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Determination of volatile nitrosamines in meat products by microwave-assisted extraction and dispersive liquid–liquid microextraction coupled to gas chromatography–mass spectrometry

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ABSTRACT

Microwave-assisted extraction (MAE) and dispersive liquid–liquid microextraction (DLLME) coupled with gas chromatography–mass spectrometry (GC–MS) were evaluated for use in the extraction and preconcentration of volatile nitrosamines in meat products. Parameters affecting MAE, such as the extraction solvent used, and DLLME, including the nature and volume of the extracting and disperser solvents, extraction time, salt addition and centrifugation time, were optimized. In the MAE method, 0.25 g of sample mass was extracted in 10 mL NaOH (0.05 M) in a closed-vessel system. For DLLME, 1.5 mL of methanol (disperser solvent) containing 20 μL of carbon tetrachloride (extraction solvent) was rapidly injected by syringe into 5 mL of the sample extract solution (previously adjusted to pH 6), thereby forming a cloudy solution. Phase separation was performed by centrifugation, and a volume of 3 μL of the sedimented phase was analyzed by GC–MS. The enrichment factors provided by DLLME varied from 220 to 342 for N-nitrosodiethylamine and N-nitrosopiperidine, respectively. The matrix effect was evaluated for different samples, and it was concluded that sample quantification can be carried out by aqueous calibration. Under the optimized conditions, detection limits ranged from 0.003 to 0.014 ng mL^{-1} for NPIP and NMEA, respectively (0.12–0.56 ng g^{-1} in the meat products).

1. Introduction

N-nitrosamines (NAms) are considered to be carcinogens and mutagenics [1,2]. In the diet, they occur as unintentional by-products of food preparation, preservation and processing [3,4], although they can also occur in the environment, and may even be formed endogenously within the human body [5]. These compounds are formed by the reaction of secondary amines with nitrosating agents, such as nitrates or nitrites, which are commonly used in the manufacture of meat products [6]. For this reason, the use of these nitrosating agents for curing meat is strictly controlled in some countries [6]. Moreover, some known inhibitors of the nitrosation reaction, such as ascorbic acid and α-tocopherol, are used in the processing of several foods [3,7]. Food irradiation at the appropriate dose has proved to be a safe way of achieving safety in this respect by reducing the NAm contents [8–10]. The meat industry produces a wide variety of products and their safety must be carefully monitored to preserve human health. The tolerance level of human exposure to the most volatile NAms has been stated to be in the range 5–10 μg kg^{-1} body weight. Indeed, a maximum level of 10 μg kg^{-1} for N-nitrosopyrrolidine (NPYR) in retail products [11] has been set in the USA and the same level for N-nitrosodimethylamine (NDMA) in Canada [12]. Whatever the case, human exposure to NAms should be reduced to the greatest extent possible and sensitive analytical methods for their determination in essential foods, including meat products, are always welcome.

The relative non-polarity, low molecular weight and sufficient vapour pressure of volatile NAms mean they can be analyzed by gas chromatography (GC) without any derivatization step prior to chromatographic separation. For this reason, GC coupled to different detection systems is the most widely used analytical technique for this purpose [13]. Though nitrogen–phosphorus detection (NPD) is specific for compounds containing nitrogen in their molecules [14,15], thermal energy analysis (TEA) has been widely used owing to its sensitivity and selectivity [8–10,12,16–21]. Nevertheless, due to its limited versatility and relatively high cost, this detector is not available in most laboratories. Mass spectrometry (MS) has also been widely coupled to GC, achieving very good sensitivity as well as unequivocal identification of the analytes [11,16–27].

The complex matrix of food samples and the low concentrations expected of the studied analytes make it necessary to include isolation and preconcentration steps in the analytical procedure. Distillation [16,21] and solvent extraction in relatively low polarity organic solvents [17], as well as the combination of both techniques

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[8,18,27], as is the case of the Official Method of analysis [28], are classical methods of NAms analyses. Solid-phase extraction has also been used [9,13,19,20,22,23,25], and has shown several advantages over the more classic extraction/preconcentration techniques. However this still requires large amounts of organic solvents, while supercritical fluid extraction (SFE) [29,30] and solid-phase microextraction (SPME) [11,12,24,26] do not. Dispersive liquid–liquid microextraction (DLLME) is a very simple and rapid technique, which has been applied for the extraction and preconcentration of both organic and inorganic compounds from aqueous samples [31–33], among its advantages over SPME being the absence of cross-memory effects and the fact the analyses take less time. As far as we know, DLLME has not, to date, been applied to NAm analyses. The use of microwaves for extraction of both organic and inorganic compounds from complex matrices is well documented [34,35]. Extraction of the analytes from the solid food matrices by means of microwave assisted extraction (MAE) in a closed vessel system is evaluated in this contribution as an alternative to the time-consuming methods, such as distillation, proposed in the literature.

The present study describes a new method for the sensitive determination of nine volatile N-nitrosamines in meat products by coupling MAE–DLLME–GC–MS: N-nitrosodimethylamine (NDMA), N-nitrosomethylethylamine (NMEA), N-nitrosodiethylamine (NDEA), N-nitrosopyrrolidine (NPYR), N-nitrosomorpholine (NMOR), N-nitrosodi-n-propylamine (NDPA), N-nitrosopiperidine (NPIP), N-nitrosodibutylamine (NDBA) and N-nitrosodiphenylamine (NDPheA).

2. Experimental

2.1. Chemicals

A nitrosamine standard mixture in dichloromethane (2000 μg mL^{-1}), containing NDMA, NMEA, NDEA, NPYR, NDPA, NPIP and NDBA was provided by Sigma (St. Louis, MO, USA). NMOR and N-DPheA were obtained individually as methanollic solutions of 5000 μg mL^{-1} from Supelco (Bellefonte, PA, USA). Diluted solutions (100 μg mL^{-1}) were prepared in methanol and stored at 4 °C in the dark. Working standard solutions were prepared daily in methanol and stored at 4 °C. Chloroform ($CHCl_3$), carbon tetrachloride (CCl_4), dichloromethane (CH_2Cl_2), 1,1,2,2-tetrachloroethane ($C_2H_2Cl_4$), acetone, methanol and acetonitrile were all of analytical grade and provided by LabScan (Dublin, Ireland). Sodium chloride, sodium hydroxide, sodium dihydrogen phosphate, phosphoric acid (85%, w/v) and hydrochloric acid (37%, w/v) were purchased from Sigma. The water used was previously purified in a Milli-Q system (Millipore, Bedford, MA, USA). The carrier gas used for GC was helium (Air Liquide, Madrid, Spain).

2.2. Instrumentation

GC analyses were performed on an Agilent 6890N (Agilent, Waldbronn, Germany) gas chromatograph coupled to an Agilent 5973 quadrupole mass selective spectrometer equipped with an inert ion source and provided with a split–splitless injection port. The helium carrier gas was maintained at a constant flow of 0.5 mL min^{-1}. An HP5MS UI (5% diphenyl 95% dimethylpolysiloxane, Agilent) capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness) was used. Injection volumes of 3 μL were used. The injection port was held at 230 °C and used in the splitless mode, applying a pressure pulse of 40 psi. The GC temperature was programmed as follows: start temperature of 70 °C (held 3 min) and increase to 140 °C at 15 °C min^{-1}, then to 200 °C at 5 °C min^{-1} and finally to 250 at 10 °C min^{-1}. The total analysis time for one GC run was 25 min. Ionization was carried out in the electron-impact (EI) mode (70 eV). The electron multiplier voltage was set automatically. The temperatures of the ion source and the transfer line were 230 and 325 °C, respectively. The identification of the compounds was confirmed by injection of pure standards and comparison of their retention index and relevant MS-spectra. The analytes were quantified in the selected ion monitoring (SIM) mode using the target ion and three qualifier ions. Identification was confirmed by the retention time of the target ion and the qualifier-to-target ion ratios (Table 1).

Table 1
Retention times and target and qualifier ions for the NAms.

Compound	t_R (min)	T	Q_1 (Q_1/T%)	Q_2 (Q_2/T%)	Q_3 (Q_3/T%)
NDMA	4.29	74	42 (90)	57 (83)	102 (10)
NMEA	6.50	88	57 (90)	86 (30)	102 (10)
NDEA	10.11	102	43 (90)	44 (25)	84 (23)
NPYR	10.97	100	86 (48)	84 (10)	42 (5)
NMOR	12.54	56	84 (86)	116 (20)	130 (16)
NDPA	13.49	130	84 (45)	86 (25)	42 (18)
NPIP	14.19	114	84 (50)	86 (12)	42 (7)
NDBA	19.20	84	57 (66)	121 (24)	141 (20)
NDPheA	20.21	168	169 (91)	167 (60)	149 (19)

An IKA A11 grinder (IKA, Staufen, Germany) was used for homogenizing some meat products. A microwave extraction labstation (Ethos Sel, Milestone, USA) provided with two magnetrons of 800 W, high pressure rotor with capacity for twelve closed vessels and automatic temperature sensor ATC-400, was used for the MAE stage. An EBA 20 (Hettich, Tuttlingen, Germany) centrifuge was used at the maximum speed recommended for the conical glass tubes, 5000 rpm.

2.3. Samples and analytical procedure

A total of twenty one meat products were obtained from a local supermarket: two canned samples (chicken meatballs and pork lean), eight cured meats vacuum plastic packed (sweet pork ham, cured pork ham, turkey ham, pork back, mortadella, mortadella with olives and Frankfurt sausages of two different trade marks), three samples of pâté glass packed (one turkey pâté and two samples of foie-gras pâté) and four types of stuffed meat (Majorcan sausage, two samples of dry cured sausage, blood sausage and four types of dry cured sausage seasoned with paprika). An aliquot of about 50 g of the minced sample was homogenized in the IKA-A11, except for the pâté and Majorcan sausage samples, whose texture was already considered homogeneous. Samples were stored in the fridge until analysis.

For the MAE step, the sample (about 0.25 g) was mixed with 10 mL of NaOH 0.05 M in the PTFE vessel and, once tightly closed, submitted to an oven programme consisting of heating to 120 °C in 5 min, a temperature which was held for 1 h. The mixture obtained was centrifuged at 5000 rpm for 3 min and the supernatant was made up to 10 mL in a calibrated flask. Two aliquots of each sample were separately submitted to MAE. For the DLLME, an aliquot of 5 mL of the extract obtained from MAE was placed in a glass centrifuge tube with conical bottom containing 0.6 g NaCl, and neutralized by adding 40 μL of HCl (5 M) before adding 0.5 mL of a phosphate buffer solution (0.1 M, pH 5). A total of 1.5 mL methanol (as dispersive solvent) containing 20 μL carbon tetrachloride was injected rapidly into the sample solution using a syringe, and the mixture was again gently shaken manually for a few seconds. A cloudy solution consisting of very fine droplets of CCl_4 dispersed throughout the sample solution was formed and the NAms were extracted into the fine droplets. After centrifugation for 5 min at 5000 rpm, the extraction solvent was sedimented in the bottom of the conical tube. The sedimented phase (volume about 17 μL) was

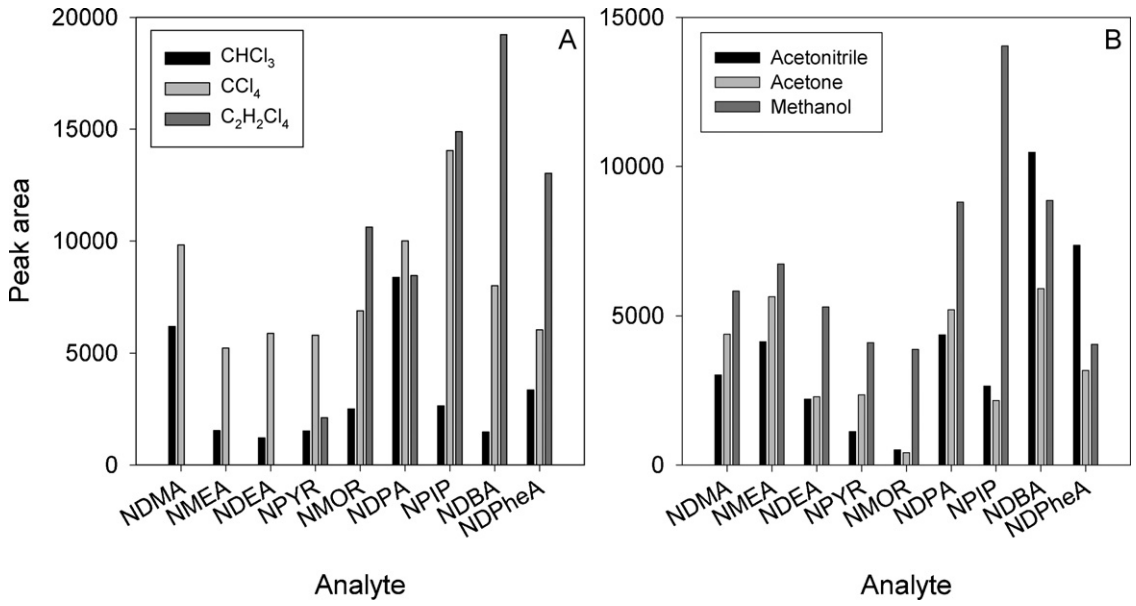

Fig. 1. Effect of the type of (A) extraction solvent and (B) disperser solvent on the sensitivity of the NAms by DLLME–GC–MS. Extraction conditions: aqueous phase volume, 5 mL; extraction solvent volume, 30 μL; disperser solvent volume, 0.5 mL; concentration of each compound, 20 ng mL^{-1}.

transferred to a 100 μL eppendorf and a 3 μL aliquot was injected in the splitless mode into the GC–MS by means of a microsyringe.

2.4. Recovery assays

Since no reference materials were available, spiked samples (pork lean, sausage and mortadella) were prepared at two concentration levels, 5 and 25 ng g^{-1}. The spiked samples were set aside for 60 min in the closed vessels and submitted to the above described extraction and preconcentration procedures. Samples were analyzed in duplicate.

3. Results and discussion

3.1. Chromatographic and detection parameters

Optimal separation conditions were obtained using an ultra inert HP-5MS capillary column. NDMA eluted when the oven temperature was increased from 70 to 140 °C, NMEA, NDEA, NPYR, NMOR, NDPA and NPIP when the temperature was increased to 200 °C, and finally NDBA and NDPheA were eluted in the last ramp temperature. The helium gas flow rate was varied between 0.5 and 4 mL min^{-1} and best results were obtained at a constant flow-rate of 0.5 mL min^{-1}. When the influence of the injection volume was studied between 1 and 4 μL in the splitless mode, sensitivity increased with the injection volume but no significant differences were attained between 3 and 4 μL. Therefore 3 μL was selected as the injection volume. Generally, when high volumes are to be injected in the splitless mode, a pressure pulse is applied during the injection to improve sensitivity and repeatability since, in this way the sample is introduced more rapidly into the column. In this case, the best results were obtained applying 40 psi pressure pulse for 1 min at the beginning of the injection. The effect of the injection temperature was studied between 200 and 300 °C. No significant differences were observed between 230 and 270 °C for most compounds, and so 230 °C was the value adopted. Table 1 shows the retention times, as well as the target and the three qualifier ions, selected for the nine NAms studied in the chromatographic conditions finally used in the SIM mode.

3.2. DLLME parameters

The parameters affecting the DLLME procedure – the extraction and disperser solvents, as well as their volumes, the addition of salt and the centrifugation time – were optimized. For this purpose, 5 mL of an aqueous solution containing analyte concentrations of about 20 ng mL^{-1} was used and 3 μL of the settled phase was injected into the GC.

The correct selection of the extraction solvent to be used must take into account several properties: higher density than water, high extraction capability, low solubility in water and good chromatographic behaviour. Bearing these factors in mind, carbon tetrachloride (CCl_4), chloroform ($CHCl_3$), dichloromethane (CH_2Cl_2) and 1,1,2,2-tetrachlorethane ($C_2H_2Cl_4$) were examined using 30 μL of the extraction solvent and 0.5 mL methanol as the disperser solvent. The high solubility of dichloromethane in water (13 g L^{-1} at 20 °C) prevented the sedimented phase from being discernible, as has been previously reported [36]. The sedimented phase was discernible with $C_2H_2Cl_4$, but this solvent provided a wide chromatographic peak which masked NDMA, NMEA and NDEA. Fig. 1A shows the results obtained when using 1,1,2,2-tetrachlorethane, chloroform and carbon tetrachloride, the best extraction results being obtained when carbon tetrachloride (solubility in water at room temperature is below 1.0 g L^{-1}) was used as extraction solvent. The main parameter to bear in mind when selecting the disperser solvent is its miscibility in the extraction solvent and the aqueous phase. Acetonitrile, methanol and acetone have this property, and were tested in this study using 0.5 mL of each one and 30 μL of carbon tetrachloride as the extraction solvent. As shown in Fig. 1B, methanol provided the best sensitivity for all compounds, except for the two least volatile NAms, and was therefore selected.

The influence of the carbon tetrachloride volume was studied in the 5–50 μL range. Peak areas increased for all compounds with increasing extraction solvent in the range 5–20 μL (Fig. 2A). On further increasing the volume of extraction solvent peak areas decreased as a consequence of dilution, and so 20 μL was selected (the volume of the sedimented phase was 17 ± 1 μL after extraction and centrifugation). The volumes assayed for the disperser solvent were in the range 50–2000 μL. According to Fig. 2B, the extraction

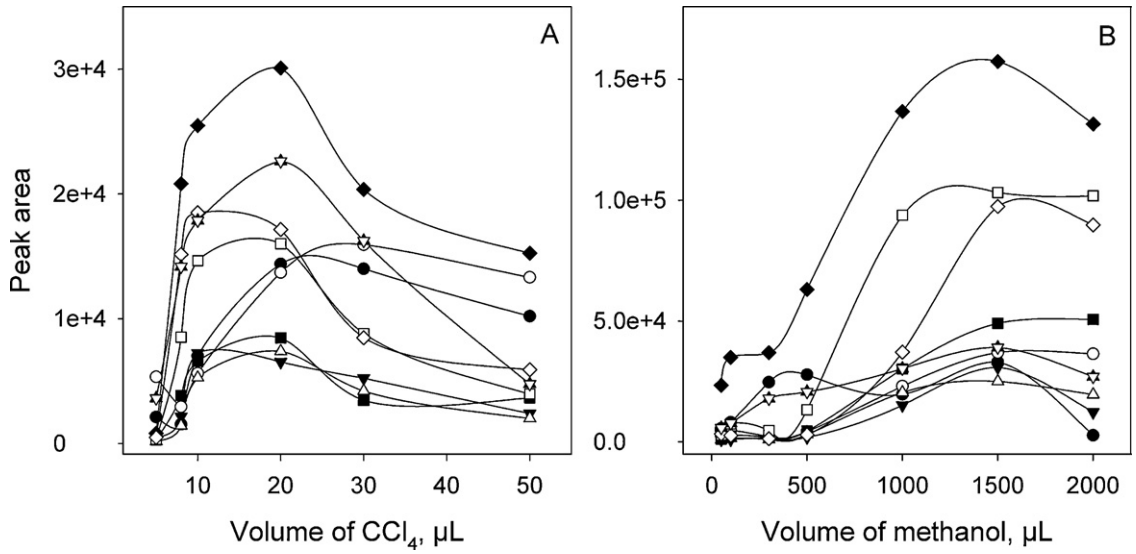

Fig. 2. Influence of the volume of (A) carbon tetrachloride and (B) methanol on the peak area of the NAms by DLLME–GC–MS. Extraction conditions: aqueous phase, 5 mL; concentration of each compound, 20 ng mL^{-1}. Symbols correspond to: NDMA (●), NMEA (○), NDEA (▼), NPYR (△), NMOR (■), NDPA (□), NPIP (♦), NDBA (◊) and NDPheA (▽).

efficiency increased up to 1000–1500 μL and then decreased with increasing volumes of methanol. At a low volume of disperser, the cloudy state was hardly reached, meaning that extraction recovery was low. At higher volumes of methanol, the solubility of the NAms probably increase in water, and so the extraction efficiency decreases. Highest sensitivity was attained for all the NAms when 1.5 mL of methanol was injected into 5 mL of the aqueous solution (Fig. 2B).

Sodium chloride was added to the aqueous phase to increase its ionic strength. This can reduce the solubility of the NAms, resulting in greater solubility in the organic phase. The effect of the amount of sodium chloride on extraction efficiency was studied between 0 and 24% (w/v); higher concentrations were not assayed because they would have prevented the sedimented phase from being collected. Peak area increased for all the compounds with increasing salt concentration because the solubility of the NAMs decreased in the aqueous phase, reaching a maximum at 12% (w/v) salt concentration, above which the area decreased or remained constant (Fig. 3A). The decrease for several compounds at high proportions of salts can be attributed to the fact that their mass transference kinetics slowed down owing to the higher viscosity of the aqueous phase. Therefore, 12% (w/v) was adopted for subsequent experiments. Compounds may be present in different forms when the pH of the sample solution was varied and, consequently, in most cases, sample enrichment will be influenced by the acidity. The effect of the aqueous phase pH was optimized over the range 2–8 by adding 0.5 mL of 0.1 M phosphate buffer solution. As can be observed in Fig. 3B, the best results were obtained at pH 6, this value being selected.

Extraction time in DLLME, defined as the interval between injecting the mixture of disperser and extraction solvents and before starting the centrifugation step, had no influence on extraction efficiency, which is one of the most important advantages of this technique. Nevertheless, the centrifugation time and speed,

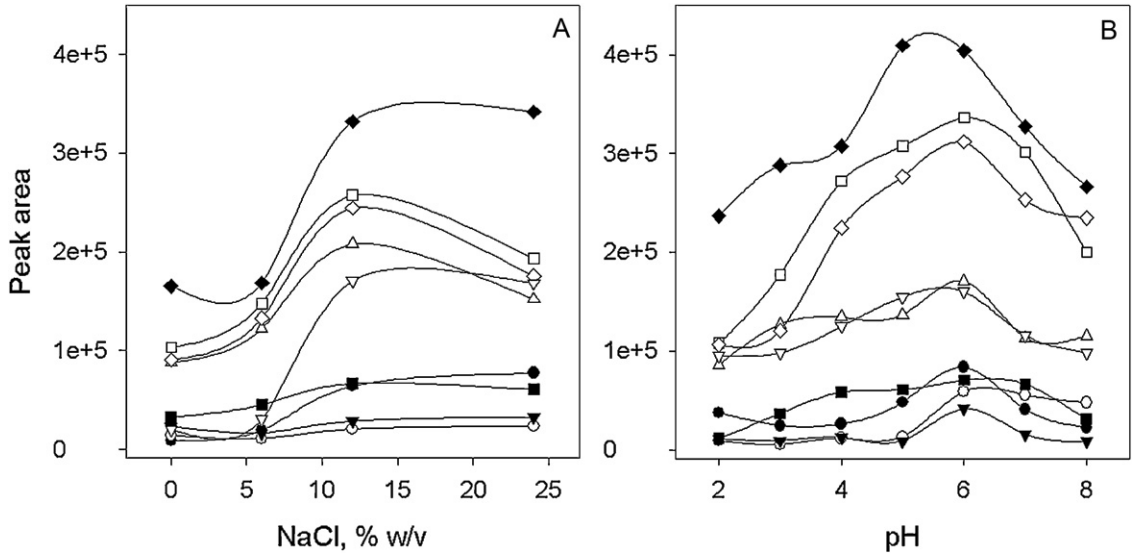

Fig. 3. Influence of (A) the salt concentration and (B) the pH of the aqueous phase on the sensitivity obtained by DLLME–GC–MS. Extraction conditions: aqueous phase, 5 mL; extraction solvent (CCl_4), 20 μL; disperser solvent (methanol), 1.5 mL; concentration of each compound, 20 ng mL^{-1}. Symbols correspond to: NDMA (●), NMEA (○), NDEA (▼), NPYR (△), NMOR (■), NDPA (□), NPIP (♦), NDBA (◊) and NDPheA (▽).

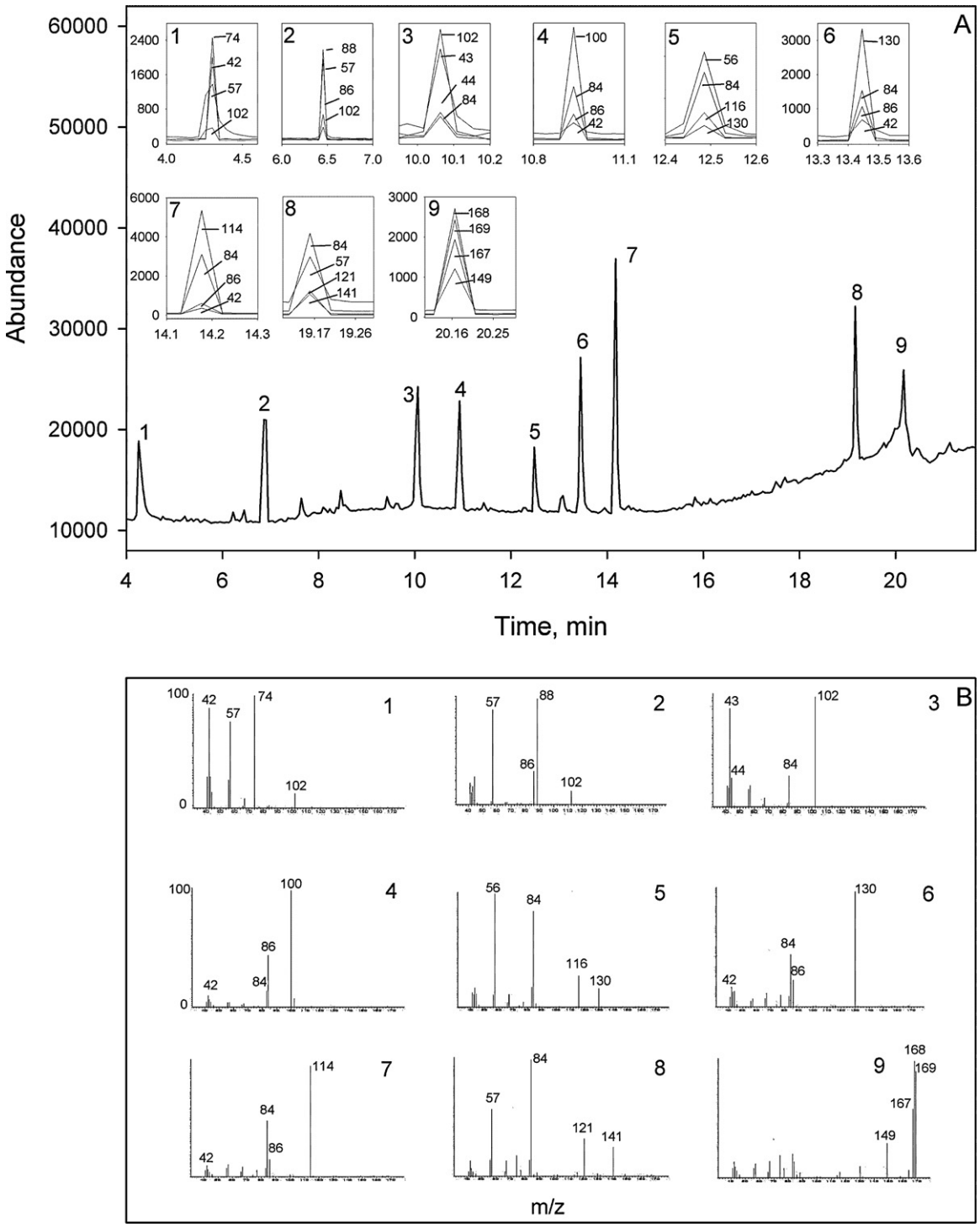

Fig. 4. (A) Elution profile obtained from an aqueous standard solution by DLLME–GC–MS and extracted ion chromatograms. Peaks correspond to: 1, NDMA; 2, NMEA; 3, NDEA; 4, NPYR; 5, NMOR; 6, NDPA; 7, NPIP; 8, NDBA; 9, NDPheA. Concentration of the NAms, 20 ng mL^{-1}. (B) Mass spectra for each analyte.

necessary to disrupt the cloudy solution and collect the sedimented phase, were evaluated in the ranges 1–10 min and 500–5000 rpm, respectively. Best results were attained by centrifuging the mixture for 6 min at the maximum speed recommended for the glass conic tubes used, 5000 rpm.

Fig. 4A shows the elution profile obtained using DLLME–GC–MS in SIM mode for a standard solution of the NAms in the selected conditions, as well as the chromatograms of the extracted ions for each one of the nine analytes. The mass spectra appear in Fig. 4B.

3.3. MAE step

Preliminary experiments were carried out to develop a simpler extraction procedure than that described in the Official Method [28], which is based on vacuum distillation for NAms in fried bacon; for this, a 0.25 g sample of a homogenized meat product was submitted to a microwave oven program in alkaline medium. The program temperature applied consisted of increasing the temperature from ambient to 120 °C in 5 min and holding for 1 h. When 10 mL NaOH (0.2 M) were added to two different samples fortified

Table 2
Effect of the NaOH concentration in the NAms extraction from meat products.

Compound	Recovery percentage, %			
	Pork lean		Chicken meatballs	
	NaOH 0.2 M	NaOH 0.05 M	NaOH 0.2 M	NaOH 0.05 M
NDMA	54	99	66	90
NMEA	69	95	27	102
NDEA	38	96	57	96
NPYR	46	97	46	94
NMOR	60	97	46	82
NDPA	36	89	44	89
NPIP	74	89	36	99
NDBA	49	93	41	96
NDPheA	62	95	68	101

at 5 ng g^{-1} concentration level for the nine NAms and the MAE step applied, recovery percentages varied between 36 and 74% (Table 2), depending on the analyte and the compound. When the NaOH concentration was decreased to 0.05 M, as shown in Table 2, recoveries in the range 82–102% were obtained, and therefore these were the conditions selected for MAE. The alkaline medium used avoids the artifactual formation of NAms during the sample extraction by blocking the N-nitrosation reactions [13].

The amount of sample that can be processed at the same time is one shortcoming of MAE in closed-vessel systems, and sample masses higher than 0.25 g are not recommended by the manufacturer of the system used in this experiment. Considering that the microwave extraction step was applied in soft conditions, no mineralization of the solid sample occurred and the resulting solution had to be centrifuged and made up to 10 mL before being submitted to DLLME.

3.4. Analytical characteristics of the method

The matrix effect was studied by comparing the slopes of aqueous standards and standard additions calibration graphs obtained for four different samples, namely pork lean, chicken meatballs, and two types of sausages. No statistically significant differences were observed, and so quantification was carried out by external calibration.

The method was validated for linearity, detection and quantification limits, selectivity, accuracy and precision. Calibration curves using DLLME–GC–MS were obtained by least-squares linear regression analysis of the peak area versus analyte concentration using five concentration levels in duplicate. The validation parameters, range of linearity and the correlation coefficients for the nine NAms are shown in Table 3. The values of r^2 were good ($r^2 > 0.999$) demonstrating excellent linearity for the range studied. The limits of detection (DL, calculated as three times the signal-to-noise ratio) are included in Table 3. The limits of quantification (QL, calculated as ten times the signal-to-noise ratio) roughly varied between 10 and 50 pg mL^{-1} (for NPIP and NMEA, respectively). The enrichment factor (EF) was calculated as the ratio between the analyte concentration in the sedimented phase after extraction and the initial concentration of analyte in the aqueous solution; values between 220 and 342 were attained. Detection limits obtained for meat product samples by MAE–DLLME–GC–MS are also shown in Table 3; the limits of quantification varied between 0.4 and 1.9 ng g^{-1} (for NPIP and NMEA, respectively). The selectivity of the method was judged from the absence of interfering peaks at the analyte elution times for blank chromatograms of different unspiked samples. No matrix compounds existed that might give a false positive signal in the blank samples.

The repeatability was calculated using the relative standard deviation from a series of ten consecutive DLLME–GC–MS analyses of two aqueous standards solutions containing the NAms at 1 and 5 ng mL^{-1}. RSD values between of 2.1 and 5.9% were obtained in all cases. When a series of ten consecutive MAE–DLLME–GC–MS analyses of lean pork fortified at about 5 (Table 3) and 25 ng g^{-1} was carried out, the RSD values varied between 2.4 and 10%.

3.5. Analysis of samples

The optimized procedure was applied to the analysis of 21 meat products and the results obtained appear in Table 4. NDMA and NPYR were the NAms most commonly found in the food samples [37], and their contents, too, were generally higher than the rest of NAms in all samples analyzed. Nevertheless, neither NDMA nor NPYR surpassed 10 ng g^{-1}, the highest value tolerated by some countries in retail food. Moreover, only five of the total samples analyzed exceeded 10 ng g^{-1} for the total NAms analyzed. None of the samples contained NMOR or NDPheA, at least above the corresponding detection limits. Fig. 5 shows the chromatogram obtained using MAE–DLLME–GC–MS in SIM mode for the dry cured sausage with paprika sample 4. The elution profile obtained for this sample as well as for the rest of meat products analyzed demonstrated the absence of interfering compounds eluting at the retention times of the analytes. The NAms were identified by comparing the retention time and considering the abundance and above mentioned specificity criteria (Table 1). The low contents obtained for total NAms indicated that the positive effects of curing agents are overwhelming; moreover, the amount of meat product to be ingested in order to attain the tolerated level would be totally unviable.

To test the accuracy of the proposed method in real samples, three different meat products (lean pork, sausage and mortadella) were fortified at two concentration levels (roughly 5 and 25 ng g^{-1}) and analyzed by the optimized MAE–DLLME–GC–MS, taking into account the known analyte contents for these samples. The results showed a mean recovery ± standard deviation of 94.5 ± 7.3% ($n = 72$).

Table 3
Analytical parameters for NAms using the proposed procedure.

Compound	Linearity (ng mL^{-1})	r^2	DL (ng mL^{-1})	EF	DL[a] (ng g^{-1})	RSD[b] (%)
NDMA	0.05–200	0.9991	0.012	287	0.48	6.1
NMEA	0.05–200	0.9990	0.014	285	0.56	7.1
NDEA	0.05–200	0.9994	0.013	220	0.52	6.5
NPYR	0.05–200	0.9990	0.010	305	0.40	8.3
NMOR	0.05–200	0.9991	0.009	326	0.35	7.4
NDPA	0.02–200	0.9997	0.004	340	0.16	10.0
NPIP	0.02–200	0.9993	0.003	342	0.12	5.9
NDBA	0.02–200	0.9991	0.004	339	0.16	8.8
NDPheA	0.05–200	0.9994	0.013	328	0.52	7.6

[a] MAE–DLLME–GC–MS.
[b] Values obtained for lean pork fortified at 5 ng g^{-1} concentration level.

Table 4
NAms contents ($ng\,g^{-1}$) obtained in the analysis of meat products by MAE–DLLME–GC–MS.

Sample	NDMA	NMEA	NDEA	NPYR	NDPA	NPIP	NDBA
Chicken meatballs	ND	ND	ND	ND	ND	0.9 ± 0.04	1.5 ± 0.2
Pork lean	1.7 ± 0.2	ND	ND	1.5 ± 0.2	ND	1.0 ± 0.1	ND
Sweet pork ham	2.6 ± 0.3	ND	2.4 ± 0.2	3.4 ± 0.3	ND	1.9 ± 0.1	ND
Cured pork ham	2.0 ± 0.3	2.5 ± 0.3	ND	2.9 ± 0.3	ND	1.8 ± 0.3	ND
Turkey ham	3.8 ± 0.3	ND	ND	2.8 ± 0.2	ND	1.2 ± 0.2	ND
Pork back	3.3 ± 0.2	ND	ND	ND	ND	1.6 ± 0.1	1.6 ± 0.04
Mortadella	1.5 ± 0.1	ND	ND	1.6 ± 0.2	1.2 ± 0.1	0.8 ± 0.09	ND
Mortadella with olives	3.4 ±0.2	ND	ND	1.4 ± 0.2	ND	0.7 ± 0.06	2.2 ± 0.1
Frankfurt sausages 1	2.0 ± 0.3	ND	ND	ND	1.9 ± 0.2	1.4 ± 0.1	ND
Frankfurt sausages 2	2.2 ± 0.1	ND	ND	2.2 ± 0.1	ND	2.3 ± 0.2	ND
Turkey pâté	2.3 ± 0.2	ND	1.9 ± 0.2	ND	ND	ND	ND
Foie-gras pâté 1	5.7 ± 0.3	3.8 ± 0.2	ND	2.8 ± 0.3	ND	0.8 ± 0.03	ND
Foie-gras pâté 2	ND	ND	ND	ND	ND	0.9 ± 0.05	ND
Majorcan sausage	3.3 ± 0.1	ND	ND	ND	ND	ND	ND
Dry cured sausage 1	2.2 ± 0.2	ND	ND	ND	ND	1.3 ± 0.1	3.3 ± 0.2
Dry cured sausage 2	2.4 ± 0.2	ND	ND	1.5 ± 0.1	ND	ND	ND
Blood sausage	3.5 ± 0.2	ND	ND	2.1 ± 0.2	ND	2.0 ± 0.2	3.4 ± 0.2
Dry cured sausage with paprika 1	4.1 ± 0.3	ND	2.8 ± 0.3	2.6 ± 0.1	ND	1.5 ± 0.2	ND
Dry cured sausage with paprika 2	3.3 ± 0.3	ND	2.2 ± 0.2	1.8 ± 0.1	ND	1.1 ± 0.1	1.9 ± 0.1
Dry cured sausage with paprika 3	4.0 ± 0.2	ND	1.9 ± 0.2	ND	ND	ND	ND
Dry cured sausage with paprika 4	3.1 ± 0.3	ND	3.6 ± 0.3	1.5 ± 0.1	1.0 ± 0.1	2.2 ± 0.2	1.2 ± 0.1

Mean value ± standard deviation ($n = 4$). ND means not detected.

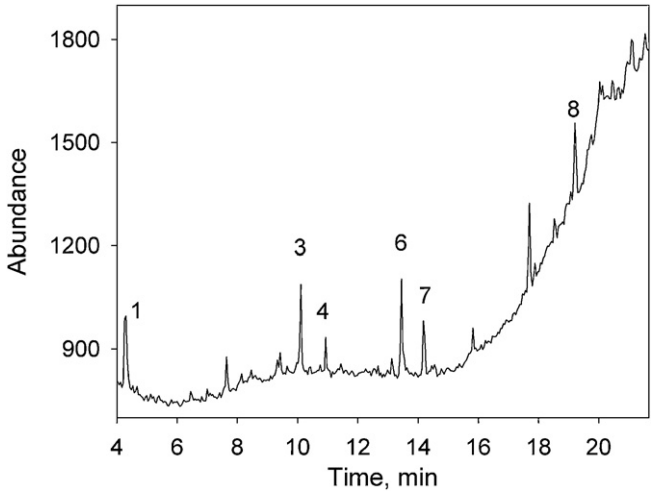

Fig. 5. MAE–DLLME–GC–MS chromatogram obtained for the dry cured sausage with paprika sample 4. Peaks correspond to: 1, NDMA; 3, NDEA; 4, NPYR; 6, NDPA; 7, NPIP and 8, NDBA.

4. Conclusion

The results obtained confirm the suitability of the simple, rapid and environmentally friendly method proposed for volatile NAms determination in meat products. The efficiency of MAE for the isolation of NAms from the complex food matrices analyzed was confirmed not only by the recovery values obtained but also by the fact that quantification could be carried out by aqueous calibration. Very good detection limits were achieved in spite of the low amount of sample which can be submitted to MAE, due to the high enrichment power of DLLME.

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