Ion-Pair High-Performance Liquid Chromatographic Determination of Biogenic Amines in Meat and Meat Products

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Twelve biogenic amines (tyramine, histamine, serotonin, β-phenylethylamine, tryptamine, octopamine, dopamine, putrescine, cadaverine, agmatine, spermine, and spermidine) were separated and quantified by a liquid chromatographic method in fresh and processed meat. The method consists of the separation of ion pairs formed between biogenic amines and octanesulfonic acid by reversed-phase column, a postcolumn derivatization with *o*-phtalaldehyde-2-mercaptoethanol, and spectrofluorometric detection. Results of the reliability study were satisfactory. The method was linear for each amine between 0.25 and 10 mg/L. Average recoveries ranged between 88 and 104%. The precision ranged between 0.75 and 9.09% (coefficient of variation) and determination limits were ≤1.5 mg/kg. The method was applied to pork and beef meat samples and to ripened and cooked meat products. Biogenic amine contents, in general, varied greatly among the different meat products as well as among samples of a single product type. Spermine and spermidine were always found in all samples. Average levels of tyramine, histamine, putrescine, cadaverine, and tryptamine were higher in samples of ripened meat products than those in fresh meat and in cooked meat products.

Keywords: *Biogenic amines; tyramine; histamine; meat; meat products; sausages*

INTRODUCTION

Biogenic amines, organic bases of low molecular weight, can be formed and degraded as a result of normal metabolic activity in animals, plants, and microorganisms. These amines are usually produced in foods by decarboxylation of amino acids (Halász et al., 1994). Toxicological problems resulting from the ingestion of foods containing relatively high levels of biogenic amines have been reviewed (Smith, 1981; Stratton et al., 1991). It is well-known that ingestion of biogenic amines may provoke hypertensive crises in patients treated with monoaminoxidase inhibitor drugs (MAOI) (Lippman and Nash, 1990). Among biogenic amines in food, histamine is potentially hazardous and is the causative agent of "histaminic intoxication" (Morrow et al., 1991). Other amines such as putrescine, cadaverine, tyramine, β-phenylethylamine, spermine, and spermidine have been described as a potentiators that enhance the toxicity of histamine (Pfannhauser and Pechanek, 1984; Taylor, 1985). Likewise, tyramine and β-phenylethylamine are thought to precipitate migraine attacks in susceptible subjects (Crook, 1981). In addition, polyamines such as putrescine, cadaverine, spermine, and spermidine may originate carcinogenic nitrosamines in the presence of nitrites (Vandekerckove, 1977; Hotchkiss et al., 1977). This is another reason for preventing the accumulation of biogenic amines in cured products.

Biogenic amines occur in a wide variety of foods, such as fish products, meat, cheese, wine, and other fermented foods (Askar and Treptow, 1986; Vidal-Carou et al., 1990a; Veciana-Nogués et al., 1989; Izquierdo-Pulido et al., 1994). Biogenic amines can be found in processed meat products as a consequence of microbial activity related to the fermentation involved in their processing, but amines can be also found in poor quality raw materials as a consequence of microbial contamination (Vidal-Carou et al., 1990b). Therefore, biogenic amines in cooked meat products (not fermented) might serve as useful indicators of the hygienic quality of the meat employed for their elaboration. However, this relationship for ripened meat products is rather complicated since the ability to produce biogenic amines of the fermentative microflora should be well-known before limits can be set for those products.

According to the few studies performed on biogenic amines in meat products, concentrations of those substances varied among the type and origin of meat products. Histamine and tyramine are the biogenic amines most often studied (Tschabrun et al., 1990; Vidal-Carou et al., 1990b). Some authors (ten Brink et al., 1990; Shalaby, 1993; Eerola et al., 1993) have recently studied some other biogenic amines, such as putrescine, cadaverine, and tryptamine, especially in ripened meat products, but studies for cooked meat products are still lacking. In general, levels reported in meat products are much higher than those reported for fish products, which have been more studied, and, moreover, legal limits or at least tolerable maximum contents of histamine have been established for them (e.g., Food Drug Administration; European Union). Despite the fact that meat products are widely consumed in Spain and in other European countries, data about biogenic amine contents on those products are scarce.

Several methods to isolate and estimate biogenic amines in food have been reported; however, some of them have limitations, such as detection of only a few amines and poor resolution. Furthermore, meat products show a complex matrix in comparison with other food products because of the high proportion of fat and protein that makes the extraction of amines more difficult. The aim of our research was to apply and to

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study the reliability of a liquid chromatographic method for biogenic amine determination in meat and meat products. The analytical method applied derived from a liquid chromatographic procedure setup by us for the biogenic amine determination in fish and fish products (Veciana-Nogués et al., 1995). Some additional changes have been made, such as the inclusion of two more biogenic amines (octopamine and dopamine) which have sometimes been reported in meat products and an extensive extraction with perchloric acid to increase the sample cleanup. The proposed method results of easy management and can be applied to fresh meat as well as to a wide range of different types of meat products. Data about biogenic amine contents in Spanish meat products are also reported.

MATERIALS AND METHODS

Reagents and Standards. Acetonitrile was of high-performance liquid chromatography (HPLC) grade (SDS, Peypin, France). Other chemicals were of reagent grade. Sodium acetate, Brij-35, 2-mercaptoethanol, and *o*-phthalaldehyde (OPT) were obtained from Merck (Darmstadt, Germany); sodium octanesulfonate, from Romil Chemicals (Cambridge, Great Britain); and boric acid and potassium hydroxide, from Panreac (Montplet & Esteban SA, Barcelona, Spain). Double-distilled water was obtained from the Milli-Q System (Millipore Corp., Bedford, MA).

Biogenic amine standards histamine (HI) dihydrochloride and tyramine (TY) free base were purchased from Merck. β-Phenylethylamine (PHE) hydrochloride, serotonin (SE) creatinine sulfate, tryptamine (TR) hydrochloride, octopamine (OC) free base, dopamine (DO) free base, cadaverine (CA) dihydrochloride, putrescine (PU) hydrochloride, agmatine (AG) sulfate, spermine (SM) tetrahydrochloride, and spermidine (SD) trihydrochloride were obtained from Sigma Chemical Co. (St. Louis, MO). A concentrated 1000 mg/L stock solution as a free base of each biogenic amine in 0.1 N HCl was prepared. A 50 mg/L intermediate solution was prepared in 0.1 N HCl from the stock solution. Calibration standards (ranging from 0.25 to 10.00 mg/L) were prepared in 0.1 N HCl from the intermediate standard solution. Then, they were filtered through a 0.45 μm filter, stored in refrigerator, and protected from light.

HPLC Analysis. The HPLC system (Waters Chromatography, Milford, MA) consisted of a Waters 600 E system controller pump, a Waters 715 autosampler, a Waters RDM postcolumn reaction equipment, and a Waters 470 spectrofluorometric detector. The waters RDM was connected to a zero dead volume mixing T installed between the column outlet and the detector. A coil of 200 cm long and 0.01 in. i.d. stainless steel tubing was used to connect the T with the detector. Data acquisition was accomplished by a system MAXIMA 820 (Waters). The separation was performed on a Nova Pack C18 column, 3.9 × 150 mm, 4 μm particle size (Waters), with a matching guard cartridge.

Mobile Phase. *(1) Eluent A.* A solution of 0.1 M sodium acetate and 10 mM sodium octanesulfonate was adjusted to pH 5.20 with acetic acid.

(2) Eluent B. Solvent B + Acetonitrile (6.6:3.4). Solvent B consisted of 0.2 M sodium acetate and 10 mM sodium octanesulfonate solution and was adjusted to pH 4.50 with acetic acid.

(3) The gradient program was implemented as follows: time = 0 min, 80% A, 20% B; time = 50 min, 20% A, 80% B; time = 52 min, 20% A, 80% B; time = 54 min, 80% A, 20% B; and time = 64 min, 80% A, 20% B. The two last steps were to reequilibrate the column to the initial conditions. The increase of eluent B was according to an exponential function of second order.

(4) Postcolumn Derivatizating Reagent. A 15.5 g sample of boric acid and 13.0 g of potassium hydroxide were dissolved in 500 mL of water. A 1.5 mL aliquot of 30% Brij-35 solution and 1.5 mL of 2-mercaptoethanol were added. Then, 0.1 g of OPT dissolved in 2.5 mL of methanol was added and the solution mixed. The derivatizating reagent was prepared fresh daily and protected from light.

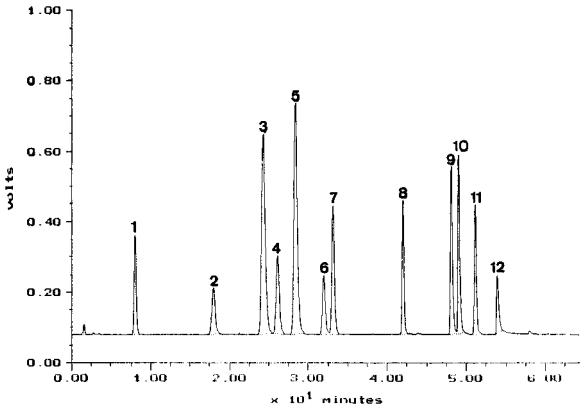

Figure 1. Chromatogram of a biogenic amine standard solution of 6 mg/L. Peak identities: OC (1), DO (2), PU (3), TY (4), CA (5), SE (6), HI (7), AG (8), PHE (9), SD (10), TR (11), and SM (12).

(5) The flow rate of the mobile phase was 1 mL/min, and the flow rate of the derivatizating reagent was 0.5 mL/min.

Mobile phases and the derivatizating reagent were filtered and degassed before use. The column and postcolumn reaction equipment were set at room temperature. Automatic injection (20 μL) of standard solutions or prepared samples was carried out when the eluate was alkaline (pH 10.50−11.00) and a steady base line was recorded. The eluate was monitored at 340 nm excitation and 445 nm emission wavelengths.

Samples. All samples were purchased from Spanish retail stores. The samples studied were pork and beef fresh meat and different meat products. We analyzed the following: (a) five samples of pork fresh meat, (b) five samples of beef fresh meat, (c) five samples of cooked ham, (c) five samples of "mortadella", (d) ten samples of ripened meat products (five samples of "chorizo" and five samples of "salchichón"). The ripened products studied consisted of mixtures of pork and beef, seasoned with salt, curing substances, and spices and packed in natural and/or artificial casings. Chorizo and salchichón are similar in composition but differ in the spices used in their production and in the way of ripening. All samples were stored at −20 °C prior to analysis.

Sample Preparation. Samples of meat and meat products (without casings or gelatin) were triturated and homogenized mechanically using a domestic meat mincer for about 1 min. Usually, 5 g of sample to 50 mL of 0.6 N perchloric acid (PCA) was used for dry cured products and 10 g of sample to 25 mL of 0.6 N PCA for fresh meat and cooked meat products. PCA must be handled with caution.

Samples were accurately weight, and 10 or 20 mL of 0.6 N PCA was added and mixed throughly over a magnetic stirring plate for 10 min. After centrifugation at 3000 rpm for 10 min and separation of the 2 phases, 10 or 20 mL of 0.6 N PCA was added to the solid residue obtained and mixed thoroughly for 10 min, and the centrifugation repeated. This operation was repeated once more with the residue obtained. The three PCA extracts were combined, and the volume was adjusted to 25 or 50 mL. If the amine contents were greater than 50 mg/kg, the sample weight/PCA extraction volume had to be decreased. Perchloric extracts were filtered through a 0.45 μm filter prior to LC analysis.

Statistical Methods. Cochran's test was performed using the statistics package Microstat (Ecosoft, Inc., 1985).

RESULTS AND DISCUSSION

Preliminary trials to optimize the HPLC method indicated that the retention times of standard biogenic amines were pH dependent; therefore, the pH of eluent A was critical. The elution program was developed to provide chromatograms of high-resolution peaks (Figure 1). Scan analysis of standard biogenic amines indicated

Table 1. Precision of the Method for Determination of Biogenic Amines in Meat and Meat Products

	fresh meat		cooked products		ripened products	
amine	$X \pm SD^a$	RSD,[b] %	$X \pm SD^a$	RSD,[b] %	$X \pm SD^a$	RSD,[b] %
TY	3.90 ± 0.17	4.35	31.90 ± 0.30	0.95	367.00 ± 15.65	4.25
HI	4.20 ± 0.23	5.45	1.50 ± 0.03	2.00	288.90 ± 11.95	4.15
SE	3.95 ± 0.19	4.80	1.15 ± 0.08	6.95	9.90 ± 0.09	0.90
OC	4.00 ± 0.29	7.20	2.00 ± 0.06	3.00	10.45 ± 0.08	0.75
DO	3.90 ± 0.27	6.90	1.30 ± 0.12	9.09	9.40 ± 0.20	2.25
PHE	3.95 ± 0.17	4.30	1.95 ± 0.02	1.00	28.25 ± 1.10	3.90
TR	4.00 ± 0.13	3.20	1.95 ± 0.10	5.10	14.75 ± 0.90	6.10
PU	3.95 ± 0.22	8.30	1.60 ± 0.03	1.90	404.25 ± 8.70	2.15
CA	4.35 ± 0.30	5.55	5.55 ± 0.06	1.20	200.45 ± 14.70	7.33
AG	4.10 ± 0.34	6.90	1.65 ± 0.02	1.05	2.60 ± 0.19	7.30
SD	6.00 ± 0.35	5.85	2.65 ± 0.03	1.15	14.10 ± 0.45	3.20
SM	19.40 ± 0.45	2.30	9.00 ± 0.15	1.65	41.80 ± 2.20	5.25

[a] Mean ± standard deviation in milligrams per kilograms ($n = 7$). [b] Relative standard deviation.

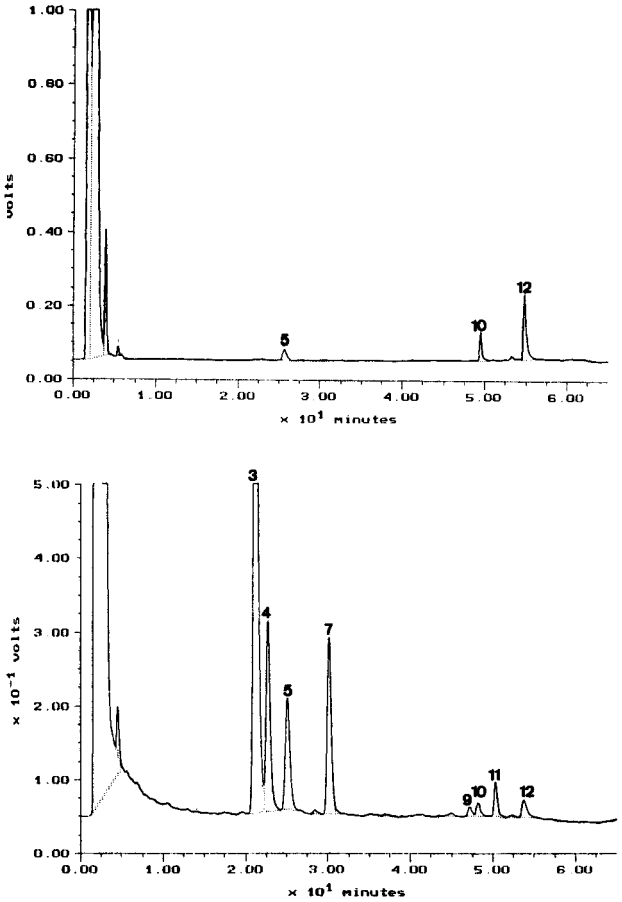

Figure 2. Chromatograms of biogenic amines in a fresh meat sample (A, top) and in a meat product sample (B, bottom). Peak identities: PU (3), TY (4), CA (5), HI (7), PHE (9), SD (10), TR (11), and SM (12).

that the detection of those compounds was optimum at 340 (excitation wavelength) and 445 nm (emission wavelength). According to literature reports, TCA, methanol, and PCA can be used to extract biogenic amines. Our preliminary work showed that more interferences were present in chromatograms when TCA or methanol was used. Figure 2 shows typical chromatograms of amines in a fresh meat sample and in a meat product sample. Chromatograms were relatively simple and identification was certain. Amine identification was made on the basis of retention time by comparison with standard solution.

Linearity. Detector response in the corresponding calibration curves (from 0.25 to 10.00 mg/L) was linear. Linearity was verified by analysis of the variance of the regression. Least-squares analysis produced a correlation coefficient of $r > 0.9990$ for HI, OC, PHE, TR, AG, PU, and CA ($p < 0.001$) and of $r > 0.9975$ for TY, SE, DO, SD, and SM ($p < 0.001$). The coefficient of determination (r^2) was better than 99.50% for all of the standard curves.

Precision. Seven determinations of the same sample were performed using the same reagents and apparatus to evaluate the method precision in different types of samples (fresh meat and cooked and ripened meat products). When some amine was not present in the sample, a known quantity of corresponding amine was spiked to the sample used for the precision study. Results of Table 1 show that a relative standard deviation (RSD) of less than 10% was obtained for all amines in all types of samples. These results were always satisfactory according to the Horwitz formula for intralaboratory studies (Horwitz, 1982).

Recovery. Recovery was tested by the standard addition procedure using two addition levels for each amine in each kind of sample (fresh meat and cooked and ripened meat products). Six determinations were carried out for each addition level. Results obtained are shown in Tables 2 and 3. By statistical analysis (Cochran's test), we verified that method accuracy did not depend on the biogenic amine content of samples. Recovery was satisfactory, since it was always higher than 88%.

Sensitivity. The determination limit was calculated using the Long and Winefordner criterion (Long and Winefordner, 1983). The blank used for this study was 0.6 N PCA, because it was not possible to obtain a sample without biogenic amines. Limits of determination were lower than 1.00 mg/kg for TY, HI, PHE, TR, OC, DO, PU, CA, and AG and lower than 1.50 mg/kg for SE and SM. These results are average values from eight tests.

Biogenic Amine Contents in Meat and Meat Products. The biogenic amine concentration was rather constant between the different samples of pork and beef meat. On the contrary, amine levels, in general, varied greatly among the types of meat products as well as among the samples of a single product type. Tables 4−6 show the range of concentrations as well as the average values and the standard deviations for each biogenic amine. The average levels of TY, HI, PU, CA, and TR were higher in samples of ripened meat products than those found in fresh meat and in cooked products.

We did not find either OC or DO in any of the analyzed samples. There are few data about the contents of those amines in meat and meat products, only the data reported by Sayem-el-Daher et al. (1984)

Table 2. Recovery of the Method for the Determination of Biogenic Amines in Meat

amine	initial contents,[a] (μg)	exptl content after addition		mean recovery, %
		level I (50 μg)	level II (100 μg)	
TY	ND	47.25 ± 2.80	98.20 ± 3.20	97.60
HI	ND	49.75 ± 4.13	104.35 ± 3.40	101.45
SE	ND	39.75 ± 3.60	94.50 ± 3.20	88.30
OC	ND	51.30 ± 1.00	97.60 ± 7.20	101.45
DO	ND	47.10 ± 2.85	98.20 ± 5.20	95.30
PHE	ND	45.60 ± 4.90	92.15 ± 1.60	94.95
TR	ND	47.85 ± 1.48	88.30 ± 4.10	90.85
PU	ND	52.15 ± 3.90	102.40 ± 3.20	104.00
CA	ND	48.85 ± 3.85	99.80 ± 6.60	99.60
AG	ND	47.70 ± 5.80	99.75 ± 4.30	100.80
SD	37.70 ± 2.30	86.45 ± 7.30	146.80 ± 6.20	104.50
SM	358.10 ± 3.00	422.05 ± 2.90	457.55 ± 5.70	103.65

[a] ND = not detected.

Table 3. Recovery of the Method for the Determination of Biogenic Amines in Cooked and Ripened Meat Products

amine	cooked products				ripened products			
	initial content,[a] μg	exptl content after addition		mean recovery, %	initial content,[a] μg	exptl content after addition		mean recovery, %
		level I[b]	level II[c]			level I[d]	level II[e]	
TY	ND	48.80 ± 0.35	91.30 ± 0.55	94.40	1265.50 ± 2.50	1676.60 ± 29.10	2180.85 ± 35.50	96.00
HI	ND	51.20 ± 0.17	92.00 ± 0.27	97.15	10.50 ± 1.25	19.35 ± 0.40	29.95 ± 1.50	95.90
SE	ND	45.05 ± 0.30	91.70 ± 0.35	90.90	ND	10.00 ± 0.25	20.05 ± 0.80	100.10
OC	ND	48.00 ± 0.42	87.90 ± 0.20	92.00	ND	9.65 ± 0.45	19.85 ± 0.85	97.90
DO	ND	50.00 ± 0.20	97.50 ± 0.30	98.70	ND	10.05 ± 0.30	20.05 ± 0.95	100.40
PHE	ND	48.10 ± 0.12	86.90 ± 0.28	91.55	25.50 ± 1.50	50.15 ± 1.40	73.05 ± 2.25	98.00
TR	ND	43.50 ± 0.25	89.50 ± 0.32	88.30	25.25 ± 2.20	49.50 ± 1.40	73.30 ± 2.20	98.40
PU	ND	49.40 ± 0.07	93.00 ± 1.40	95.95	94.75 ± 0.10	187.50 ± 7.70	283.40 ± 6.10	96.40
CA	ND	49.00 ± 0.17	91.60 ± 0.25	94.85	7.25 ± 0.75	16.10 ± 0.30	27.50 ± 1.10	97.20
AG	ND	51.00 ± 1.10	96.30 ± 1.10	99.15	14.50 ± 1.10	23.60 ± 0.70	34.35 ± 0.85	97.90
SD	17.40 ± 0.15	65.60 ± 0.10	109.00 ± 0.85	95.10	29.50 ± 0.95	53.90 ± 1.75	76.75 ± 3.50	98.40
SM	223.70 ± 1.25	279.50 ± 1.10	317.50 ± 8.00	100.05	215.35 ± 1.30	398.20 ± 10.80	567.75 ± 11.30	94.70

[a] ND = not detected. [b] 50 μg (TY, HI, SE, OC, DO, PHE, TR, PU, CA, AG, SD, SM). [c] 100 μg (TY, HI, SE, OC, DO, PHE, TR, PU, CA, AG, SD, SM). [d] 10 μg (OC, DO, SE, HI, CA, AG); 25 μg (PHE, SD, TR); 100 μg (PU); 200 μg (SM); 500 μg (TY). [e] 20 μg (OC, DO, SE, HI, CA, AG); 50 μg (PHE, SD, TR); 200 μg (PU); 400 μg (SM); 1000 μg (TY).

Table 4. Biogenic Amine Contents (mg/kg) in Pork and Beef Meat[a]

sample	*n*	TY	HI	SE	PU	CA	SD	SM
pork meat	5	ND[b]−3.5 (0.7 ± 1.6)[b]	ND	ND	ND−0.6 (0.1 ± 0.3)	ND−0.7 (0.1 ± 0.3)	2.2−4.1 (3.0 ± 0.8)	25.5−38.6 (33.5 ± 4.1)
beef meat	5	ND	ND−1.1 (0.4 ± 0.6)	ND−2.9 (1.1 ± 1.4)	ND−1.75 (0.6 ± 0.8)	ND	1.9−4.2 (3.2 ± 0.9)	28.7−44.6 (39.8 ± 6.5)

[a] Range; values in parentheses are the mean ± standard deviation. [b] ND = not detected.

Table 5. Biogenic Amine Content (mg/kg) in Cooked Meat Products[a]

samples	TY	HI	PHE	TR	PU	CA	AG	SD	SM
cooked ham (*n* = 5)	ND[b]−11.9 (2.7 ± 5.2)	ND	ND	ND	ND−3.9 (0.9 ± 1.3)	ND−0.9 (0.5 ± 0.4)	ND−0.3 (0.05 ± 0.1)	1.7−3.0 (2.2 ± 0.5)	18.1−25.4 (21.9 ± 3.5)
mortadella (*n* = 5)	ND−66.0 (18.8 ± 27.4)	ND−4.8 (1.2 ± 2.2)	ND−1.0 (0.2 ± 0.5)	ND−1.4 (0.5 ± 0.7)	ND−3.9 (1.7 ± 1.5)	0.6−7.0 (2.2 ± 2.7)	ND−7.9 (1.75 ± 3.5)	1.9−8.9 (5.2 ± 2.8)	7.8−32.2 (18.7 ± 10.1)

[a] Range; values in parentheses are the mean ± standard deviation. [b] ND = not detected.

and Tiecco et al. (1985) who found DO in some samples of ground beef and Italian salami, respectively. SE was only present in two samples of beef meat and at low levels (1.5 and 2.9 mg/kg). Only Tiecco et al. (1985) reported SE in some samples of salami. PHE was not detected in meat and cooked ham samples and only was found in a sample of mortadella. However, this amine was detected in 80% of samples of ripened meat products ranging between not detected and 35.00 mg/kg. Therefore, this fact could indicate a relationship between PHE and the ripening process.

The polyamines SD and SM were the only amines found in all tested samples, and SM levels were always much higher than those of SD. No remarkable differences for polyamine concentrations between the pork and beef meat and between cooked ham and mortadella were found. Levels of SD were very similar in pork meat (3.0 ± 0.8 mg/kg), in beef meat (3.2 ± 0.9 mg/kg), and in ripened (3.8 ± 2.1 mg/kg), and in cooked meat products (3.70 ± 2.50 mg/kg). SM levels in pork meat (33.5 ± 4.1 mg/kg) and beef meat (39.80 ± 6.50 mg/kg) were higher than those in ripened and cooked meat products (18.0 ± 0.8 and 20.3 ± 7.3 mg/kg, respectively). SM and SD have been found as naturally occurring amines in fresh and pork beef meat (Maijala and Eerola, 1993; Szerdahely et al., 1994; Halász et al., 1994). Therefore, their presence in meat products could be due to the meat employed as the main raw material. The lower levels observed in processed products would be due to the dilution involved in their preparation, with other ingredients. AG, another polyamine, was not detected either in fresh meat or in chorizo. Levels of AG in mortadella and salchichón were very similar (between not detected and 8.0 mg/kg), while in cooked

Table 6. Biogenic Amine Content (mg/kg) in Ripened Meat Products[a]

sample	TY	HI	PHE	TR	PU	CA	AG	SD	SM
chorizo	76.5−477.8	ND[b]−108.3	ND−7.7	5.7−65.1	31.6−361.9	3.9−34.9	ND	1.4−7.9	15.4−37.8
($n = 5$)	(324.6 ± 159.5)	(34.8 ± 44.4)	(2.1 ± 3.3)	(39.8 ± 23.8)	(156.1 ± 123.9)	(20.0 ± 11.3)		(4.4 ± 3.0)	(22.4 ± 9.9)
salchichón	67.5−465.2	2.35−87.3	2.7−34.7	12.9−47.4	85.9−184.5	2.1−68.5	ND−7.7	2.5−3.9	8.9−19.4
($n = 5$)	(300.1 ± 148.9)	(25.0 ± 35.3)	(18.6 ± 12.3)	(28.2 ± 12.7)	(134.4 ± 45.4)	(25.3 ± 28.6)	(1.6 ± 3.4)	(3.2 ± 0.7)	(13.7 ± 5.2)

[a] Range; values in parentheses are the mean ± standard deviations. [b] ND; not detected.

ham they were clearly lower (between not detected and 0.3 mg/kg). No information is available about the origin and significance of this amine in meat products; however, we have verified its formation during the early steps of fish spoilage (Veciana-Nogués, 1993).

TR was not found in any sample of fresh meat and cooked ham. In ripened meat products, TR was detected in all samples with an average value of 34.0 ± 18.9 mg/kg. TR was also detected in two samples of mortadella but at really low levels (0.8 and 1.4 mg/kg). Therefore, TR is only significantly associated with ripened products.

TY, HI, PU, and CA were the amines that showed more differences among fresh meat and cooked and ripened products. In fresh meat, TY and CA were only detected in one sample of pork meat, and HI was found in two samples of beef meat and PU in one pork meat sample and in two beef meat samples. However, levels of these amines were always very low (<4 mg/kg). TY and PU were found in 70% of cooked meat products; CA was found in 90% and HI in 30% of the samples. The average level of TY was relatively high (18.8 ± 27.4 mg/kg) in mortadella, while CA, PU, and HI levels were below 8 mg/kg. The presence of these amines in cooked meat products would be related with the use of low hygienic quality meat (main raw material), since cooked meat products elaboration does not include any step where biogenic amine formation could be expected.

In chorizo and salchichón, TY, PU, and CA were always detected, while HI was found in 90% of samples. TY was the prevailing amine although its levels widely fluctuated, from 67.5 to 477.8 mg/kg. PU was also found at high levels in chorizo (156.1 ± 123.9 mg/kg) and in salchichón (134.4 ± 45.4 mg/kg). Average values of CA and HI were alike in both types of ripened products, 22.7 ± 20.7 mg/kg for CA and 29.9 ± 38.1 mg/kg for HI. Similar or even higher levels of these amines have been found by other authors in other fermented sausages from different countries (Vandekerckove, 1977; Tiecco et al., 1985; ten Brink et al., 1990). Only data about TY and HI in chorizo and salchichón have been previously reported (Santos-Buelga et al., 1981; Vidal-Carou et al., 1990b), and our data are in agreement with those reported.

It seems accepted that TY, PU, CA, and HI can be formed during the fermentation involved in the ripened meat products elaboration (Bauer et al., 1989; ten Brink et al., 1990; Maijala and Eerola, 1993). Nevertheless, it is not still clear what microorganisms are implicated in biogenic amine formation. The fermentative microflora and/or natural or contaminant microorganisms present in the meat employed as raw material could be related to amine formation. On the other hand, HI, TY, CA, and PU are the biogenic amines usually related with fish and meat spoilage (Taylor, 1986; Sayem-el-Daher et al., 1984).

From a public health point of view, the biogenic amine levels found in fresh meat and cooked products seemed to be low to produce direct or indirect toxicological effects. However, the relatively high amine levels found in cured meat products corroborate the exclusion of those products in the MAOI diets. Furthermore, some cured meat products showed histamine levels close to the threshold value of 10 mg/100 g of food reported as the value to induce histaminic intoxication symptons (Stratton et al., 1991).

The proposed method allows a complete pattern of biogenic amines (aromatic, diamines, and polyamines)

in a single run. The simple preparation of the sample and the automatic derivatization of the amines considerably reduce time and effort. Furthermore, the method can be applied to fresh meat as well as to cooked and ripened meat products.

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