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Determination of biogenic amines in fresh and processed meat by suppressed ion chromatography-mass spectrometry using a cation-exchange column

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Abstract

A new method for simultaneous determination of underivatized biogenic amines based on the separation by cation-exchange chromatography and suppressed conductivity coupled with mass spectrometry detection has been developed. The method has been applied to the analysis of cadaverine, putrescine, histamine, agmatine, phenethylamine and spermidine in processed meat products. The amines were extracted from muscle tissue with methanesulfonic acid without any additional derivative step or sample clean-up. Biogenic amines were separated by the IonPac CS17 column, a cation-exchange column used with gradient elution, and detection was done by suppressed conductivity and mass spectrometry. Tyramine was simultaneously analysed by using a spectrophotometer (275 nm) before the suppressed conductivity detection. Linearity of response was obtained in the range 0.25–25 $\mu g\,mL^{-1}$. The detection limits ranged from 23 $\mu g\,L^{-1}$ for putrescine to 155 $\mu g\,L^{-1}$ for spermidine (suppressed conductivity) and from 9 $\mu g\,L^{-1}$ for agmatine to 34 $\mu g\,L^{-1}$ for spermidine (MS). Average recoveries from meat samples ranged from 85 to 97% and coefficients of variation ranged from 4.5 to 9.7%. The analysis of biogenic amines in fresh and processed meats (dry-cured, cooked and fermented products) can be used as a quality marker of raw material and for studying the relationship between their changes and the fermentation process involved in dry sausage ripening.

Keywords: Biogenic amines; Cation-exchange chromatography; IC-MS; Dry-cured meat; Fermented meat products

1. Introduction

Biogenic amines (cadaverine, putrescine, spermidine, histamine, phenethylamine, agmatine and tyramine) are organic compounds present in living organisms at low levels where they are responsible for many essential functions. Their occurrence in food, especially in fish, cheese and meat products, varies by a great extent depending on technological processes and microbial factors. In fact their generation mainly depends on the actions of microbial decarboxylases and in a minor role on the endogenous amino acid decarboxylase activities.

Biogenic amines in food and meat products are related both to food spoilage and food safety. Their presence is of health concern in meat products because of their toxicological implication [1–5] and of their role as potential quality indicators.

The accumulation in foods of aromatic amines such as histamine, tyramine and phenethylamine are undesirable due to their vasoactive and/or psychoactive effects [6,7]. Although other biogenic amines are much less toxic than histamine and tyramine, secondary amines such as cadaverine and putrescine could undergo nitrosation reactions during the curing

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process and can form carcinogenic compounds, such as nitrosamines [8,9].

The determination of biogenic amines in fresh and processed foods is of great interest not only due to their toxicity, but also because they can be a useful index of spoilage or ripening. Histamine, putrescine and cadaverine have been suggested as spoilage indicators in fresh food, above all in fish and meat products, as their presence is closely related to gram negative bacteria. Tyramine is usually found in fermented and long-ripened foods, such as dry-cured sausages, where amino-decarboxylase microflora may be introduced as a starter culture, may be part of the natural population of the food, or may be coming from accidental process contamination [10–12]. During manufacturing of dry-cured and cooked meat products several technological factors, such as pH, temperature and salt concentration are key factors in the onset and the rate of amino enzymatic reactions and their synergic effect. Thus, biogenic amine profile could be an important index in quality assurance of processed meat products.

Various HPLC methods were developed for analysis of such kind of biogenic amines in foods, but due to lack of a suitable chromophore or fluorophore group for direct detection, aliphatic amines cannot be detected with the required sensitivity with common HPLC spectrophotometric detectors such as UV or fluorescence detectors. For this reason, almost all the proposed methods of detection were indirect with precolumn derivatization to form dansyl (Dns) derivatives, fluorescent derivatives, benzoyl derivatives, etc. [4,13–17]. These methods suffer from various drawbacks such as cumbersome sample preparation, by-products interference, complex instrumentation, skilled operator and/or long time of analysis.

Liquid chromatography coupled with pulsed amperometric detection (PAD) or integrated pulsed amperometric detection (IPAD) using noble-metal electrodes in alkaline media, is a sensitive and selective method for the detection [18–21], but it requires a post-column addition of a pH modifier. Suppressed conductivity was recently used for the determination of histamine in tuna [22]. Side by side with classical analytical techniques, an increasing number of methods involve the use of capillary electrophoresis [23–27].

On these premises, this paper describes the use of a cation-exchange column coupled with UV, suppressed conductivity and mass spectrometric detection in series to analyse biogenic amines in meat products (fresh and dry-cured sausage, dry cured and cooked ham). At the same time we wish to give an overview on possible detection schemes for the determination of biogenic amine profile, and noticeably the use of a MS detection as a confirmatory device. In fact, suppressed conductivity is the most used detection technique for determination of cations by IC, UV can give selectivity towards classes of molecules (aromatic for instance), MS would confirm the results obtained with those common techniques.

2. Experimental

2.1. Materials and chemicals

Methanesulfonic acid (MSA) eluent was generated by an eluent generator EG50 (Dionex, Sunnyvale, CA, USA) equipped with an EGC-MSA cartridge.

Cadaverine, putrescine, histamine, agmatine, phenethylamine, spermidine and tyramine were reagent grade (Novachimica, Cinisello, MI, Italy). Methanesulfonic acid (MSA; >99% pure) was supplied by Sigma–Aldrich (Poole, UK). Water for chromatography and solutions was purified ($18\,M\Omega\,cm^{-1}$ quality) by a Milli-Q system (Millipore, Bedford, MA, USA).

2.2. Chromatographic separation

Chromatographic analyses were performed on a DX-600 Ion Chromatograph (Dionex) equipped with GS50 Gradient Pump, LC25 column compartment, AS50 autosampler, ED50A electrochemical detector, PDA100 photodiode array detector and MSQ single quadrupole mass spectrometer. A Dionex IonPac CG17 50 mm × 2 mm guard column coupled with an IonPac CS17 2 mm × 250 mm column (macroporous 7 μm 55% cross-linked poly(ethylvinylbenzene–divinylbenzene) grafted with carboxylated functional groups) was used with methanesulfonic acid (MSA) eluent. Background conductivity was suppressed with a CSRS Ultra-II 2 mm suppressor. All measurements were made at 40 °C and all samples were filtered through 0.2 μm filters. Dionex Chromeleon 6.60 chromatography software controlled data collection and the operation of all components in the system.

2.3. MS detection

MS detection was carried out by a single stage quadrupole detector (Thermo Finnigan MSQ, Dionex). The MS was operated in the positive electrospray (ESI+) ionization mode at 3.0 kV. Probe temperature was set at 350 °C, cone voltage was 50 V.

$[Cadaverine + H]^+$ has been detected at a mass-to-charge ratio 89 *m/z*, $[putrescine + H]^+$ 103 *m/z*, $[histamine + H]^+$ 112 *m/z*, $[agmatine + H]^+$ 131 *m/z*, $[phenethylamine + H]^+$ 122 *m/z* and $[spermidine + H]^+$ 146 *m/z*. Chromatographic conditions are summarized in Table 1.

2.4. Preparation of standard solutions and samples

Biogenic amines (BA) stock solutions ($1\,mg\,mL^{-1}$) were prepared in 0.01 mol/L MSA, stored at 4 °C and protected from light. Working standard solutions for calibration were daily prepared by dilution of concentrated solutions with ultra pure water.

Biogenic amines were extracted from meat samples with $0.1\,mol\,L^{-1}$ MSA solution. To 10 g of blended meat sample,

Table 1
Chromatographic conditions for IC-MS determination of biogenic amines

Column	IonPac CG17 50 mm × 2 mm; IonPac CS17 250 mm × 2 mm	
	Time	MSA (mM)
Eluent	−7	3
	0	3
	6	3
	26	30
	40	30
Temperature (°C)	40	
Flow rate (mL min^{-1})	0.38	
Injected volume (μL)	5	
Detection	Suppressed conductivity, CSRS Ultra-II, external water mode	
	UV 276 nm	
	MS ESI+: 3.0 kV, cone 50 V, probe temperature: 400 °C, dwell time: 0.2 s	
SIM	Analyte	*m/z*
	[Putrescine + H]$^+$	89
	[Cadaverine + H]$^+$	103
	[Histamine + H]$^+$	112
	[Phenethylamine + H]$^+$	122
	[Agmatine + H]$^+$	131
	[Spermidine + H]$^+$	146

40 g of 0.1 mol L^{-1} MSA solution were added (dilution 1:5, w/w), the mixture was homogenised (14,000 rpm, 1 min) and then centrifuged at 10,000 rpm for 20 min at low temperature (4 °C) in order to separate the fat. The aqueous phase was then removed and filtered through a 0.2 μm IC-grade PTFE filter (IC-Millex, Millipore, Milan, Italy) for chromatographic analysis.

Dry cured sausages samples were diluted again with water before the chromatographic analysis (final dilution = 1:25). In order to verify the analytical performances of the method on meat matrix, several fresh and processed meat samples (dry cured, fermented and cooked) were purchased in Italian markets.

3. Results and discussion

3.1. Ion-exchange chromatography of biogenic amines

A typical chromatographic separation is shown in Fig. 1 for a standard mixture of seven amines by UV, suppressed conductivity and MS detection at all considered SIMs, using the gradient elution program described in Table 1 (concentrations relative to the LOQ of the suppressed conductivity detection). The standard mixture contains those polyamines and biogenic amines expected to be naturally found in meat products (polyamines) and also other amines that are related to microbial growth (putrescine, cadaverine, histamine and tyramine). In this case all the considered amines were separated in 40 min (total run time). It is to be stressed that tyramine cannot be detected by MS if this device follows a suppressed conductivity detector because tyramine is removed by the suppressor. Tyramine could be detected by MS only when the separator column is directly connected to the mass detector, however, in this case you should find another eluent because MSA eluent is not compatible with the mass spectrometric detector. A test with a MS compatible acid – such as formic acid – enabled tyramine detection; but this eluent did not allow a full separation of all the other amines. Furthermore the relative high concentration of formic acid (30 mM) – necessary for the tyramine elution – reduces in mass spectrometry both the sensitivity and the ionization of the analyte (tyramine).

3.2. Chromatographic performance

Repeatability, linearity and sensitivity of the method were examined. The repeatability was expressed as relative standard deviation (RSD) of peak area of each amine both in conductivity (putrescine, cadaverine, histamine, phenethylamine, agmatine and spermine) and spectrophotometric (λ = 275 nm for tyramine) detection mode and was tested by seven identical injections of the test solution (5 mg L^{-1} standard solution). The repeatability of each peak with regard to conductometric detection was between 1.2 and 3.7% and 1.3% for the tyramine peak.

Peak area was linear between 0.25 and 25 μg mL^{-1} for putrescine, cadaverine and histamine, while for phenethylamine, agmatine and spermidine it was found to be linear in the range 1–25 μg mL^{-1}. Linearity range for tyramine determination by spectrophotometry was between 1 and 25 μg mL^{-1}. Data obtained from the external calibration curves were submitted to linear regression analysis and correlation coefficients between 0.97 and 0.99 were obtained. Repeatability, linearity and detection limits of biogenic amines for a signal-to-noise ratio of 3 are reported in Table 2.

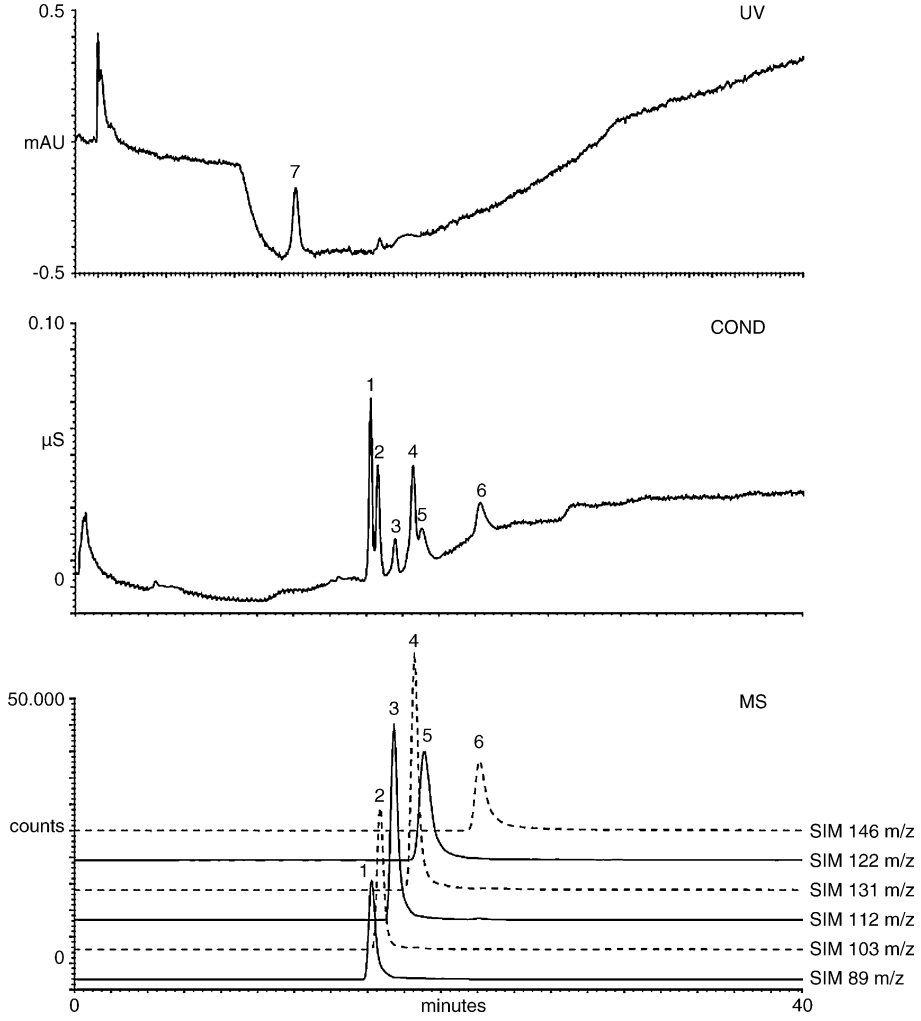

Fig. 1. Chromatograms of a standard solution of biogenic amines (0.5 mg L^{-1} each). Chromatographic conditions as in Table 1. Peaks: 1, putrescine; 2, cadaverine; 3, histamine; 4, agmatine; 5, phenetylamine; 6, spermidine; 7, tyramine.

3.3. Recovery

The recoveries of amines from fresh and processed meat were also investigated. Recovery was tested by standard addition procedure using two addition levels for each amine in fresh (1.0 and 10.0 mg kg^{-1}) and processed (10.0 and 25.0 mg kg^{-1}) meat samples. Results were summarized in Table 3.

The recovery of the method was tested using different type of meat products such as fresh meat, dry cured meat and fermented meat in order to evaluate possible interferences coming from fat substances content, non-protein nitrogenous compounds (free amino acids and low-weight peptides) and salt content. Absence of interferences in fresh and processed samples was confirmed by MS detection for all the samples. The average recoveries ranged from 84% for cadaverine in a

Table 2
Repeatability, linearity and detection limits of biogenic amines

Analyte	Area (RSD, %)[a]	Linearity range (mg L^{-1})	LOD (µg L^{-1})			LOQ (µg L^{-1})		
			UV	COND	MS	UV	COND	MS
Tyramine	1.30	1–25	135	–	–	410	–	–
Putrescine	1.20	0.25–5	–	25	22	–	70	64
Cadaverine	1.30	0.25–5	–	35	15	–	106	44
Histamine	1.50	0.25–5	–	107	11	–	323	32
Phenethylamine	2.50	1–20	–	117	9	–	350	27
Agmatine	2.50	1–20	–	123	19	–	371	58
Spermidine	3.70	1–20	–	155	33	–	465	100

[a] $n = 7$.

Table 3
Precision and recovery of the method for determination of biogenic amines in fresh and processed meat samples

Analyte[a]	Sample		Sample + 1 mg kg^{-1}		Sample + 10 mg kg^{-1}	
	Found[b]	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Raw meat						
Tyramine	2.1	4.5	90	5.9	90	5.4
Putrescine	5.3	6.3	85	7.2	88	6.4
Cadaverine	2.7	7.5	92	6.9	90	6.5
Histamine	0.2	5.2	91	5.6	91	5.0
Phenethylamine	–	–	88	7.0	89	6.7
Agmatine	–	–	93	6.7	95	7.3
Spermidine	4.2	9.7	87	8.8	85	8.9

Analyte[a]	Sample		Sample + 10 mg kg^{-1}		Sample + 25 mg kg^{-1}	
	Found[b]	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Dry cured sausage						
Tyr	143.7	4.7	95	4.8	95	4.9
Put	127.3	6.1	92	5.6	89	5.7
Cad	68.8	8.3	84	5.2	90	5.1
His	–	–	91	5.0	94	4.8
PhEth	4.4	7.4	89	6.1	92	5.8
Agm	12.5	6.3	91	6.3	95	6.1
Spermd	6.4	7.9	95	6.8	85	6.5
Dry cured ham						
Tyr	64.3	3.7	97	5.1	94	4.9
Put	12.4	5.9	92	5.8	88	4.7
Cad	4.1	6.4	86	5.4	84	5.1
His	–	–	89	5.8	93	5.1
PhEth	5.9	3.7	93	6.7	94	6.1
Agm	8.3	5.4	95	6.5	91	5.8
Spermd	9.1	8.7	83	7.1	86	6.2

[a] Tyr, tyramine; Put, putrescine; Cad, cadaverine; His, histamine; PhEth, phenethylamine; Agm, agmatine; Spermd, spermidine.
[b] Values in mg kg^{-1}.

dry-cured sausage sample to 97% for tyramine in a dry-cured ham sample. Concerning the complexity of the meat matrix, these recoveries values could be considered satisfactory both in fresh and in processed meat samples.

As already stated, putrescine, cadaverine and histamine are the most frequently monitored in meat (and food products in general) as they are indexes intrinsic or induced toxicity, therefore, we evaluated that the correlation between suppressed conductivity and mass spectrometric detection was better than 0.92.

3.4. Real samples

The IC-MS method was applied to analyse several meat samples to verify the analytical suitability of the method and to examine the biogenic amines levels in raw and processed meat products purchased from Italian retail stores. Typical chromatograms of meat samples, fresh meat, dry-cured sausages, dry-cured and cooked ham were shown in Figs. 2–4. Median values and the range of contents of biogenic amines found in several fresh and processed meat

Table 4
Level of biogenic amines (mg kg^{-1}) in Italian meat products

Analyte	Fresh pork meat, $N = 44$		Dry-cured sausage, $N = 33$		Dry-cured ham, $N = 43$		Cooked ham, $N = 18$	
	Median	Range	Median	Range	Median	Range	Median	Range
Tyramine	2	0–56	140	10–408	38	4–171	11	6–108
Putrescine	9	0–16	108	12–364	6	1–237	32	2–139
Cadaverine	1	0–7	80	10–248	1	0–5	5	1–12
Histamine	1	0–6	1	1–4	1	0–7	1	0–11
Phenethylamine	0.4	0–2	2	1–6	7	1–19	0.5	0–2
Agmatine	3	0–14	10	1–25	1	0–5	3	1–15
Spermidine	3	0–37	5	2–35	3	1–35	5	1–18

Median and range (min–max) of each kind of meat were reported.

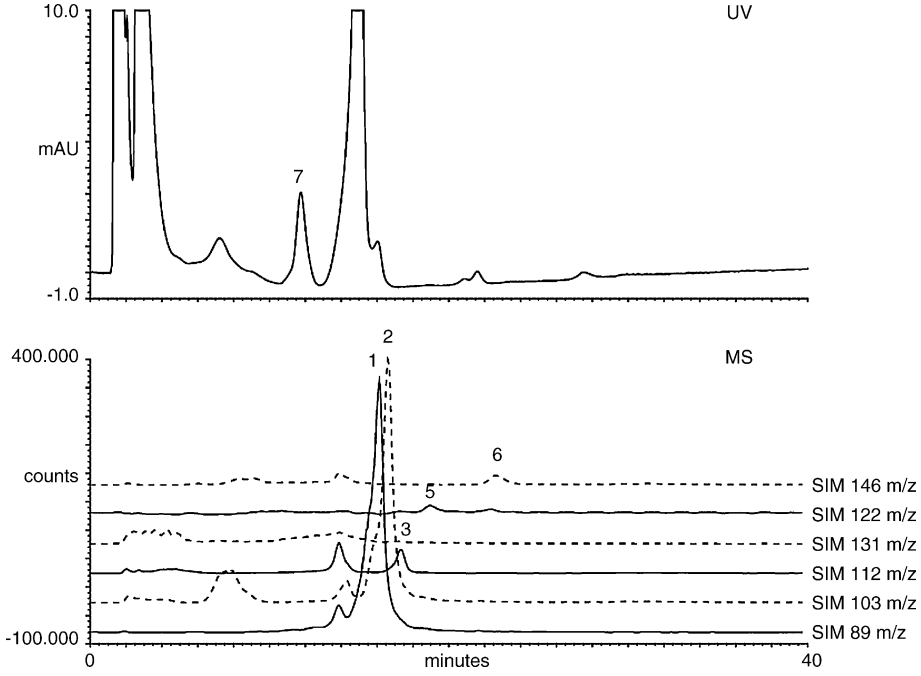

Fig. 2. Chromatograms of a dry-cured sausage sample. Chromatographic conditions as in Table 1. Peaks: 1, putrescine (207 mg kg^{-1}); 2, cadaverine (128 mg kg^{-1}); 3, histamine (5.6 mg kg^{-1}); 5, phenethylamine (2.9 mg kg^{-1}); 6, spermidine (4.3 mg kg^{-1}); 7, tyramine (123 mg kg^{-1}).

samples collected during a screening in Italian market retails are shown in Table 4.

Biogenic amines content in meat samples varies by a great extent, depending on manufacturing process (biogenic amines in processed food were commonly higher than in fresh samples) and on microbial factors. Generally, higher concentrations of all the amines are observed in dry-cured sausages, as expected, because during the fermentation step the microbial strains (added as starter cultures or naturally selected from endogenous populations) can sustain the decarboxylase activities [28]. Tyramine, putrescine and cadaverine were the most important amines found in fermented meat samples, ranging from 10 to 408 mg kg^{-1} for tyramine, from 12 to 364 mg kg^{-1} for putrescine and from 10 to 248 mg kg^{-1} for cadaverine, although in variable amounts depending on the samples. The variability of amine contents in meat samples, in

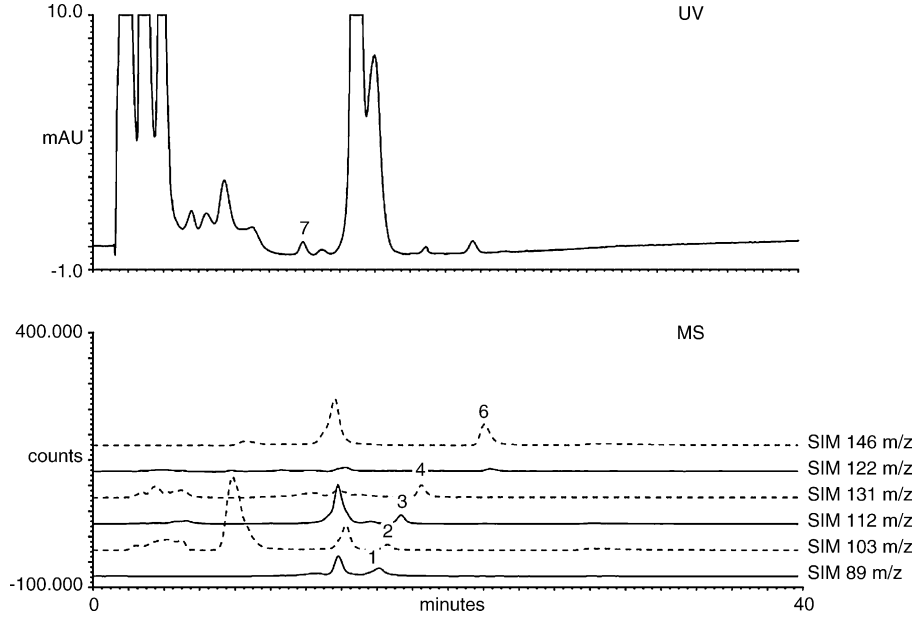

Fig. 3. Chromatograms of a dry-cured ham sample. Chromatographic conditions as in Table 1. Peaks: 1, putrescine (5.5 mg kg^{-1}); 2, cadaverine (2.0 mg kg^{-1}); 3, histamine (2.1 mg kg^{-1}); 4, agmatine (4.9 mg kg^{-1}); 6, spermidine (7.7 mg kg^{-1}); 7, tyramine (13.7 mg kg^{-1}).

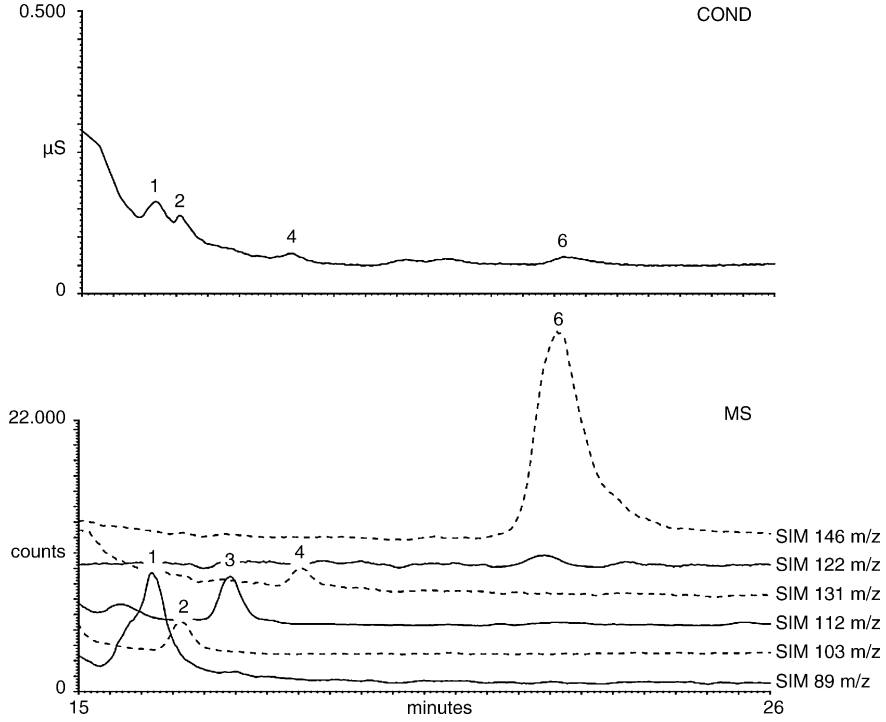

Fig. 4. Chromatograms of a cooked ham sample. Chromatographic conditions as in Table 1. Peaks: 1, putrescine (2.5 mg kg^{-1}); 2, cadaverine (0.38 mg kg^{-1}); 3, histamine (0.31 mg kg^{-1}); 4, agmatine (0.07 mg kg^{-1}); 6, spermidine (4.2 mg kg^{-1}).

particular in fermented and dry-cured sausages, could be explained on the basis of the technological process, raw material hygienic quality and contaminations accidentally occurring during the process [29–31].

Biogenic amine amounts in dry-cured (Fig. 3) and cooked ham (Fig. 4) are generally much lower than in fermented sausage as well as their microbial contamination is generally more controlled. Putrescine, cadaverine and histamine were present at low levels or even absent, however, noticeable level of tyramine in dry-cured ham was observed [13,32–34]. In Fig. 4 (conductivity detector), a small amount of agmatine (peak 4), closely related to microbial contamination [35], in a cooked ham sample was observed, also confirmed by MS detector. As expected small amount of spermidine was found in all the meat samples both fresh and processed.

Data resulting from the screening was in agreement with others and show that the biogenic amine content depends on the manufacturing process and microflora growth. Dry-cured sausage manufacturing (fermentation step is needed) affects biogenic amines occurrence both during the fermentation step and ripening time, while cooking manufacturing inhibits most of the microbial growth after pasteurization. Thus, biogenic amines detected in cooked meat products could be coming from an earlier spoilage.

4. Conclusions

The proposed method for direct determination of cadaverine, putrescine, histamine, agmatine, phenethylamine, spermidine and tyramine in meat products by cation-exchange separation coupled with spectrophotometric, suppressed conductivity and mass spectrometric detection avoids the need of long and cumbersome derivatization procedures and improves selectivity in real samples with good reproducibility and recoveries. In particular, fat content and non-protein nitrogenous substances (low weight peptides and free amino acids) do not interfere with the determination of the mentioned amines. The proposed method provides a number of advantages for the amount of information obtained for biogenic amines in a single run, including the application to a larger number of analytes, simple extraction procedure and clean-up, elution at low acid concentration, improved chromatographic separation, long term stability of MS signal thanks to the use of a suppressor device before the MS ionization.

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