Formation of Amadori Compounds in Dehydrated Fruits

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The presence of Amadori compounds in commercial dehydrated fruits has been shown through HPLC analysis of the corresponding 2-furoylmethyl-amino acids obtained by acid hydrolysis. Furosine (2-furoylmethyl-lysine) was the main 2-furoylmethyl derivative observed in dried figs and apricot samples, whereas in prunes and dates similar amounts of furosine and 2-furoylmethyl-γ-aminobutyric acid were detected. A considerable variation of 2-furoylmethyl-amino acid contents among commercial raisin samples was observed. 2-Furoylmethyl-γ-aminobutyric acid and 2-furoylmethyl-arginine, the most abundant 2-furoylmethyl-amino acids, ranged between 9.9 and 75.8 mg/100 g sample and 10.0 and 62.5 mg/100 g sample, respectively. Most of the Amadori compounds present in raisins seem to have originated during the commercial shelf life period rather than during processing. Determination of 2-furoylmethyl-amino acids could be used as a method of controlling commercial dehydrated fruit and selecting storage conditions.

Keywords: *Amadori compounds; 2-furoylmethyl-amino acids; storage; dehydrated fruits; raisins*

INTRODUCTION

Dehydrated fruits are an important nutritional source, and they are very popular in many countries. Raisins are the dried fruit produced in the greatest quantity worldwide. One of the most significant features of raisins is their low moisture content which helps preserve them; nevertheless, quality changes can occur during dehydration or storage because of browning reactions (*1*). These modifications can be caused by the enzyme polyphenoloxidase, as well as by nonenzymatic processes. Caramelization of sugars and Maillard reactions between reducing carbohydrates and free amino groups of amino acids, peptides, and proteins are the main types of nonenzymatic browning (*2*).

The Maillard reaction, one of the most important processes that takes place during processing or storage of many foods, is controlled by different parameters such as pH, water activity, time, and temperature (*3*). Amadori compounds formed during the first step of the Maillard reaction are considered as precursors of the color, aroma, and flavor of processed foods, and determination of their levels provides a sensitive indicator for early detection of these changes (*4*). Recently, a sensitive method has been described for the determination of Amadori compounds based on the HPLC analysis of the 2-furoylmethyl amino acids formed during their acid hydrolysis. These derivatives have been proposed as quality indicators in orange juice and tomato products (*5*, *6*). In this work, analysis of the 2-furoylmethyl amino acids has been used to study the presence of Amadori compounds in dehydrated fruits and their formation during preparation and storage of raisins. Their usefulness as indicators of the early stages of the Maillard reaction was also explored.

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MATERIALS AND METHODS

Commercial Samples. Nine commercial raisin samples of different varieties (Moscatel ($n = 3$), Sultana ($n = 2$), Corinto ($n = 1$), and unspecified ($n = 3$)) and commercial prune, fig, date, and dried apricot samples were purchased in a local market. "Thompson Seedless" grape samples were stored at 5 °C before the drying process.

Synthesis of 2-Furoylmethyl Amino Acids. Amadori compounds were synthesized by mixing 6 mmol of glucose and 1 mmol of the corresponding L-amino acid (alanine, arginine, γ-aminobutyric acid, and proline) with 1 g of microcrystalline cellulose (Merck). Each mixture was dissolved in 5 mL of 0.01 N phosphate buffer (pH 7.0) and lyophilized. Samples were stored at 50 °C in a desiccator with a saturated K_2CO_3 solution ($a_w = 0.44$). Formation of Amadori compounds was followed by TLC (*7*), and the storage was stopped when a yellow-brown color appeared (4 days for alanine and γ-aminobutyric acid, 6 days for arginine, and 7 days for proline). The 2-furoylmethyl amino acids were obtained by hydrolysis of the corresponding Amadori compounds in 7.95 N HCl for 24 h at 110 °C.

Drying of Grape Samples. Thompson Seedless grapes were submitted to a pretreatment of dipping in an alkaline solution of 0.3% NaOH (93 °C) for 3 or 4 s in order to remove their waxy layer and increase their drying rate (*8*). Berries were washed with water to eliminate the alkali solution and then were cut from the bunch with their cap-stems. Drying was carried out at 65 °C, in a heater supplied with a current of air, during 24 h. Samples were taken at 0, 14, 17, 21, and 24 h and the loss of water was determined by weight. Berries dried for 24 h were kept in polyethylene bags and stored at room temperature for six months.

Chromatographic Analysis. *Sample Preparation.* Portions of dehydrated fruit (10 g) mixed with 20 mL of water were homogenized and centrifuged at 7000*g* for 30 min at 5 °C, and the aqueous solution was used for all chromatographic analyses. All analyses were performed in duplicate.

Determination of Carbohydrates. The aqueous solution (0.5 mL) was mixed with 2 mL of 1% methyl-α-D-galactopyranoside as internal standard, diluted to 25 mL with 70% methanol, and filtered through Whatman no. 40 filter paper. A 1-mL aliquot of the filtrate was evaporated under vacuum and then treated with 100 μL of anhydrous pyridine (Merck), 100 μL of trimethylsilylimidazole (Sigma), and 100 μL of trimethyl-

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Table 1. Variation of Water Content of Grapes Air-Dried at 65 °C

time of treatment (h)	water content (%)
control	79.10−80.42
14	42.01−45.59
17	28.21−32.87
21	17.06−28.38
24	10.26−16.17

chlorosilane (Sigma) at room temperature. After derivatization, 0.1 mL of hexane and 0.2 mL of water were added to the mixture, and 1 μL of the upper layer was injected into a gas chromatograph (*9*). The analysis was performed on a Varian gas chromatograph (model 3380) with flame ionization detector and a 3 m × 1.0 mm i.d. stainless steel column (Chrompack) packed with 2% OV-17 on nonsilanized 120/140 Volaspher A-2 (Merck). The temperature of the injector and detector was 300 °C, and the oven temperature was programmed from 180 °C to 270 °C at a rate of 6 °C/min with an initial holding at 180 °C for 6 min. Nitrogen was used as carrier gas, and injections were made in the split mode, with a split ratio of 1:5. Chromatographic peaks were measured using a HP Chem-Station acquisition system.

The identity of each silylated carbohydrate was confirmed by GC−MS. Mass spectra were obtained using a Fisons gas chromatograph (model 8030) coupled to a Fisons quadrupole mass detector (MD800) and a fused silica capillary column (50 m × 0.25 mm i.d.) coated with methyl silicone. The temperature of the injector and detector was 290 °C, and the oven was programmed at a initial temperature of 210 °C during 30 min followed by an increase of 20 °C/min to 270 °C. Helium was used as carrier gas, and injections were made in the split mode (1:40).

Determination of Amino Acids. Primary free amino acid analysis was performed by HPLC using a Beckman liquid chromatograph controlled by a System Gold software data system. A 0.25-mL aliquot of the aqueous solution was diluted to 5 mL with 0.4 M borate buffer, pH 10. Samples were submitted to an automatic precolumn derivatization with *o*-phthaldialdehyde (OPA) (*10*) The separation of amino acids was carried out on a Novapak C-18, 60 Å, 4-μm column (150 × 3.9 mm i.d.). Detection was performed by fluorescence using excitation and emission wavelengths of 340 and 425 nm, respectively. To determine arginine (main amino acid), it was necessary to dilute the solutions five times with borate buffer.

Determination of 2-Furoylmethyl Amino Acids. Portions (3 mL) of the aqueous solution were hydrolyzed with 7.1 mL of 11.4 N HCl at 110 °C for 24 h. The hydrolysate was filtered through Whatman no. 40 filter paper, and 0.5 mL of the filtrate was applied to a previously activated Sep-pak C_{18} cartridge (Millipore). 2-Furoylmethyl amino acid was eluted with 3 mL of 3 N HCl, and 50 μL was injected in the chromatograph. Analysis of 2-furoylmethyl amino acids was performed by an ion-pair RP-HPLC method (*11*) using a C_8 (Alltech furosine-dedicated) column (250 × 4.6 mm i.d.) and a variable wavelength detector at 280 nm (LDC Analytical, SM 4000). Acquisition and processing of data were achieved with System Gold software (Beckman Instrument). Calibration was performed by the external standard method using a commercial standard of pure 2-furoylmethyl-lysine (furosine) (Neosystem Laboratories, Strasbourg, France).

Identity of synthesized 2-furoylmethyl amino acids was confirmed by HPLC−mass spectrometry using a Hewlett-Packard 1100 HPLC−MS operating in electrospray ionization mode under atmospheric pressure and positive polarity (APIES) and coupled with a UV−Vis detector at 280 nm. The analysis was performed on the previously mentioned C_8 Alltech column maintained at 37 °C and using 2% acetic acid as mobile phase. The flow rate was 0.6 mL/min, and the injected volume was 75 μL. Fragmentor voltage was set at 40 V after optimization. The nebulizer pressure was 40 psi and the capillary voltage was 4000 V. Drying gas rate was 10 L/min at 320 °C. Total ion current chromatograms were acquired.

Table 2. Changes in the Major Amino Acid Content during Storage of Raisins at Room Temperature (data are expressed as mg/100 g of product)

	time of storage (months)		
amino acids	0	3	6
aspartic acid	19.09 (0.52)[a]	9.43 (0.76)	8.06 (0.39)
serine	19.76 (0.71)	12.67 (0.76)	10.63 (0.22)
histidine	14.31 (0.03)	15.53 (0.15)	10.56 (0.18)
threonine	25.45 (1.02)	13.52 (0.03)	11.71 (0.36)
arginine+alanine	164.69 (13.24)	145.68 (0.58)	122.33 (0.99)
γ-aminobutyric acid	89.71 (2.75)	43.27 (1.30)	36.72 (1.25)
tyrosine	16.37 (1.52)	10.20 (0.12)	14.88 (0.41)
valine	12.55 (0.38)	5.47 (0.41)	3.93 (0.13)
phenylalanine	21.83 (0.70)	12.54 (0.06)	7.18 (0.04)
leucine	25.41 (0.91)	15.78 (0.07)	10.08 (0.36)
ornithine	13.44 (0.07)	6.43 (0.60)	4.23 (0.05)

[a] Relative standard deviation (%).

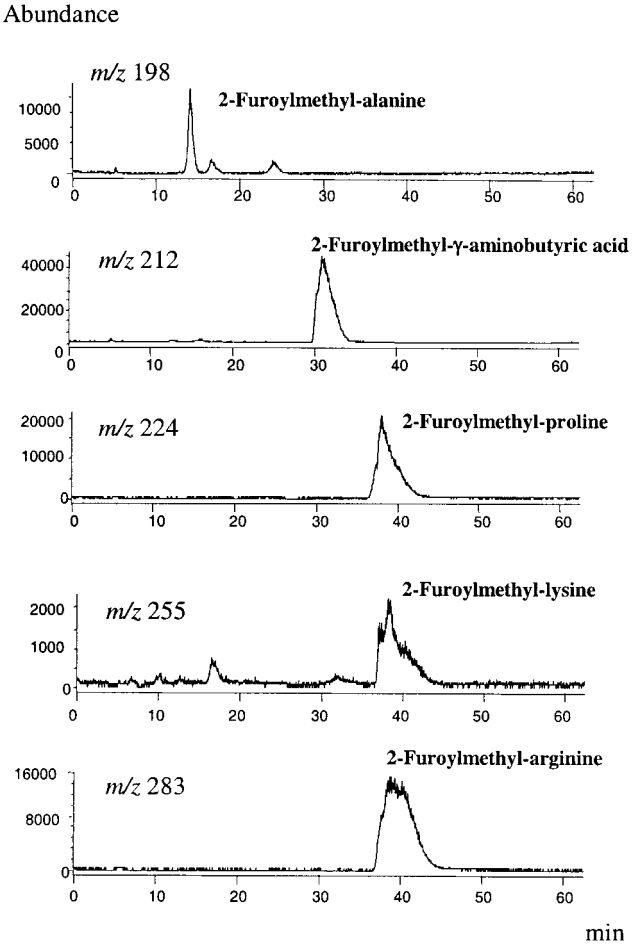

Figure 1. HPLC−MS ion chromatograms of 2-furoylmethyl amino acids in a commercial raisin sample reconstructed from total ion current profile. Mobile phase was 2% acetic acid.

Identification of 2-furoylmethyl amino acids in dehydrated fruits was achieved by comparison of their retention times and MS data with those of the synthesized reference compounds.

RESULTS AND DISCUSSION

TLC analysis showed the formation of the corresponding Amadori compounds of arginine, γ-aminobutyric acid, alanine, and proline with R_f values of 0.15, 0.16, 0.21, and 0.25 respectively, according to previous studies (*6*). The order of elution of synthesized 2-furoylmethyl amino acids on HPLC−UV analysis (*11*) was 2-furoylmethyl -alanine (7.4 min), 2-furoylmethyl-pro-

Table 3. Formation of 2-Furoylmethyl Amino Acids during Storage of Raisins at Room Temperature

months of storage	2-furoymethyl derivatives (mg/100 g of raisins)				
	alanine	proline	GABA	lysine	arginine
0	none	none	tr[b]	none	tr
3	2.66 (0.19)[a]	1.85 (0.19)	3.09 (0.11)	1.44 (0.14)	11.11 (0.32)
6	3.29 (0.25)	5.60 (0.41)	13.96 (1.77)	n.d.	43.97 (5.23)

[a] Relative standard deviations (%). [b] tr, traces; n.d., not detected.

Table 4. Content of 2-Furoylmethyl Amino Acids (mg/100 g of raisins) in Commercial Raisins

origin	2-furoylmethyl derivative of				
	alanine	proline	γ-aminobutyric acid	lysine	arginine
unspecified 1	10.02 (1.45)[a]	8.06 (0.50)	40.48 (4.35)	20.00 (1.81)	62.46 (4.33)
unspecified 2	6.65 (0.32)	3.38 (0.35)	11.31 (0.40)	11.35 (0.45)	30.08 (0.84)
unspecified 3	[b]tr	3.64 (0.40)	13.12 (1.56)	8.89 (0.57)	11.30 (1.28)
moscatel 1	0.00	2.36 (0.10)	9.97 (0.89)	7.24 (0.36)	10.01 (0.25)
moscatel 2	0.00	2.81 (0.44)	10.48 (1.28)	7.53 (0.19)	10.62 (0.67)
moscatel 3	15.87 (0.48)	8.13 (0.02)	75.80 (5.69)	31.16 (2.43)	62.25 (5.95)
sultana 1	6.22 (0.69)	5.82 (0.16)	21.36 (2.85)	18.98 (0.76)	40.20 (3.81)
sultana 2	10.02 (0.39)	8.06 (0.48)	40.48 (1.90)	20.00 (2.43)	62.46 (3.24)
corinto 1	5.27(0.46)	2.33 (0.13)	25.88 (1.19)	18.93 (0.15)	32.21(2.48)

[a] Relative standard deviations (%). [b] tr, traces.

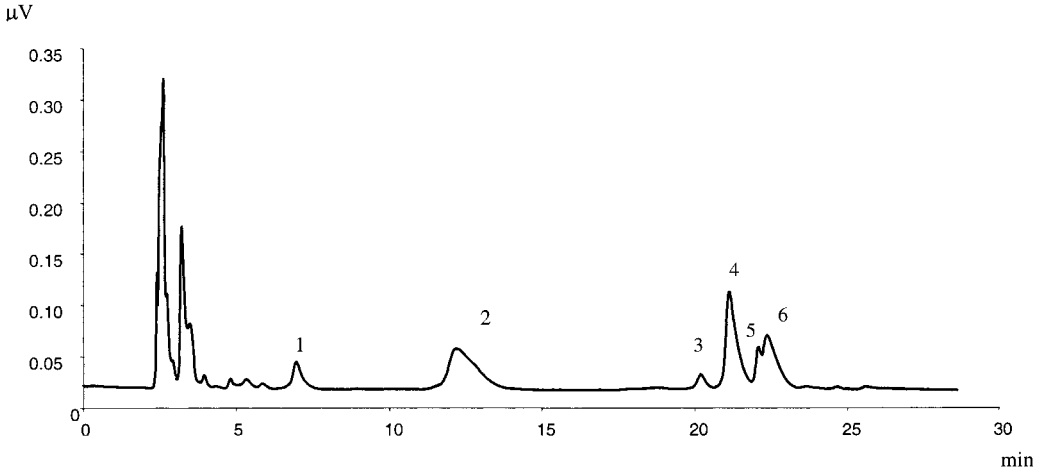

Figure 2. HPLC chromatogram of 2-furoylmethyl amino acids in a commercial raisin sample: (1) 2-furoylmethyl-alanine, (2) unknown, (3) 2-furoylmethyl-proline, (4) 2-furoylmethyl-γ-aminobutyric acid, (5) 2-furoylmethyl-lysine (furosine), (6) 2-furoylmethyl-arginine.

line (20.4 min), 2- furoylmethyl-γ-aminobutyric acid (21.5 min), furosine (22.4 min), and 2-furoylmethyl-arginine (22.9 min) as previously reported by del Castillo et al. (*5*). The identity of 2-furoylmethyl amino acids was also confirmed by the presence of their M+H ion on HPLC−MS analysis. 2-Furoylmethyl derivatives of proline, arginine, and lysine coeluted in the same peak but they were characterized from their M+H ions.

Dehydration Assays. Table 1 shows the water content of grapes during the drying process. At 24 h samples achieved a moisture level between 10.26% and 16.17%, which is the range usually found in commercial raisins.

Fructose, glucose, and *myo*-inositol were identified by retention times and chromatographic patterns, and confirmed by GC−MS. Other peaks corresponding to *scyllo*-inositol, sorbitol, and sucrose were also identified at concentrations below the limit of quantification. Appreciable changes were not detected in the carbohydrate fraction during the dehydration process.

During dehydration of grapes, the total amino acids content decreased slowly, reaching a loss of 20.04% at 24 h. Amino acids, which showed a major decrease, were glutamine, glutamic acid, tryptophan, ornithine, and histidine (96.15%, 76.34%, 62.04%, 59.83%, and 52.50%, respectively). The diminution observed in glutamic acid and ornithine could be in part due to a conversion into arginine that takes place during the manufacture of raisins (*12*).

Presence of 2-furoylmethyl amino acids was not detected in control grapes samples, and only traces of 2-furoylmethyl-arginine and 2-furoylmethyl-γ-aminobutyric acid were observed at the end of the drying process.

Storage Assays. During storage at room temperature, carbohydrate content decreased, and the most significant change occurred within 3 and 6 months. The diminution observed in fructose and glucose was about 42% and 28%, respectively. *Myo*-inositol did not show changes during this storage.

Table 2 shows the diminution of major amino acids during raisin storage. Total free amino acid content showed a decrease of 32.12% and 44.38% at 3 and 6 months of storage, respectively, which may be attributed to the low a_w of samples which favors Maillard reaction at room temperature. At the end of the storage process the most abundant amino acids were arginine+alanine, and γ-aminobutyric acid. Valine, ornithine, and phenylalanine showed the greatest decrease (68.67%, 68.51%, and 67.10%, respectively).

Table 5. Content of 2-Furoylmethyl Amino Acids in Dehydrated Fruits (mg/100 g product)

	2-furoylmethyl derivative of			
sample	alanine	proline	γ-aminobutyric acid	lysine
dried apricots	0.0	5.73 (0.18)	3.59 (0.08)	7.74 (0.17)
dates	0.0	2.88 (0.04)	17.89 (0.29)	16.30 (2.82)
prunes	4.54 (0.64)[a]	3.61 (0.47)	21.61 (1.98)	20.62 (1.76)
figs	0.0	4.18 (0.13)	7.81 (0.55)	14.20 (2.30)

[a] Relative standard deviations (%).

Table 3 shows the formation of 2-furoylmethyl amino acids during storage of raisin samples. Freshly processed raisins showed only traces of 2-furoylmethyl-arginine and 2-furoylmethyl-γ-aminobutyric acid, however, at the third month of storage, a noticeable amount of 2-furoylmethyl amino acids corresponding to alanine, proline, γ-aminobutyric acid, lysine, and arginine were detected. 2-Furoylmethyl-arginine was the most abundant compound (55% of total), whereas furosine accounted for only about 7% of the total. At the end of the storage period studied, a considerable increase of the 2-furoylmethyl amino acids was observed. The 2-furoylmethyl-arginine peak increased considerably at six months of storage and overlapped the furosine peak.

Commercial Dehydrated Fruits. Amadori compounds corresponding to alanine, arginine, γ-aminobutyric acid, lysine, and proline were found in commercial raisins. Figure 1 shows the chromatograms corresponding to the 2-furoylmethyl amino acid M+H ions, and the chromatographic profile of these compounds used in quantitative analysis appears in Figure 2. Peak number 2 probably corresponds to a compound with M+H ion at *m*/*z* 215, the identity of which has not been confirmed. Table 4 shows the content of 2-furoylmethyl amino acids found in commercial raisins. Major 2-furoylmethyl amino acids were those of arginine and γ-amino butyric acid in all samples studied. There were wide variations between samples, with 2-furoylmethyl-γ-aminobutyric acid and 2-furoylmethyl-arginine varying from 9.97 to 75.80 mg/100 g of product and from 10.01 to 62.46 mg/100 g of product, respectively. Because drying of grapes did not cause appreciable formation of 2-furoylmethyl amino acids, the observed variations among samples could be mainly attributed to different storage conditions.

2-Furoylmethyl amino acids were also measured in different dehydrated fruits: dates, figs, apricots, and prunes. The presence of 2-furoylmethyl-proline, 2-furoylmethyl-γ-aminobutyric acid, and furosine was observed in all dried fruits analyzed (Table 5). 2-Furoylmethyl-alanine was detected only in prunes, and traces of 2-furoylmethyl-arginine were found in dates. Furosine was the main compound found in figs and dried apricots. For prunes and dates the main compound was 2-furoylmethyl-γ-aminobutyric acid.

Present results seem to indicate that formation of Amadori compounds takes place mainly during the commercial shelf life period. Determination of 2-furoylmethyl amino acids could be used as a method of controlling commercial dehydrated fruit and selecting storage conditions.

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