at a flow

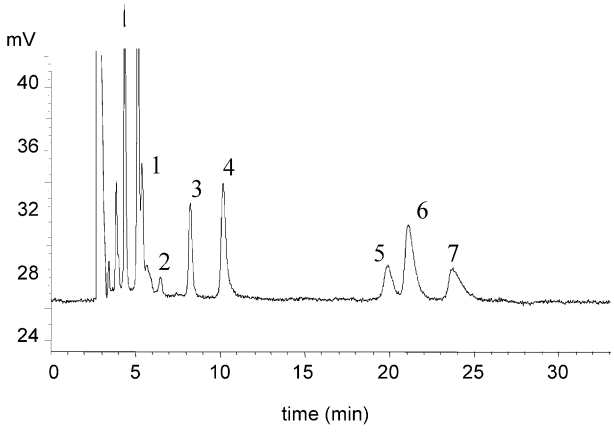

Fig. 1. HPLC–UV chromatogram of a mixture of acid hydrolysates of Amadori compounds 1: 2-FM-Asp; 2: 2-furoylmethyl pyrrolidone carboxylic acid (2-FM-PCA); 3: 2-FM-Ala; 4: Unknown; 5: 2-FM-GABA; 6: 2-FM-Pro; 7: 2-FM-Arg and furosine. (Mobile phase: 2% acetic acid in water.)

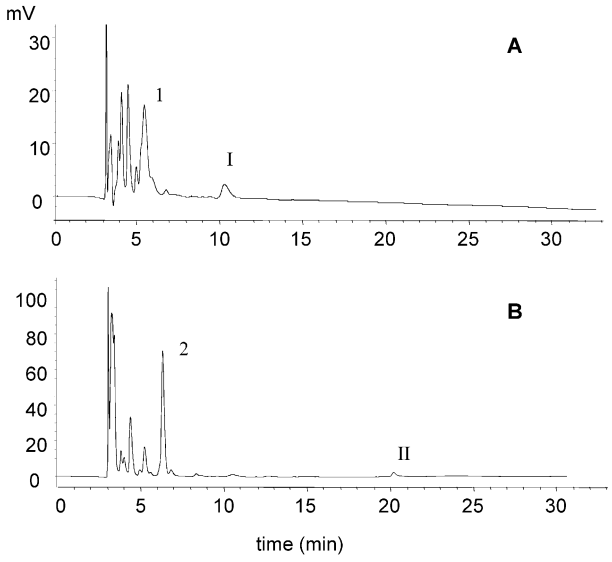

Fig. 2. HPLC–UV chromatograms of the acid hydrolysates of the Amadori compounds of Asn (A) and Glu (B). 1: 2-FM-Asp, 2: 2-FM-PCA, I and II: Unknown.

rate of 1 ml/min. DAD signal was recorded at 280 nm. Mass spectrometer values of needle potential, gas temperature, drying gas and nebuliser pressure were adjusted to 4000 V, 330 °C, 10 l/min and 50 psi, respectively. A fragmentor potential of 80 V was selected, since it provided the best sensitivity with reference compounds. Scan range was from 50 to 500 umas.

3. Results and discussion

3.1. HPLC–MS method optimisation

Standards of furosine, 2-FM-Arg and 2-FM-GABA were used to optimise the method of analysis. Acetic acid was used as eluent, since it provided both good

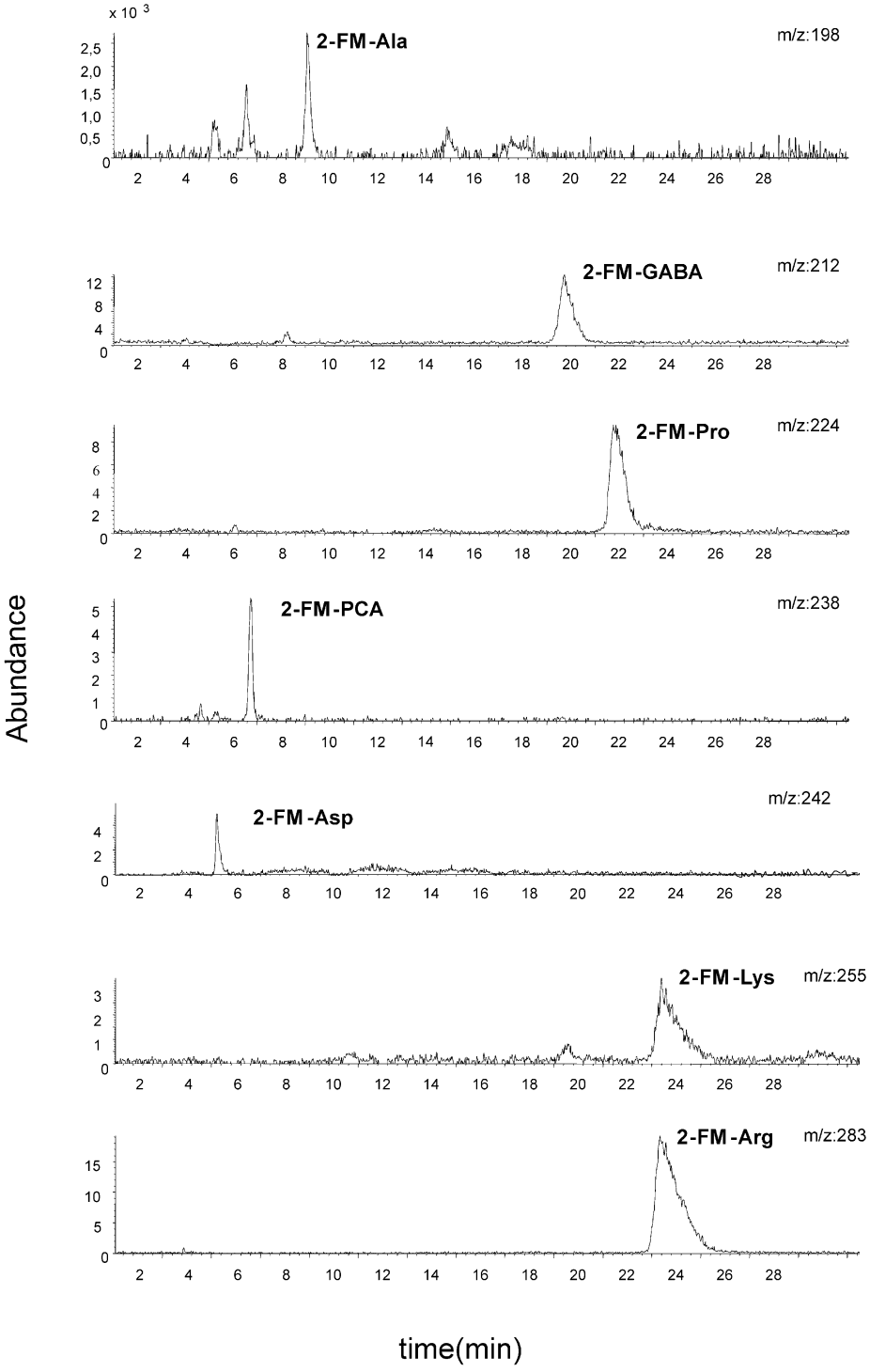

Fig. 3. Specific ion chromatograms extracted from ion total current trace, of 2-FM-AA found in hydrolysed dehydrated orange juice stored at 50 °C and $a_w = 0.44$ for 14 days.

chromatographic resolution and ion formation efficiency. Although the use of acetic acid can produce acetate adducts (Friedrich, Eberhardt, & Galensa, 2000), these were not detected in the positive ionisation mode. The influence of acetic acid on the chromatographic resolution was studied by varying its concentration between 0.4 and 3%. Table 1 shows the retention time and the efficiency for 2-FM-GABA, 2-FM-Arg and furosine using 1 and 2% acetic acid as mobile phase. The assays showed that the best results were obtained using 2% acetic acid, which produced an acceptable efficiency and reasonable time of analysis of

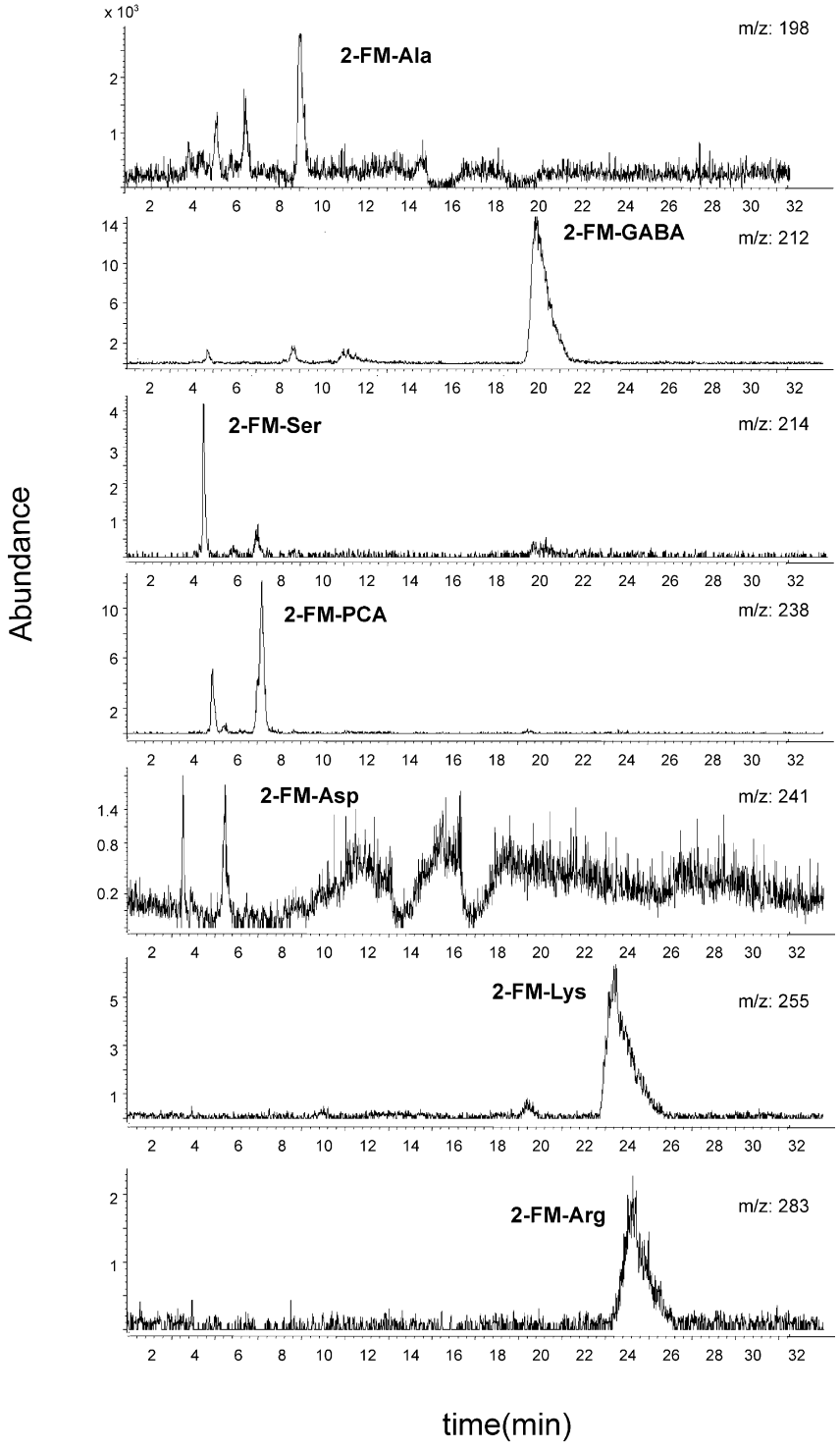

Fig. 4. Specific ion chromatograms extracted from ion total current trace, of 2-FM-AA found in dehydrated tomato product stored at 50 °C and $a_w = 0.44$ for 11 days.

2-FM-AA. Besides, these chromatographic conditions did not produce background signals and improved ionisation of 2-FM-AA. Under these conditions, standards of furosine and 2-FM-Arg coeluted as one peak but the spectral peak patterns allowed the detection of both compounds.

The temperature of analysis was tested in a range from 32 to 50 °C (Table 1). Since the increase of the temperature did not improve the efficiency, 32 °C was selected as the temperature of analysis.

The use of a fragmentor voltage of 80 V allowed an acceptable ionisation without undesirable fragmentation. The yield of quasimolecular ion production in positive mode was higher than in the negative mode and then the first condition of analysis was selected.

Scan mode was used in order to get qualitative information about all sample components. API–ES (using selected fragment profiles) and UV detection limits (280 nm) were calculated for the 2-FM-AA standards. UV values were about 10 times lower, because of the higher noise and lower specificity of the MS scan mode detection.

3.2. Identification of 2-FM-AA synthesised

The identity of 2-FM-AA synthesised was assigned by the presence of their quasi-molecular ion (M + H). Fig. 1 shows the HPLC–UV chromatogram of a mixture of 2-FM-AA eluted with 2% acetic acid. 2-FM-Thr (228 m/z) and 2-FM-Ser (214 m/z) eluted at 4.1 and 4.3 min, respectively. The corresponding HPLC-UV chromatograms of the acid hydrolysates of the Amadori compounds derived from Asn and Glu are shown in Fig. 2. In previous studies (del Castillo et al., 1999; Sanz et al., 2000), peaks I and II were tentatively assigned as the corresponding 2-FM-AA; however, analysis by HPLC–MS indicates that these peaks did not correspond to 2-FM-AA and no MS signals of 2-FM-Asn or 2-FM-Glu were obtained. However, the MS signals corresponding to 2-FM-Asp and 2-FM-PCA were detected. It is known that under acidic conditions Asn and Glu may be converted into Asp and PCA, respectively (Eichner, Schräder, & Lange, 1996; chap. 4); thus, acid hydrolysis of the Amadori compounds derived from Asn and Glu may give rise to the formation of 2-FM-Asp and 2-FM-PCA, respectively.

3.3. Identification of 2-FM-AA in stored dehydrated orange juice and tomato product

The HPLC–MS chromatograms of ion mass extracted from total ion current trace recorded from the dehydrated orange juice sample are shown in Fig. 3. The method allowed the detection of seven compounds: 2-FM-Ala (198 m/z), 2-FM-GABA (212 m/z), 2-FM-Pro (224 m/z), 2-furoylmethyl-pyrrolidone carboxylic acid (2-FM-PCA; 238 m/z), 2-FM-Asp (242 m/z), furosine (255 m/z) and 2-FM-Arg (283 m/z). With respect to our previously published results (del Castillo et al., 1999), three new compounds, 2-FM-Asp, 2-FM-PCA and furosine, were found. The presence of 2-FM-Asn and 2-FM-Glu was not confirmed by HPLC–MS analysis.

HPLC–MS of the 2-FM-AA of acid hydrolysates of dehydrated tomato sample (Fig. 4) shows the presence of seven compounds which have been identified as: 2-FM-Ser, 2-FM-Asp, 2-FM-PCA, 2-FM-Ala, 2-FM-GABA, 2-FM-Lys and 2-FM-Arg. In a previous work (Sanz et al., 2000), we detected (by HPLC–UV analysis in this sample), eight 2-FM derivatives of Ser, Thr, Glu, Ala, Asp, Asn, GABA and Lys. The tentative assignment was carried out by comparison with retention times of synthesised compounds. In the present work, as in the case of dehydrated orange juice, 2-FM-Asn and 2-FM-Glu were not detected. The ion corresponding to 2-FM-Thr was not found; however, two new compounds, 2-FM-PCA and 2-FM-Arg were obtained. In the previously cited work (Sanz et al., 2000), we assigned (by the HPLC–UV analysis) 2-FM-Ser, 2-FM-Thr and 2-FM-Glu as on single peak at a retention time of 5.1 min. Now, the HPLC–MS analysis showed that this peak corresponds only to 2-FM-PCA.

The acid hydrolysis of food samples is necessary for the formation of 2-FM-AA and the HPLC chromatogram of this reaction mixture contains a considerable number of peaks, which makes the analysis very complicated. Present results show that HPLC–MS makes it possible to correctly identify the 2-FM-AA present in processed foods. This is very important since these compounds have been recently proposed as new indicators of processed foods.

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References

del Castillo, M. D., Corzo, N., & Olano, A. (1999). Early stages of Maillard reaction in dehydrated orange juice. *Journal of Agricultural and Food Chemistry*, *47*, 4388–4390.

del Castillo, M. D., Villamiel, M., Corzo, N., & Olano, A. (2000). Use of 2-furoylmethyl derivatives of GABA and Arginine as indicators of the initial steps of Maillard reaction in orange juice. *Journal of Agricultural and Food Chemistry*, *48*, 4217–4220.

Eichner, K., Schräder, Y., & Lange, M. (1996). Early detection of changes during heat processing and storage of tomato products. In *Chemical markets for processed and stored foods* (pp. 32–44).

Friedrich, W., Eberhardt, A., & Galensa, R. (2000). Investigation of proanthocyanidins by HPLC with electrospray ionization mass spectrometry. *European Food Research Technology*, *211*, 56–64.

Pellegrino, L., Resmini, P., & Luf, W. (1995). Assessment (indices) of heat treatment of milk. In *Heat damage changes in milk* (pp. 409–447). International Dairy Federation (2nd ed.). Brussels.

Sanz, M. L., del Castillo, M. D., Corzo, N., & Olano, A. (2000). Presence of 2-furoylmethyl derivatives in hydrolysates of processed tomato products. *Journal of Agricultural and Food Chemistry*, *48*, 468–471.

Villamiel, M., Arias, M., Corzo, N., & Olano, A. (1999). Use of different thermal indices to assess the quality of pasteurised milks. *Zeitschrift fur Lebensmittel Untersuchung und Forschung A*, *208*, 169–171.