Evolution of Free Amino Acids and Amines during Ripening of Iberian Cured Ham

Juan J. Córdoba, T. Antequera Rojas, C. García González, J. Ventanas Barroso, C. López Bote, and Miguel A. Asensio*

Food Science and Technology, Faculty of Veterinary Science, Universidad de Extremadura, 10071 Cáceres, Spain

Fifty-eight hams were obtained from Iberian pigs and were traditionally processed into dry cured products. Free amino acids and amines from semimembranosus and biceps femoris muscles were detected and quantified by HPLC. The larger increases of free amino acids took place in the drying cycle. Glutamic acid, alanine, leucine, and glycine were the free amino acids present in higher quantities in the final product. The amines with higher concentrations were not in the toxic range. Most amino acids increased according to their proportion in porcine skeletal muscle, except for some basic amino acids that may be involved in the formation of volatile compounds and amines.

Keywords: *Amino acids; amines; ham; proteolysis; HPLC*

INTRODUCTION

Iberian cured ham, the most valuable meat product of Spain, is produced from uncooked hams of Iberian breeds of hogs, following a traditional method that requires at least 18 months. For the first 4 months the hams are held under controlled low temperature and high relative humidity, while for the last 14 months they are left exposed to the natural environmental conditions found in the mountain ranges of Spain. The traditional method begins with meat being chilled to allow salt equalization, followed by a period of controlled increasing temperatures and decreasing relative humidity to environmental levels. This increase in temperature and decrease in humidity is completed in 1–2 months in a drying room during the summertime and 12 additional months in a cellar environment. Currently, dry-cured hams tend to be processed under refrigeration with no drying stage, which influences the quality of the final product.

During ripening of this and other types of cured hams, protein hydrolysis takes place. Increases in non-protein nitrogen have been reported in the Parma ham (Cantoni and Cattaneo, 1974), Serrano cured ham (Flores *et al.*, 1984), American country style ham (McCain *et al.*, 1968), and Iberian cured ham (Ventanas *et al.*, 1992). Amino acids constitute the main fraction of non-protein nitrogen in the previously mentioned cured hams (Ambanelli *et al.*, 1969; McCain *et al.*, 1968; Ventanas *et al.*, 1992). Free amino acids are highly correlated with flavor development in aged ham (McCain *et al.*, 1968). These amino acids have been reported as precursors of sour, sweet, and bitter taste (Kato *et al.*, 1989). In addition, some of them could contribute to aromatic compounds formed by different pathways such as the Strecker degradation or its reaction with reducing compounds in the Maillard reaction (Baines and Mloztiewicz, 1984). On the other hand, amino acid degradation to amines would affect not only the flavor but also the health of the consumer if biogenic amines are formed.

* Author to whom correspondence should be addressed (telephone 07 34 27 257124/257125; fax 07 34 27 257110).

In spite of the relevance to flavor of all these compounds, very little is known about the conditions that rule their production in dry-cured hams. The purpose of this study was to measure the concentration of different free amino acids and amines at different stages of Iberian cured ham processing, which could provide a scientific basis for the factors responsible for their release.

MATERIALS AND METHODS

Processing of the Hams. Fifty-eight hams obtained from Iberian pigs (160 kg live weight), which were fattened in pasture with acorns as the basic food source, were processed into cured hams according to the traditional method (Ventanas and Córdoba, 1992). Average contents of fat and protein were relatively close to 9% and 21% for the semimembranosus and approximately 10% and 19% for the biceps femoris (Córdoba *et al.*, 1990), respectively. The steps including the number of hams (n) removed from the curing process for sampling at each stage were as follows:

Green State (G). The hams were held for 48 h at 0–4 °C after slaughter, then partially skinned (except for the lower leg), keeping the hoof, bones, and nearly all fat intact (n = 10).

Salting (S). The hams were thoroughly rubbed with sea salt containing 1% potassium nitrate and placed in piles with alternate beds of hams and sea salt for 15 days at 4 °C and 90% relative humidity (n = 8).

Postsalting 1 (PS1). The hams were brushed to free them from the salt left on their surface and held at 0–4 °C and 90% relative humidity for 60 days (n = 6).

Postsalting 2 (PS2). The hams were left for 45 days in a chamber, where the temperature was increased 2–3 °C per week and relative humidity was decreased 1–2% per week, reaching 18 °C and 80%, respectively (n = 8).

Drying (D). During the summertime, the hams were kept at environmental conditions for 45 days in drying rooms, where the temperatures reached up to 30 °C and relative humidity ranged from 45% to 70%, as has been previously described (Antequera *et al.*, 1992) (n = 8).

Ripening. The hams were left to ripen for 12 additional months in a cellar with a relative humidity of 60–70% and temperatures ranging from 12 to 20 °C, depending on the time of the year. Two samplings were carried out in this stage: one as half-ripened hams (HR, n = 8), after the first 6 months in the cellar, and the second, at the end of the cellar stage, as fully ripened hams (FR, n = 10).

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Analytical Methods. Whole semimembranosus and biceps femoris muscles were excised from the hams and ground prior to analysis.

Amino Acids. As an internal standard 10 μL of a solution of norleucine (10 mg/mL) was added to samples before homogenization. For deproteinization, a 10 g sample was homogenized in a Sorvall omnimixer with 5% sulfosalicylic acid for 1 min. The homogenates were maintained at 2 °C for 17 h. They were then centrifuged at 15300*g* for 10 min and filtered through Whatman No. 54 paper. After the pH of the filtrates was adjusted to 6 with 4 N NaOH, amino acid derivatization was carried out with phenyl isothiocyanate (PITC) according to a modified method used by Yang and Sepúlveda (1985). Fifty microliters of filtrate was mixed with 200 μL of PITC solution (ethanol–water–triethylamine–PITC 7:1:2:1) for 10 min, dried in a Speedvac (Savant Instruments) set at 1800 mTorr and low drying time (no heating) for 20 min, and reconstituted in 500 μL of 0.5 M sodium phosphate buffer, pH 7.4, and 5% acetonitrile for analysis.

The PITC derivates were detected on a Beckman liquid chromatograph equipped with two pumps (Model 110B) and a UV detector (Model 166). The column was a Supelcosil LC-18 containing octadecyldimethylsilyl, 25 cm × 4.6 mm (5 μm particle size) from Supelco. The temperature was controlled to 35 °C. The eluents used were (A) 0.03 M sodium acetate and 0.05% triethylamine, pH 6.80, and (B) 90:10 acetonitrile–water. To achieve the amino acid separation, the flow rate was set to 1 mL/min and the following gradient was performed: initial 3.2% B for 0.5 min, linear change to 4.5% B in 5 min, linear change to 10% B in 9.5 min, linear change to 19% B in 7 min, linear change to 27% B in 10 min, linear change to 99% B in 5 min, wash with this percentage of B for 15 min and equilibrate at 3.2% B. The detection was carried out at 254 nm. Twenty microliters of standard or sample was injected into the system. For amino acid identification, solutions (1 mg/mL) of standard amino acids (Sigma Chemical Co., St. Louis, MO, no. LAA-21), including L-Ala, L-Arg HCl, L-Asn, L-Asp, L-Cys, L-Cis, L-Glu, L-Gln, Gly, L-His HCl, Pro-(4-OH), L-Ile, L-Leu, L-Lys HCl, L-Met, L-Phe, L-Pro, L-Ser, L-Thr, L-Trp, L-Tyr and L-Val, were used. The concentration of different amino acids was calculated from the standard curves of the pure amino acids prepared and derivatized simultaneously with samples and run under identical conditions.

Amines. Amines were extracted from 10 g samples with 2:1 chloroform–butanol after deproteinization with 40 mL of 0.6 N $HClO_4$ and derivatized with dansyl chloride according to the method of Spinelly *et al.* (1974) and modified by Edwards *et al.* (1983). One milliliter of a solution of 500 mg/mL of 1,7-diaminoheptane was added to the samples before homogenization as an internal standard. Amine derivatives were analyzed in a Beckman liquid chromatograph as previously described. The detection was performed at 254 nm. Chromatographic conditions consisted of a solvent system of two eluents: (A) 0.01 M sodium acetate, pH 4.2, containing 5% tetrahydrofuran; and (B) 90:10 acetonitrile–tetrahydrofuran. The flow rate used was 1 mL/min, and the following gradient was performed: first, 50% B after 27 min; second, linear gradient from 50% to 90% B in 5 min; maintenance at 90% B isocratic flow for 10 min; and final return of the system to 50%B in 5 min. Twenty microliters of sample or standard was injected into the system. Putrescine, tryptamine, cadaverine, histamine, tyramine, spermine, and spermidine were identified by injection of standards (Sigma, no. P7505, T9628, C5659, H7250, T2879, S2876, and S2501). The concentrations of different amines were calculated from standard curves of the pure amines prepared and derivatized simultaneously with samples and run under identical conditions.

Moisture and Salt Contents. Moisture content was analyzed according to the method described in ISO method 1442 (ISO, 1973). To estimate the salt content, chlorides were extracted with water–ethanol (60:40 v/v) and quantified by the Carpentier Volhard method (AOAC, 1984).

Statistical Analysis. Statistical analysis of data was carried out by one-way analysis of variance, and means were separated by Tukey's honest significant difference test using a StatGraphics software package from Statistical Graphics Corp. (Rockville, MD).

RESULTS AND DISCUSSION

All free amino acids detected in the green stage increased significantly ($p < 0.05$) during ripening in both the semimembranosus and the biceps femoris (Table 1). Alanine and glutamic acid were the free amino acids present in higher amounts ($p < 0.05$) in the final product, followed by leucine, glycine, and lysine. Alanine and glutamic acid also have been found to be present in higher levels in other types of cured hams, such as American country style (McCain *et al.*, 1968), Parma (Ambanelli *et al.*, 1969), and Serrano (Toldrá *et al.*, 1992). With regard to the lengths of the different stages, the fastest increase in the concentration of any of the studied amino acids took place in the drying cycle (Table 2). It is in the drying and salting stages of the processing of Iberian ham when NPN increases at a maximum rate and the most marked reduction of myofibrillar proteins takes place (Ventanas *et al.*, 1992; Córdoba *et al.*, 1994). After drying, amino acid nitrogen, instead of peptide nitrogen, is the major non-protein nitrogen fraction (Ventanas *et al.*, 1992). The relatively high temperatures reached at this stage probably stimulated proteolysis. In Parma ham, Baldini *et al.* (1977) found the highest amounts of free amino acids at the end of processing, when the temperature reached maximum values (around 20 °C). In the final product the amounts of amino acids found, in this study, were higher than those reported by Ambanelly *et al.* (1969), Giolitti *et al.* (1971), and Baldini *et al.* (1977) in Parma hams. The longer processing time of Iberian cured ham (18 *vs* 12 months for Parma ham) and the higher temperatures reached during drying (30 *vs* 18–20 °C for Parma ham) could explain the higher proteolysis in the Iberian hams.

Irrespective of the muscle studied, most amino acids showed average increases of approximately 1–4 mmol/100 g of dry matter (DM) during processing (Figure 1). This was a 20–60-fold increase from the values in raw pork with arginine in the lower limit. The exceptions were asparagine and histidine, with increases during processing of only 0.1 and 0.2 mmol/100 g DM, respectively, and also tryptophan, which reached a final concentration of approximately 800 times that found in raw pork. The increase of histidine, asparagine, and arginine in the fully ripened ham was lower than expected, according to their ratio in muscle proteins from pig (Zarkadas *et al.*, 1988). These three amino acids are characterized by their intense reaction with reducing compounds in the Maillard condensations (Ashoor and Zent, 1984; Wong and Stanton, 1989). Reducing compounds, such as hexanal, nonanal, and heptanal, are formed during ripening of Iberian ham (García *et al.*, 1991; Antequera *et al.*, 1992). In addition, the major formation route of aldehydes such as 2-methylbutanal, 3-methylbutanal, phenylacetaldehyde, and α-dicarbonyls (2,3-butandione and 2,3-pentandione) was reported in Iberian (García *et al.*, 1991) and Parma (Barbieri *et al.*, 1992) hams and seems to be the oxidative deamination–decarboxylation of amino acids via the Strecker degradation (Ventanas *et al.*, 1992). Although the optimum conditions for these reactions (temperature about 90 °C and pH values near neutrality) are different from those of the processing of Iberian ham, the high free amino acid content together with the

Table 1. Means and Standard Deviations of Free Amino Acid Contents from Semimembranosus and Biceps Femoris Muscles at Different Stages of Processing[a]

amino acid	green	salting	postsalting 1	postsalting 2	drying	half-ripened	fully ripened
			A. Semimembranosus Muscle				
Asp	43.5 ± 0.6^{1}	48.0 ± 4.6^{1}	178.2 ± 7.2^{2}	215.2 ± 7.1^{3}	509.4 ± 11.8^{4,a}	674.9 ± 4.7^{5,a}	1048.3 ± 11.2^{6,a}
Glu	126.5 ± 3.5^{1}	234.0 ± 9.1^{2}	661.1 ± 23.9^{3}	764.5 ± 28.8^{4}	2539.2 ± 45.4^{5}	3428.6 ± 66.9^{6}	3992.3 ± 48.6^{7,a}
Ser	56.8 ± 1.4^{1}	47.7 ± 2.0^{2}	259.4 ± 9.0^{3}	361.8 ± 12.0^{4}	1053.7 ± 61.0^{5,a}	1268.8 ± 50.5^{6}	2033.7 ± 31.1^{7}
Asn	12.4 ± 1.0^{1}	21.7 ± 0.5^{2}	29.9 ± 0.5^{3}	54.6 ± 0.8^{4}	94.5 ± 7.2^{5}	114.1 ± 4.2^{5}	130.2 ± 1.8^{6}
Gly	109.5 ± 2.4^{1}	120.3 ± 3.6^{1}	201.0 ± 6.0^{2}	258.8 ± 5.9^{3}	1419.8 ± 67.6^{4}	1828.7 ± 67.9^{5,a}	3031.5 ± 66.7^{6,a}
Gln	39.4 ± 0.8^{1}	43.8 ± 1.3^{1}	147.9 ± 3.6^{2}	244.1 ± 8.2^{3}	990.8 ± 56.8^{4,a}	1691.1 ± 57.9^{5,a}	1730.3 ± 30.1^{5,a}
Arg	50.1 ± 0.9^{1}	48.1 ± 4.9^{1}	155.1 ± 4.7^{2}	250.1 ± 8.2^{3}	689.3 ± 12.4^{4,a}	752.1 ± 25.6^{5,a}	1004.3 ± 13.7^{6,a}
His	51.0 ± 1.3^{1}	68.0 ± 1.7^{2}	185.8 ± 5.7^{3}	202.3 ± 6.9^{3}	265.8 ± 4.3^{4}	278.6 ± 13.4^{4,a}	276.2 ± 6.0^{4,a}
Thr	40.9 ± 0.9^{1}	70.3 ± 0.7^{2}	197.1 ± 6.3^{3}	242.3 ± 11.8^{4}	882.1 ± 77.8^{5}	1028.9 ± 35.4^{6}	1816.2 ± 28.7^{7}
Ala	229.2 ± 4.3^{1}	231.2 ± 12.1^{1}	546.0 ± 19.6^{2}	712.7 ± 32.2^{3}	1961.6 ± 52.4^{4}	3723.7 ± 88.9^{5}	4762.1 ± 50.6^{6}
Pro	96.1 ± 1.9^{1}	109.5 ± 7.9^{1}	446.1 ± 15.0^{2}	595.6 ± 21.4^{3}	1209.9 ± 20.3^{4,a}	1705.3 ± 29.8^{5}	2043.5 ± 18.7^{6}
Tyr	34.2 ± 0.4^{1}	35.9 ± 4.5^{1}	155.5 ± 7.9^{2}	199.2 ± 7.1^{3}	901.1 ± 56.4^{4}	1328.2 ± 47.7^{5}	1610.6 ± 26.7^{6}
Val	50.8 ± 0.9^{1}	78.9 ± 27.7^{1}	352.0 ± 14.4^{2}	648.1 ± 24.1^{3}	1688.2 ± 100.3^{4}	1968.4 ± 69.9^{5}	2345.0 ± 41.0^{6,a}
Met	32.8 ± 0.7^{1}	53.9 ± 1.7^{2}	112.4 ± 4.2^{3}	139.7 ± 4.4^{4}	671.5 ± 8.5^{5,a}	721.1 ± 12.1^{6,a}	937.2 ± 16.1^{7}
Ile	39.3 ± 0.7^{1}	60.8 ± 1.1^{2}	105.7 ± 2.7^{3}	274.4 ± 8.7^{4}	1556.7 ± 107.6^{5}	1987.2 ± 20.2^{6}	2219.8 ± 22.4^{7}
Leu	64.1 ± 1.2^{1}	60.2 ± 11.9^{1}	121.0 ± 2.4^{2}	364.6 ± 14.3^{3}	1368.2 ± 31.7^{4}	2001.2 ± 24.4^{5}	3096.7 ± 31.1^{6}
Phe	39.6 ± 0.7^{1,a}	48.5 ± 10.1^{1}	166.0 ± 5.2^{2}	254.7 ± 7.6^{3}	917.0 ± 55.8^{4,a}	1300.6 ± 50.0^{5}	1472.4 ± 28.2^{6}
Trp	1.2 ± 0.1^{1}	3.5 ± 0.5^{2}	9.9 ± 0.5^{3}	58.6 ± 1.0^{4}	225.3 ± 12.3^{5}	728.3 ± 21.6^{6,a}	963.4 ± 17.2^{7,a}
Lys	49.5 ± 0.1^{1}	83.1 ± 2.1^{2}	263.2 ± 9.2^{3}	354.1 ± 14.2^{4}	1626.7 ± 99.6^{5}	2363.7 ± 77.4^{6}	2825.8 ± 47.6^{7,a}
total	1366.9	1467.4	4292.3	6193.4	20570.8	28893.5	37339.5
			B. Biceps Femoris Muscle				
Asp	44.4 ± 1.1^{1}	48.0 ± 4.2^{1}	182.9 ± 7.0^{2}	227.3 ± 5.9^{3}	415.6 ± 17.3^{4,b}	596.8 ± 15.4^{5,b}	990.0 ± 9.9^{6,b}
Glu	138.6 ± 1.6^{1}	245.0 ± 11.6^{2}	728.7 ± 28.2^{3}	887.9 ± 22.9^{4}	2665.8 ± 30.9^{5}	3100.1 ± 151.9^{6}	4447.6 ± 53.7^{7,b}
Ser	50.9 ± 6.7^{1}	49.2 ± 1.7^{1}	267.6 ± 8.9^{2}	401.7 ± 12.0^{3}	823.6 ± 45.7^{4,b}	1205.1 ± 49.3^{5}	2118.1 ± 28.8^{6}
Asn	13.5 ± 0.8^{1}	23.3 ± 0.4^{2}	25.8 ± 1.1^{2}	145.9 ± 0.9^{3}	104.0 ± 6.3^{4}	118.3 ± 2.7^{4}	131.7 ± 2.0^{5}
Gly	122.3 ± 2.0^{1}	128.8 ± 4.8^{1}	208.5 ± 7.7^{2}	282.1 ± 12.0^{3}	1397.2 ± 46.1^{3}	1532.3 ± 76.0^{3,b}	2759.2 ± 40.7^{4,b}
Gln	40.0 ± 0.8^{1}	43.6 ± 0.8^{2}	152.8 ± 5.1^{3}	280.0 ± 7.7^{4}	1179.0 ± 51.0^{5,b}	1783.0 ± 87.3^{6,b}	1955.0 ± 30.3^{6,b}
Arg	51.7 ± 0.7^{1}	59.0 ± 0.9^{2}	214.5 ± 6.2^{3}	291.6 ± 10.2^{4}	561.9 ± 27.0^{5,b}	684.8 ± 37.1^{6,b}	934.7 ± 21.4^{7,b}
His	55.4 ± 1.0^{1}	69.2 ± 3.0^{2}	208.1 ± 6.2^{2}	222.2 ± 7.3^{2}	225.8 ± 19.4^{2}	191.1 ± 21.2^{2,b}	238.5 ± 5.7^{2,b}
Thr	46.5 ± 0.6^{1}	73.6 ± 2.9^{2}	200.7 ± 6.1^{3}	281.7 ± 9.9^{4}	802.1 ± 20.6^{5}	932.3 ± 39.1^{6}	1776.7 ± 24.6^{7}
Ala	253.5 ± 3.5^{1}	286.6 ± 26.7^{1}	563.5 ± 9.6^{2}	828.9 ± 21.2^{3}	1956.0 ± 73.9^{4}	3598.6 ± 166.1^{5}	4553.4 ± 73.0^{6}
Pro	104.6 ± 1.3^{1}	127.2 ± 11.3^{2}	498.7 ± 17.7^{3}	684.8 ± 17.4^{4}	1032.4 ± 70.0^{5,b}	1618.0 ± 43.0^{6}	1962.9 ± 25.9^{7}
Tyr	33.4 ± 0.6^{1}	40.0 ± 1.8^{2}	151.7 ± 7.4^{3}	234.1 ± 7.4^{4}	932.0 ± 43.6^{5}	1284.4 ± 67.2^{6}	1625.9 ± 23.5^{7}
Val	50.4 ± 0.3^{1}	67.1 ± 10.0^{2}	374.5 ± 13.0^{3}	714.7 ± 23.8^{4}	1637.9 ± 78.2^{5}	1880.1 ± 97.0^{5}	2184.9 ± 31.6^{6,b}
Met	35.2 ± 0.4^{1}	54.2 ± 1.6^{2}	121.6 ± 5.8^{3}	144.4 ± 3.4^{4}	568.6 ± 27.5^{5,b}	619.1 ± 20.3^{5,b}	949.8 ± 10.8^{6}
Ile	41.7 ± 0.2^{1}	58.9 ± 2.4^{2}	114.5 ± 2.5^{3}	316.7 ± 9.8^{4}	1435.6 ± 66.8^{5}	1925.5 ± 40.7^{6}	2188.1 ± 25.3^{7}
Leu	67.4 ± 0.8^{1}	89.4 ± 8.5^{2}	111.3 ± 3.8^{3}	369.1 ± 20.0^{4}	1535.6 ± 90.2^{5}	2068.3 ± 112.2^{6}	3244.2 ± 43.2^{7}
Phe	41.4 ± 0.4^{1,b}	66.1 ± 0.7^{2}	189.0 ± 6.9^{3}	304.4 ± 8.4^{4}	709.9 ± 48.2^{5,b}	1295.3 ± 71.2^{6}	1398.5 ± 14.7^{6}
Trp	1.0 ± 0.0^{1}	3.3 ± 0.1^{2}	9.7 ± 1.5^{3}	60.8 ± 1.0^{4}	213.0 ± 12.0^{5}	604.5 ± 27.2^{6,b}	911.3 ± 13.9^{7,b}
Lys	48.9 ± 0.5^{1}	87.4 ± 0.3^{2}	296.1 ± 8.7^{3}	410.2 ± 9.3^{4}	1570.2 ± 76.6^{5}	2256.1 ± 61.2^{6}	3003.6 ± 43.5^{7,b}
total	620.8	1619.9	4619.2	7086.5	19766.2	27293.7	37374.1

[a] Results are expressed in micromoles per 100 g of dry matter. Values with different numbers as superscript along a row are significantly different ($p < 0.05$). Values for the same amino acid and stage with different letters as superscript are significantly different ($p < 0.05$).

Table 2. Daily Increases (Micromoles per 100 g of Dry Matter per Day) of Free Amino Acids (Average for the Two Muscles) in the Different Stages of Processing

amino acid	salting	postsalting 1	postsalting 2	drying	half-ripened	fully ripened
Asp	0.27	1.78	1.39	5.36	0.95	1.77
Glu	7.13	6.07	4.38	39.47	3.62	4.42
Ser	−0.36	2.87	3.94	12.38	1.63	3.88
Asn	0.63	0.07	0.75	1.09	0.09	0.07
Gly	0.57	1.07	2.19	25.29	1.49	5.62
Gln	0.27	1.42	3.72	18.28	3.34	2.07
Arg	0.18	1.75	2.87	7.88	0.51	1.16
His	1.03	1.71	0.51	0.75	−0.06	0.10
Thr	1.88	1.69	2.10	12.89	0.76	3.78
Ala	1.17	3.94	7.20	26.40	9.30	4.61
Pro	11.87	4.72	5.59	10.69	2.95	1.58
Tyr	0.28	1.54	2.11	15.55	2.13	1.44
Val	1.49	3.87	10.61	21.81	1.43	1.58
Met	1.34	0.84	0.83	10.62	0.27	1.27
Ile	1.29	0.67	6.18	26.68	2.51	1.15
Leu	0.60	0.55	8.36	24.11	3.18	5.26
Phe	1.12	1.60	3.40	11.86	2.65	0.64
Trp	0.15	0.09	1.66	3.54	2.44	1.26
Lys	2.40	2.59	3.42	27.03	3.89	2.80

low water activity may facilitate their development. Therefore, these reactions can also contribute to the smaller increase of histidine, asparagine, and arginine.

On the other hand, the 800-fold increase for tryptophan is partially due to its low concentration in the free form found in raw pork, given that its overall increase throughout the ripening process is lower than that of most amino acids (Figure 1). On the other hand, the low ratio at which tryptophan has been reported in proteins from porcine skeletal muscle (Zarkadas *et al.*, 1988) suggests that the released amount of this amino acid exceeds expectations. The explanation for this unexpected increase during ripening could come from a specific tryptophan aminopeptidase activity. It could also be related with its synthesis through the chorismic acid pathway (Crawford, 1989) by *Aspergillus* spp., *Penicillium* spp., *Saccharomyces* spp., or *Lactobacillus* spp., all of them growing on Iberian hams (Francisco *et al.*, 1981; Monte *et al.*, 1986). In any case, these data have to be carefully considered given the sensitivity of tryptophan to low pH values.

When the evolution of amino acids was compared in the two muscles studied, no significant difference was observed in the concentration for any of the amino acids tested during the first stages of processing (salting and postsalting). However, at the end of drying (D) the content of aspartic acid, serine, arginine, proline, methionine, and phenylalanine was higher ($p < 0.05$) in

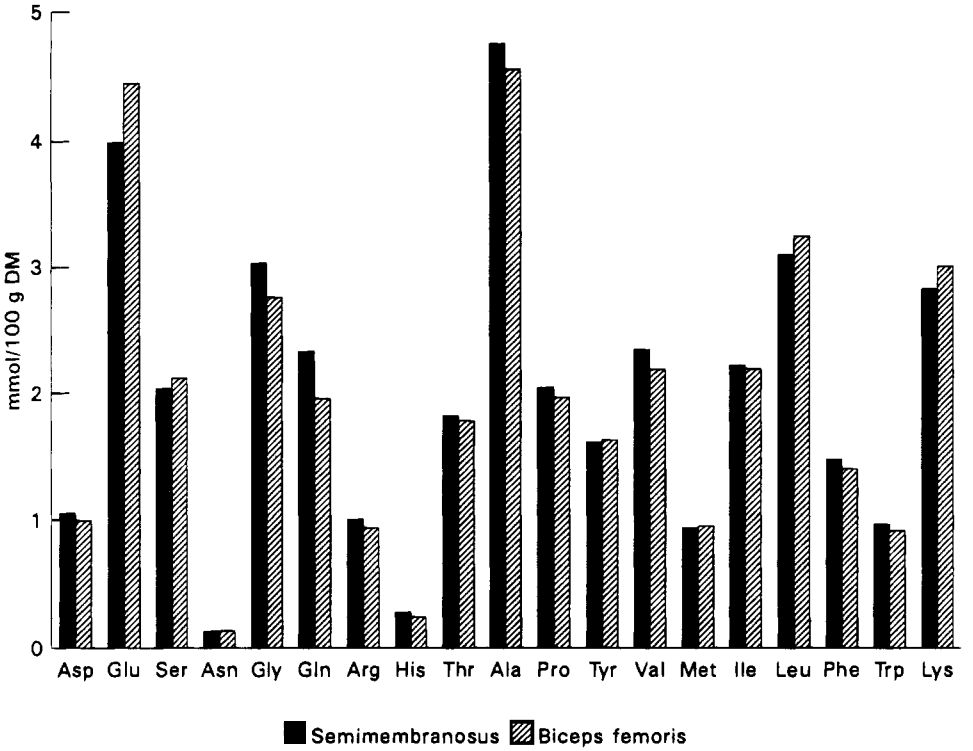

Figure 1. Total increase from uncured to end of processing in the content of the free amino acids studied.

Table 3. Means and Standard Deviations of Moisture and Salt Contents from Semimembranosus (SM) and Biceps Femoris (BF) Muscles during Ripening of Iberian Ham[a]

	moisture (%)		NaCl (%)	
stage	SM	BF	SM	BF
green state	68.01 ± 1.88^{1}	71.43 ± 0.78^{1}	nd[b]	nd
salting	$65.24 \pm 2.21^{1,2}$	$69.26 \pm 2.37^{1,2}$	$6.41 \pm 0.45^{1,a}$	$1.68 \pm 0.08^{1,b}$
postsalting 1	61.48 ± 2.55^{2}	$67.76 \pm 1.32^{2,3}$	5.11 ± 0.31^{1}	$3.23 \pm 0.57^{1,2}$
postsalting 2	$57.34 \pm 2.47^{2,3,a}$	$64.92 \pm 1.47^{3,b}$	4.46 ± 0.30^{1}	$3.57 \pm 0.35^{1,2}$
drying	$55.76 \pm 4.03^{3,a}$	$63.74 \pm 2.78^{3,b}$	4.51 ± 0.09^{1}	$4.76 \pm 0.35^{1,2}$
half-ripened	$48.02 \pm 4.49^{4,a}$	$54.66 \pm 3.61^{4,b}$	4.71 ± 0.35^{1}	5.35 ± 0.50^{2}
fully ripened	$40.71 \pm 2.28^{5,a}$	$48.44 \pm 1.66^{5,b}$	5.45 ± 0.54^{1}	5.85 ± 0.74^{2}

[a] Values with different numbers as superscript along a column are significantly different ($p < 0.05$). Values with different letters along a row as superscript are significantly different ($p < 0.05$). [b] nd, not determined.

the semimembranosus than in the biceps femoris. In the drying stage only glutamine showed a lower concentration for the semimembranosus than for biceps femoris. During cellar stages (HR and FR) again aspartic acid, arginine, and methionine, together with glycine, histidine, valine, and tryptophan, resulted in significantly ($p < 0.05$) higher concentrations in the external (semimembranosus) than in the internal muscle (biceps femoris). On the other hand, glutamine as well as glutamic acid and lysine showed significantly ($p < 0.05$) lower concentrations in the external muscle. Given that these differences between muscles were found when salt was equalized in both muscles, and moisture was 12% higher in the biceps femoris than in the semimembranosus (Table 3), it is unlikely that physicochemical conditions explain these differences. It is possible that specific exopeptidases from muscle tissue may be responsible for these differences. Since molds growing on ham surface have proved to be proteolytic (Huerta *et al.*, 1987), this activity can also contribute to these differences.

In relation to amines, a similar evolution was observed in semimembranosus and biceps femoris (Table 4). Putrescine, histamine, and tyramine increased significantly ($p < 0.05$) during ripening; spermidine showed a nonsignificant increase, while spermine decreased ($p < 0.05$) through processing. Cadaverine was found only in trace amounts in all stages of processing, and tryptamine was not detected in any of the samples analyzed. The amines with a concentration of over 2 mmol/100 g of DM in the final product were putrescine, histamine, and spermine. In general, the values found in this research were higher for putrescine and histamine, similar for tyramine, and lower for cadaverine and spermidine than those reported in Parma (Cantoni *et al.*, 1974), American country style (Zee *et al.*, 1983), and Serrano (Santos-Buelga *et al.*, 1981) cured hams. The strong decrease of spermine throughout the ripening could be related to microbial utilization for biological reactions, as described by Tabor and Tabor (1985).

The higher significant ($p < 0.05$) increases in the concentration of putrescine and histamine took place in the cellar stage with no significant difference between muscles (Table 4). In spite of the relatively large amounts of arginine, histidine, tyrosine, lysine, and tryptophan, which are released during processing, the concentrations of amines putrescine, histamine, tyramine, cadaverine, and tryptamine were always below 20% of the respective amino acids (Tables 1 and 4).

Although the concentrations of biogenic amines described in this research are neither in the range of toxic levels nor enough to reach values at which uptake

Table 4. Amine Contents from Semimembranosus (SM) and Biceps Femoris (BF) Muscles in the Different Stages of Processing[a]

	amines									
	PUT		His		Tyr		SPD		SPM	
stage	SM	BF	SM	BF	SM	BF	SM	BF	SM	BF
green	2.2 ± 1.1^{1}	2.0 ± 0.7^{1}	$14.7 \pm 2.7^{1,2}$	$15.3 \pm 1.0^{1,2}$	2.6 ± 0.1^{1}	2.8 ± 0.1^{1}	4.5 ± 0.1^{1}	3.7 ± 1.0^{1}	68.7 ± 3.8^{1}	64.0 ± 11.6^{1}
salting	2.2 ± 0.2^{1}	1.1 ± 0.1^{1}	12.4 ± 0.6^{1}	13.8 ± 0.7^{1}	4.2 ± 0.4^{1}	3.3 ± 0.2^{1}	3.3 ± 0.1^{1}	4.2 ± 0.2^{1}	$58.5 \pm 4.6^{1,2}$	62.5 ± 3.5^{1}
postsalting 1	5.7 ± 0.3^{2}	3.9 ± 0.4^{2}	12.0 ± 0.6^{1}	12.6 ± 0.2^{1}	4.8 ± 0.2^{1}	4.2 ± 0.1^{1}	6.6 ± 0.5^{1}	5.9 ± 0.5^{1}	59.2 ± 4.9^{1}	65.3 ± 0.7^{1}
postsalting 2	$9.5 \pm 3.1^{2,3}$	$7.0 \pm 1.5^{2,3}$	10.1 ± 0.4^{1}	12.8 ± 0.9^{1}	4.2 ± 0.0^{1}	4.5 ± 0.8^{1}	6.1 ± 0.1^{1}	6.9 ± 0.6^{1}	50.5 ± 1.9^{2}	59.1 ± 3.1^{1}
drying	16.0 ± 5.3^{3}	11.1 ± 1.3^{3}	17.2 ± 1.6^{2}	20.1 ± 3.2^{2}	4.2 ± 1.1^{1}	3.9 ± 0.8^{1}	$7.6 \pm 1.8^{1,2}$	$8.8 \pm 1.3^{1,2}$	$41.8 \pm 9.9^{2,3}$	51.5 ± 6.0^{1}
half-ripened	33.8 ± 4.8^{4}	24.4 ± 3.0^{4}	40.6 ± 5.1^{3}	30.8 ± 5.3^{2}	11.20 ± 3.5^{2}	8.6 ± 3.7^{1}	9.2 ± 1.8^{2}	10.2 ± 1.2^{2}	27.1 ± 3.8^{3}	34.5 ± 4.9^{2}
fully ripened	47.5 ± 4.8^{4}	36.1 ± 6.4^{4}	56.3 ± 11.1^{3}	33.9 ± 9.3^{2}	11.5 ± 4.9^{2}	14.0 ± 10.3^{1}	5.5 ± 0.5^{1}	6.0 ± 0.9^{1}	13.6 ± 1.6^{4}	19.1 ± 3.0^{3}

[a] Results are expressed in micromoles per 100 g of dry matter. Values with different superscripts along a column are significantly different ($p < 0.05$). No significant differences were found between muscles.

(Lyons *et al.*, 1983) and metabolism (Hui and Taylor, 1985) of histamine in rats are modified, it is not ruled out that such levels may be reached in Iberian cured hams produced in uncontrolled processes, especially for sensitive people.

Conclusion. From these results it can be concluded that amino acid liberation during ripening of cured ham is not selective and most amino acids increase according to their proportion in porcine skeletal muscle. Basic amino acids show lower increases, probably due to their involvement in derived reactions such as formation of volatile compounds and amines. Given that the rate of amino acid production is strongly influenced by temperature, carrying out the drying stage before ripening in the cellar, as performed in the traditional processing of Iberian cured ham, seems to be the most crucial stage for the development of compounds highly correlated to flavor in aged ham.

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