Presence of 2-Furoylmethyl Derivatives in Hydrolysates of Processed Tomato Products

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Acid hydrolysis of Amadori compounds yields the corresponding 2-furoylmethylamino acids (2-FM-AA) that can be analyzed by ion-pair HPLC. The relative proportions of the different 2-FM-AA present in the hydrolysates of tomato products were determined to assess their usefulness as indicators of quality. In the lyophilized tomato samples stored at 50 °C and $a_w = 0.44$ the formation of 2-FM derivatives of alanine, γ-aminobutyric acid (GABA), asparagine, aspartic acid, glutamic acid, lysine, serine, and threonine was detected. In commercial tomato products the most abundant 2-FM-AA was 2-FM-GABA (from traces to 26.4 mg/100 g of dry matter) followed by 2-FM-lysine (furosine). Differences in 2-FM-AA contents among samples may be related to processing and storage conditions.

Keywords: *2-Furoylmethylamino acids; processed tomato products*

INTRODUCTION

Changes occurring in food constituents during processing and storage comprise a considerable number of reactions due to the complex chemical heterogeneity of foods as well as the different types of processes applied in the food industry. The Maillard reaction plays an important role in food technology because it takes place in a considerable number of foods and can give rise not only to the formation of desired colors and flavors but also to the deterioration of food quality and the production of potentially toxic compounds. Amadori compounds (N-substituted 1-amino-1-deoxy-2-ketoses) formed during the early stages of the Maillard reaction are precursors of numerous important compounds responsible for flavors and brown polymers. They also cause the loss of nutritional values of amino acids and proteins (Friedman, 1996). The presence of free Amadori compounds in foods has been widely described in various vegetable products such as freeze-dried apricot and peach purées (Anet and Reynolds, 1957), tobacco (Mills et al., 1969), wine (Hashiba, 1978), green tea (Anan, 1979), and tomato (Reutter and Eichner, 1989). Because the Amadori compounds are formed before the occurrence of sensory changes, their determination provides a very sensitive indicator for early detection of quality changes caused by the Maillard reaction (Olano and Martinez-Castro, 1996).

ϵ-*N*-(2-Furoylmethyl)-L-lysine (furosine), formed by acid hydrolysis of the Amadori compound ϵ-*N*-(1-deoxy-D-fructosyl)-L-lysine, has been widely used for the evaluation of lysine damage in a number of foods as well as biological materials. Molnár-Perl et al. (1986) determined furosine in tomato powder, soybeans, barley, and malt. Later, Hidalgo et al. (1998) determined the furosine content in several tomato products for evaluating heat damage during processing.

Previous studies in tomato products showed the presence of Amadori compounds from alanine, aspartic acid, γ-aminobutiric acid (GABA), glycine, glutamic acid, leucine, serine, threonine, and valine (Eichner et al., 1990, 1995), so acid hydrolysis should produce, besides furosine, other 2-furoylmethylamino acids (2-FM-AA).

Therefore, the purpose of this work was to determine the presence of 2-FM derivatives in the hydrolysate of tomato products and their applicability as indicators of quality changes caused by the Maillard reaction.

MATERIALS AND METHODS

Commercial Samples. Ten commercial samples of different tomato products were purchased at local markets: one sample of whole peeled tomatoes, two samples of tomato pulp, one tomato juice from concentrate, one ketchup, one sample of double-concentrated tomato paste (28%), and four tomato sauces.

Lyophilized Tomato Samples. Aliquots (5 g) of tomato pulp (7 °Brix) 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 for 11 days. Samples were taken at 0, 4, 7, and 11 days of storage. Before analysis, samples were reconstituted to initial volume.

Synthesis of *N*-(1-Deoxy-D-fructosyl)-GABA. The compound was obtained by following the procedure described by Finot and Mauron (1969). A mixture of 3.25 g of glucose, 0.50 g of GABA (in molar relation 6:1), and methanol was refluxed for 4 h followed by cooling and evaporation under vacuum. The reaction mixture was dissolved in a minimum amount of water, and the Amadori compound was isolated using a column of Dowex 50Wx4 (pyridinium) eluted with water to remove the excess of glucose and then with 0.2 M pyridine/formic acid buffer, pH 3.25. The presence of the Amadori compound in the eluted fractions was shown by TLC on silica gel G 60 F254 aluminum plates using ninhydrin as detection reagent.

Synthesis of 2-Furoylmethyl-GABA (2-FM-GABA). The procedure for the synthesis was similar to the method proposed by Finot et al. (1968) to obtain furosine: 600 mg of *N*-(1-deoxy-D-fructosyl)-GABA was treated with 500 mL of 7.95 N HCl for 24 h under refluxing conditions. The HCl was evaporated under vacuum at 40 °C, and the dried residue was dissolved in a minimum quantity of water and placed on a 345 cm^3 column of Dowex 50Wx4 ion-exchange resin in acid form. The mixture of the reaction was eluted with 2 N HCl, and fractions

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of 20 mL were collected. The presence of 2-FM-GABA was detected by TLC on silia gel G 60 F254 aluminum plates using ninhydrin as reagent. Fractions containing the product were combined and lyophilized. The compound was characterized by TLC, HPLC, NMR, and FABMS (del Castillo et al., 1999).

Model Systems. Three millimoles of D-glucose, 0.5 mmol of the corresponding L-amino acid (alanine, aspartic acid, asparagine, glutamic acid, serine, or threonine), and 8 mL of citrate buffer 0.1 M, pH 4.4, were refluxed until a yellow-brown color appeared (from 3 to 9 h) and the presence of the Amadori compounds was detected by TLC. The reaction mixture was then hydrolyzed with 7.95 N HCl to obtain the corresponding 2-FM-AA.

Analytical Methods. Dry matter content of samples (grams per 100 g of product) was determined following AOAC Official Gravimetric Method 964.22 (AOAC International, 1990).

°Brix was determined using a refractometer ATAGO type 500 following AOAC Official Method 970.59 (AOAC International, 1990).

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

High-Performance Liquid Chromatography (HPLC). (a) Determination of Amino Acids. Determination of amino acids in lyophilized tomato samples was carried out by HPLC using a Waters liquid chromatograph controlled by a Maxima 820 chromatography workstation. Before HPLC analysis, samples were reconstituted and centrifuged at 15616*g* for 30 min at 5 °C, and free amino acids were determined in supernatant previously diluted (1:40) with borate buffer 0.4 M, pH 10. Samples were submitted to an automatic precolumn derivatization with *o*-phthaldialdehyde (OPA) (González de Llano et al., 1991). The separation of amino acids was performed in a Novapak C-18, 60 Å, 4 μm column (3.9 cm × 150 mm). Detection was performed by fluorescence using wavelengths of excitation and emission at 340 and 425 nm, respectively.

(b) Determination of 2-FM Derivatives. An ion-pair RP-HPLC method (Resmini et al., 1990) was used to analyze 2-FM derivatives using a C8 column (250 mm × 4.6 mm i.d.) (Alltech furosine-dedicated) thermostated at 35 °C, with a linear binary gradient. A Dionex chromatograph (DX-300) and variable wavelength detector at $\lambda = 280$ nm (LDC Analytical, SM 4000) were used. Acquisition and processing of data were achieved in an HP Chem Station (Hewllett-Packard).

Model systems (1.3 mL) were hydrolyzed with 3 mL of 11.4 N HCl. Tomato samples were hydrolyzed according to the procedure of Hidalgo et al. (1998): 2 g of tomato product with °Brix <12 was mixed with 6 mL of 10.6 N HCl and then hydrolyzed at 110 °C for 24 h in a Pyrex screw-cap vial with a PTFE-faced septum; 0.5 g of tomato products with °Brix >12 was diluted with 1.5 g of distilled water prior to acid hydrolysis. The hydrolysates were 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; 2-FM derivatives were eluted with 3 mL of 3 N HCl, and 20 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) and 2-FM-GABA. A single calibration curve linear over the studied range (0.21−3.6 mg/L) was obtained:

$$y = 1292.5x + 856.06 \qquad r^2 = 0.998$$

Limit of quantification was 0.12 mg/L for the standard solutions. Quantitative determination was performed by assuming the same response factor for all 2-FM derivatives.

Thin-Layer Chromatography (TLC). Isolation of Amadori compound and 2-FM derivative was followed by TLC performed on silica gel G 60 F254 aluminum plates using as solvent system pyridine/acetic acid/water, 90:10:20 v/v/v. Plates were sprayed with 0.2% ninhydrin in acetone, followed by heating at 120 °C for 2−5 min.

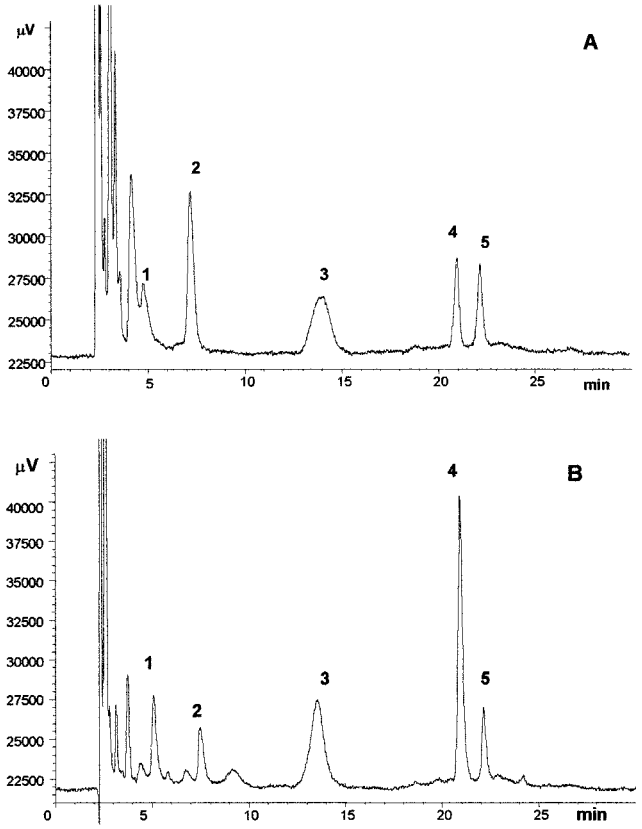

Figure 1. HPLC chromatograms of 2-FM derivatives ($\lambda = 280$ nm): (A) mixture of 2-FM derivatives of (1) serine, threonine, and glutamic acid; (2) alanine; (3) aspartic acid and asparagine; (4) GABA; and (5) lysine; (B) lyophilized tomato stored for 11 days at 50 °C ($a_w = 0.44$) [peak numbering as in (A)].

Detection of Amadori compounds in model systems (carbohydrates and amino acids) and in lyophilized tomato samples stored at 50 °C and 0.44 water activity during 11 days was carried out by TLC on cellulose aluminum plates. Solvent system was *n*-butanol/pyridine/water, 2:3:1 v/v/v. Plates were sprayed with 0.5% 2,3,5-triphenyl-2*H*-tetrazolium chloride in 0.5 mol/L sodium hydroxide (Reutter and Eichner, 1989).

RESULTS AND DISCUSSION

Model Systems. The formation of Amadori compounds in model systems was followed by TLC. R_f values from 0.04 for fructosylaspartic acid to 0.28 for fructosylthreonine were observed. Each Amadori compound formed in the corresponding model system was hydrolyzed and then analyzed by HPLC separately to determine the retention times. The HPLC chromatogram of a mixture of 2-FM-AA is shown in Figure 1A. The 2-FM derivatives of different amino acids eluted in the following order: serine and threonine ($t_r = 5.1$ min), alanine ($t_r = 7.4$ min), aspartic acid and asparagine ($t_r = 13,5$ min, and GABA ($t_r = 20.9$ min). Furosine showed a peak at $t_r = 22.1$ min. The hydrolysate of the model system containing glutamic acid showed two peaks: a major component (98.9%) at 5.1 min and a small peak at 20.9 min. Previous studies have shown that under acidic conditions the Amadori compound fructose−glutamic acid is converted into fructose−pyrrolidone−carboxylic acid (Eichner et al., 1995), so the major peak observed in the acid hydrolysate of the model system containing glutamic acid may correspond to 2-FM pyrrolidonecarboxylic acid.

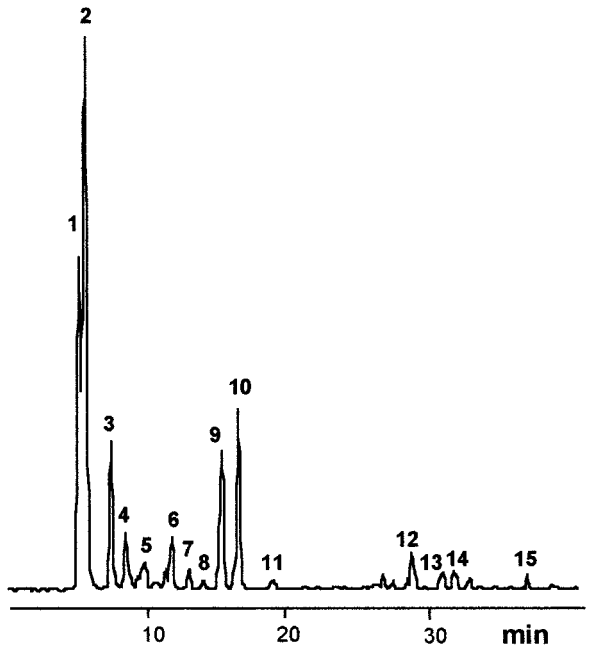

Figure 2. HPLC chromatogram of OPA amino acids of lyophilized tomato pulp: (1) aspartic acid; (2) glutamic acid; (3) asparagine; (4) serine; (5) glutamine, histidine; (6) glycine, threonine; (7) arginine; (8) β-alanine; (9) α-alanine; (10) GABA; (11) tyrosine; (12) phenylalanine; (13) isoleucine; (14) leucine; (15) lysine.

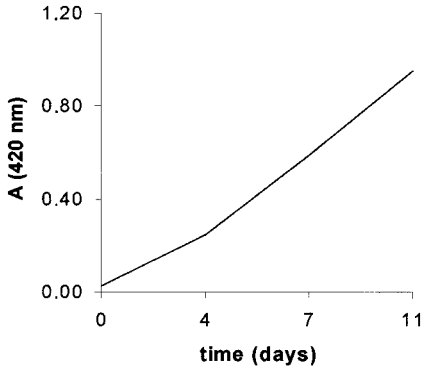

Figure 3. Development of color (absorbance 420 nm) during storage at 50 °C and $a_w = 0.44$ of a reconstituted lyophilized tomato sample.

Formation of 2-FM-AA during Storage of Lyophilized Tomato Pulp. Figure 2 shows the HPLC chromatographic profile of the free amino acids present in a tomato pulp sample. Major components are glutamic acid, aspartic acid, asparagine, GABA, and alanine. During storage under controlled conditions (50 °C, $a_w = 0.44$) the total amino acid content decreased progressively from 6.42 to 2.75, 1.70, and 1.01 g/L after 4, 7, and 11 days, respectively. The decrease of the major amino acids represented >84.5% of the total loss.

During storage, changes in color intensity were observed (Figure 3), increasing progressively. These changes correlated with the observed amino acid decrease and may be mainly caused by Maillard reaction.

The formation of 2-FM derivatives during storage of lyophilized tomato samples is shown in Table 1. Before storage, HPLC analysis showed the presence of small amounts of furosine and traces of 2-FM-GABA. At the fourth day of storage furosine and 2-FM-GABA increased and peaks with retention times corresponding to the 2-FM derivatives of alanine, aspartic acid, asparagine, glutamic acid, serine, and threonine also appeared in the chromatogram. During prolonged storage furosine increases slowly, whereas the concentrations of the other 2-FM derivatives increase rapidly. Figure 1B shows the chromatographic profile obtained at the end of the storage; the presence of the different 2-FM derivatives can be observed.

Table 1. Formation of 2-FM Derivatives of Amino Acids (Milligrams per 100 g of Dry Matter) during Storage of Lyophilized Tomato at 50 °C ($a_w = 0.44$)

days	serine + threonine + glutamic acid	alanine	aspartic acid + asparagine	GABA	lysine
0	0.0	0.0	0.0	tr[a]	7.3
4	103.5	60.9	300.5	245.4	70.9
7	119.7	71.2	336.7	250.6	74.5
11	136.8	92.0	320.4	300.5	81.8

[a] tr, trace.

Table 2. Contents (Milligrams per 100 g of Dry Matter) of 2-FM-AA Found in Hydrolysates of Commercial Tomato Products ($n = 3$)

sample	2-furoylmethyl-GABA	2-furoylmethyl-lysine (furosine)
whole peeled tomato	0.00	0.00
tomato pulp I	0.00	5.10 (9.03)
tomato pulp II	3.84 (0.38)[a]	8.50 (5.46)
juice	19.33 (2.59)	10.15 (4.72)
ketchup	12.68 (3.07)	9.01 (1.52)
double-concentrated tomato paste	87.55 (5.63)	42.81 (8.66)
tomato sauce I	20.75 (5.18)	16.97 (4.45)
tomato sauce II	16.67 (2.52)	12.59 (8.64)
tomato sauce III	21.23 (2.46)	14.79 (5.24)
tomato sauce IV	26.44 (3.71)	17.57 (4.57)

[a] Numbers in parentheses are the relative standard deviations (in %).

Formation of 2-FM-AA in Commercial Tomato Products. The presence of 2-FM-AA compounds other than furosine was also observed in samples of different commercial tomato products (Table 2). As expected, natural products showed the lowest amount of 2-FM-AA, and the higher values were observed in concentrated tomato paste. Except for natural tomato pulp, in all processed tomato products the amount of 2-FM-GABA was considerably higher than the amount of furosine. The variations observed among samples may be attributed to differences in the processing conditions.

Conclusions. Although a detailed kinetic study of the formation of 2 FM-AA would be needed as a basis for formulating criteria to distinguish different types of processed products, the present preliminary results illustrate the potential usefulness of 2-FM-AA compounds as indicators of heat and storage damage of tomato products.

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