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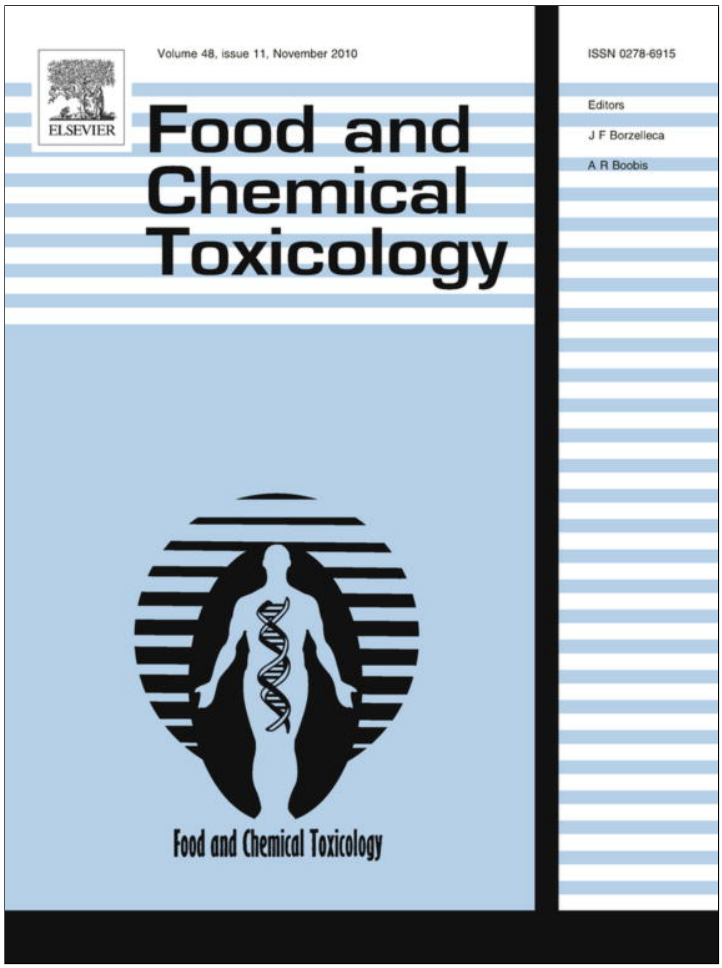

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Determination of volatile nitrosamines in various meat products using comprehensive gas chromatography–nitrogen chemiluminescence detection

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ABSTRACT

An optimized method was developed for the extraction, pre-concentration and analysis of nitrosamines (NAs) in various meat products. Values of reproducibility, linearity, limit of detection (LOD) and limit of quantitation (LOQ) for six NA standards (*N*-nitrosodimethylamine, *N*-nitrosodiethylamine, *N*-nitrosodi-*n*-propylamine, *N*-nitrosopyrrolidine, *N*-nitrosopiperidine, *N*-nitrosodi-*n*-butylamine) were determined. The LODs using this method were between 1.66–3.86 and LOQs between 6.96–16.71 μg L^{-1}. The screening of four different types of meat samples (sausage, salami, sucuk and doner kebab) showed that all samples contained levels of various NAs, identified with high confidence using comprehensive gas chromatography (GCxGC) and a fast responding element specific nitrogen chemiluminescence detector (NCD). The sum of the six NAs were highest in the doner kebab samples, being between 0.51–16.63 μg kg^{-1} and were lowest in the sausage samples at 0.45–2.93 μg kg^{-1}. The described method is simple, rapid, selective and sensitive.

1. Introduction

Nitrite and nitrate are commonly used in the formulation of meat products because they prevent formation of botulinal toxins by *Clostridium botulinum*, produce a desirable colour, flavour and texture in these products, and also prevent development of rancidity, off-odours and off-flavours during storage. Nitrite can react readily with amines and amides to form *N*-nitrosamines (NAs), most of which are known to be highly carcinogenic and mutagenic (Byun et al., 2004; Domanska-Blicharz et al., 2005). Nitrate does not react in this way, and is in itself relatively nontoxic, but nitrate has to be considered in this context because it can be reduced to nitrite by bacterial action during processing and storage. The nitrite in meat products is a primary problem in the formation of carcinogenic volatile nitrosamines under high-temperature conditions (Byun et al., 2004). The formation of nitrosamines in meat products depends on the cooking method, cooking temperature and time, residual and added nitrite concentration, presence of nitrosamine precursors, catalysts and inhibitors, preprocessing procedures and conditions, the smoking process and storage conditions (Byun et al., 2004; Yurchenko and Molder, 2007).

Sucuk and doner kebab are two examples of meat products which are part of traditional Turkish cuisine. Sausage and salami are also widely consumed in Turkey.

Doner kebab is made from intact or ground muscle. Ground meat or intact pieces are marinated with red or black pepper, salt, diced onion or onion powder, diced tomatoes or tomato sauce, olive oil, lemon juice, vinegar, milk or milk powder, yoghurt, and egg at 4 °C for 12 h (Vazgecer et al., 2004). They are then shaped into a cone and impaled on a doner kebab stick. The fat content of doner kebab ranges between 20% and 40%. Raw doner is fixed on a vertical spit and slowly rotated to roast the surface evenly, in front of a heating element powered by electric, gas or charcoal, and it is cut into thin slices as it is gradually broiled. When the meat surface is cooked, doner kebab is shaved off (Kilic, 2009). It should be noted that as doner is a fresh product, unlike sucuk, sausage and salami, no nitrate or nitrite is added during production.

The popularity of doner kebab is spreading throughout the world and consumption has hugely increased in the last 10 years. It was therefore considered an important product to study. According to the literature, the nitrosamine content of doner kebab has not been studied before.

Sucuk; a dry, uncooked, cured, fermented sausage, produced from beef or water buffalo meat; is one of the most widely consumed traditional Turkish meat products. It consists of ground meat, sheep tail fat, and a curing ingredient (nitrite or nitrate) with various spices including cumin, garlic, salt, black and red pepper. This mixture is stuffed into a sausage casing and hung for fermentation at 22–23 °C (the ripening period) which takes place using either the naturally present microorganisms or added starter cultures. It is allowed to dry for several weeks at ambient temperature

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and humidity (Bozkurt and Erkmen, 2002). Sucuk requires drying periods in which both the temperature (22–25 °C) and humidity (80–90%) are carefully controlled.

Salami is usually produced from beef, but chicken and other poultry meats have also been used in its manufacture. Fresh meat and fat is minced using a cutter. Spices, salts, some ingredients (skimmed milk powder, etc.), additives (e.g. nitrates, nitrites, antioxidants, spices, etc.) and sometimes starter cultures are added and the mixture is minced. After the mixture has been stuffed into casings, the salami are ripened at 15 °C for several days and then at 9 °C for up to 3 months (Todorov et al., 2007).

Sausage is usually made from ground meat, ground fat, salt and spices (sometimes with other ingredients such as herbs). Typically the sausage is formed in a casing. Sausages may be preserved by curing, drying in cool air, or smoking. Nitrates and nitrites are commonly added as additives.

NAs are found in the environment and in various food products such as tinned foods (Domanska-Blicharz et al., 2005), fish products (Yurchenko and Molder, 2006), beer (Perez et al., 2008) and meat products (Byun et al., 2004; Yurchenko and Molder, 2007). Because NAs are potent carcinogens, effective in most animal species and in body organs, e.g. stomach, liver, kidney, urinary bladder, pancreas and others (Byun et al., 2004), efforts have been made to understand human exposure to carcinogenic NA compounds. The tolerable level of human exposure to the most volatile NAs has been found to be in the range of 5–10 $\mu g\,kg^{-1}$ of body weight. This demands analytical techniques with the lowest possible detection limits (Ventanas and Ruiz, 2006). In literature the determination of volatile NAs in meat or seafood products has been carried out by various analytical methods, including gas chromatography with a mass selective detector (GC–MS) (Byun et al., 2004; Yurchenko and Molder, 2006, 2007; Ventanas and Ruiz, 2006; Filho et al., 2003), GC coupled to a thermal energy analyser (Byun et al., 2004), and micellar electrokinetic chromatography (Filho et al., 2003). The thermal energy analyser mentioned is another name for the nitrogen chemiluminescence detector (NCD). It is the only study in literature analyzing NA from meats using GC–NCD. They found low concentrations of *N*-nitrosodimethylamine and *N*-nitrosopyrrolidine in their meat samples.

The use of element specific detectors for chromatography is a key approach in analytical chemistry. Conventional universal detectors such as the MS (Adam et al., 2007) operating in full scan mode are not sensitive enough to achieve detection on organic nitrogen compounds at low concentrations. The flame ionisation detector (FID) (Yan, 2006) is universal in its response, and with reasonable sensitivity, but provides no independent confirmation of analyte identification. Chemiluminescent detectors which are specific for nitrogen are especially desirable for this purpose. The GC–NCD technique is not new, but is becoming increasingly popular for certain niche applications. GC–NCD has recently been used to analyse organic nitrogen compounds from diesel fuel (Wang et al., 2004), fish products (Yurchenko and Molder, 2006) and wastewater (Grebel and Suffet, 2007). NCD is a powerful GC detector developed for selective detection of organic nitrogen compounds (Yan, 2006).

Comprehensive GCxGC is a multi-dimensional GC technique that has an increased separation power. Comprehensive GCxGC has advantages over one-dimensional GC (1-D GC). If the two techniques are compared under optimized conditions, sensitivity and resolution are much better in GCxGC. The limits of detection (LODs) are typically three to five times higher and the number of satisfactorily resolved peaks is much greater (up to 10 times) in GCxGC compared with 1-D GC (Ozel et al., 2006; Adam et al., 2007; Adahchour et al., 2008). Adam et al. (2007) have analyzed various ON compounds from diesel feedstocks using GCxGC with NCD and/or MS detector.

NCD has previously been applied for NAs in only limited applications. We believe that there is significant scope for the use of NCD coupled with GCxGC separation. The nature of GCxGC requires detectors that have high speed responses in order to describe the typical peaks widths of 100–300 mS. The particular aim of this study has been to examine the performance of a fast responding NCD coupled with GCxGC for the analysis of NAs in meat products.

2. Materials and methods

2.1. Samples

All meat products were purchased from supermarkets or local doner kebab shops in Gaziantep, Turkey. six samples of sucuk were purchased, five of sausage, five of salami and six of doner kebab, totalling 22 samples in all. All doner kebabs had been roasted directly with a gas flame, rather than electricity or charcoal, and had been freshly prepared without nitrate/nitrite additives. Sausage, sucuk and salami were selected arbitrarily from the market and they all contained nitrite and nitrate in their ingredients list. Extraction of NAs was performed on the day of purchase for all samples. The extracts were stored in amber vials at −4 °C until analysis.

Meat products are complex food matrices containing water, carbohydrates, proteinaceous material, fat and various other additives. Hence samples from different sources can vary widely in composition. Meat products; namely sucuk, sausage, salami and doner kebab; were analysed in this study for their concentrations of six NAs. Three of them, sucuk, sausage and salami, as they are fermented and stored for more than 3 months prior to consumption, are known to contain nitrite and nitrate in their composition.

2.2. Chemicals and standards

Florisil from Sigma–Aldrich (Dorset, UK), Florisil water sep-pak cartridges from Waters (Milford, MA, USA) and Extrelut from Merck (Darmstadt, Germany) were purchased for the sample preparation. All solvents (methanol, dichloromethane, hexane) were supplied by Sigma–Aldrich (Dorset, UK). All products were reagent-grade. All NAs (*N*-nitrosodimethylamine (NDMA), *N*-nitrosodiethylamine (NDEA), *N*-nitrosodi-*n*-propylamine (NDPA), *N*-nitrosopyrrolidine (NPYR), *N*-nitrosopiperidine (NPIP), *N*-nitrosodi-*n*-butylamine (NDBA)) were purchased from Sigma–Aldrich (Dorset, UK).

Stock standard solutions containing 10 $g\,L^{-1}$ of each NA were prepared in methanol and stored in amber vials at −4 °C.

2.3. Sample preparation

Extraction of NAs was performed using the same method as Yurchenko and Molder (2007) with a slight modification at the end. A two-step solid-phase extraction using Extrelut and Florisil sorbents was used for sample cleaning. Each sample (6.0 ± 1.0 g) was minced and mixed with 0.1 mol L^{-1} NaOH (6 mL). For the first step, 6 g of Extrelut was placed at the bottom of the glass column (30 cm × 1.5 cm) and wetted with 20 mL hexane/dichloromethane 40:60 (v:v). Then, the sample was eluted with two 20 mL portions of hexane/dichloromethane solution 40:60 (v:v). The eluate was collected in a 50 mL concentrator flask and evaporated under a nitrogen stream. During the second step, 1 g of Florisil was placed at the bottom of the Florisil cartridge (6.5 cm × 1.3 cm) and wetted with 6 mL dichloromethane/methanol 95:5 (v:v). Then the sample solution was eluted with 6 mL dichloromethane/methanol solution 95:5 (v:v). The solution was evaporated under a nitrogen stream to about 1 mL. However, it was noticed that this solution contained fat which might have caused problems with GC injection. Therefore, we modified the extraction technique by adding another step. The fat was removed using 3 mL methanol. This was repeated three times. The combined methanol extracts were concentrated to about 100 μL under a nitrogen stream. The prepared solution was transferred to the GC auto sampler vial. Extractions were performed in triplicate.

2.4. Chromatographic analysis

The GCxGC–NCD system consisted of an Agilent 7890 gas chromatograph and an Agilent 255 Nitrogen Chemiluminescence detector (Agilent Technologies, Palo Alto, CA, USA). The modulator between first and second GC columns was based on a Leco (Cheshire, UK)) liquid nitrogen two stage cold jet system. The first column was a DB5 (30 m × 0.32 mm i.d. × 0.25 μm film thickness) and the second column a RXI-17 (1.5 m × 0.10 mm i.d. × 0.10 μm film thickness). The DB5 column was obtained from J & W Scientific (Folsom, CA, USA) and the RXI-17 column was purchased from Thames Restek (Saunderton, UK).

Data from the NCD was collected at 50 Hz over the entire course of the analysis. The modulator secondary oven was operated at +15 °C above the GC oven temperature. The first dimension separation axis extended to 840 s and the second dimension axis ended at 5 s.

Helium was used as a carrier gas. The initial temperature of the first dimension column was 60 °C for 1 min and the subsequent temperature programme was a heating rate of 10 °C min^{-1} until 180 °C was reached and held isothermally for a further 1 min. The initial temperature of the second dimension column was 75 °C for 1 min and a 10 °C min^{-1} heating rate was used until 195 °C was reached and held isothermally for a further 1 min. Peak identification was made using individual standards.

Table 1
First and second retention times of nitrosamines, LOD and LOQ of the applied method, linear range and its R^2 for the standard curves prepared from each nitrosamine standard using GCxGC–NCD.

Parameter	NDMA	NDEA	NDPA	NPYR	NPIP	NDBA
1st RT (s)	335	435	585	585	625	760
2nd RT (s)	1.66	1.68	1.74	2.02	1.98	1.76
$LOD_{analyte}$ (μg L^{-1})	3.86	2.32	1.61	2.15	1.98	2.46
$LOQ_{analyte}$ (μg L^{-1})	16.71	10.04	6.96	9.30	8.56	10.63
Linear range (μg L^{-1})	4–200	2–300	2–300	2–250	2–200	2–175
R^2	0.9675	0.9824	0.9936	0.9688	0.9732	0.9716

The sample (1 μL) was directly injected using a splitless method into the GCxGC–NCD using a Gerstel automated liquid injector (Gerstel, Mulheim an der Ruhr, Germany). A one-dimensional GC–NCD chromatogram was collected for comparison with GCxGC–NCD.

2.5. GCxGC–NCD optimization

Our NCD system was used in NA mode. The conditions for the Agilent 255 NCD were as follows: Dual plasma burner, 140 torr burner pressure, four psi gas flow through the ozone generator and zero hydrogen flow rate (NA mode does not use hydrogen gas). *N*-Nitroso compounds, commonly known as NAs, have a generic structure of R–N–NO. The N–NO bond is typically the weakest in the molecule, and therefore may be selectively broken under moderate conditions. Optimization of the response was carried out with samples (1 μL) containing 50 μg of each of the six nitrosamine standards per litre of methanol solution. The system normally works at 900–1000 °C. However, it has been suggested by the manufacturer to use it between 350 and 500 °C when in the nitrosamine mode. In this study, we optimized oxygen flow rate and burner temperature for six nitrosamine standards.

2.6. Validation of the method

Optimization and validation parameters were investigated using the solutions of NA standards in methanol. To optimize the method under analytical control, LOD, LOQ, reproducibility and linear range experiments were performed. The efficiency of solid-phase extraction for the separation of NAs of meat products was determined by applying the full procedure to three replicate samples of sausage. The samples were spiked with the six NA standards to obtain the NA concentrations

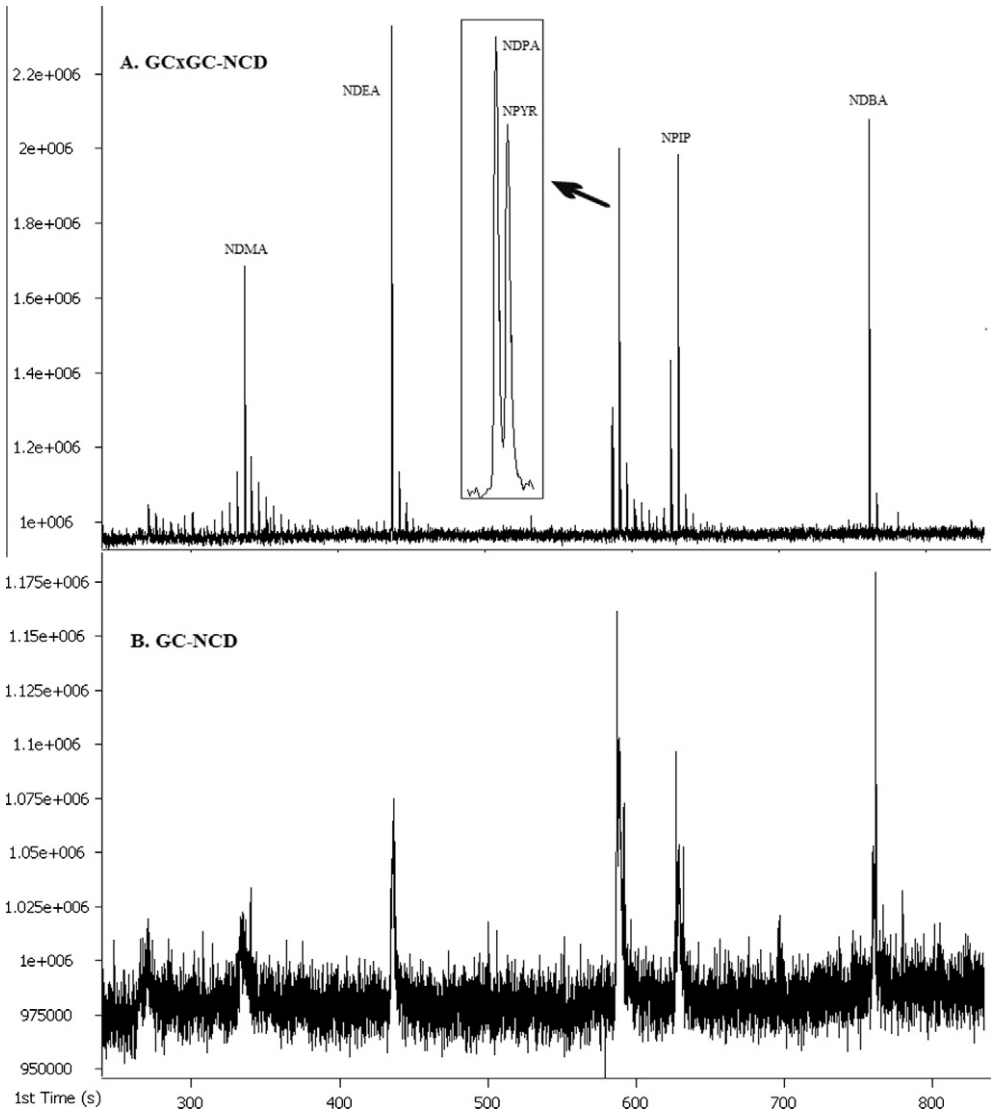

Fig. 1. (A) GCxGC–NCD chromatogram of six nitrosamine standards (each 50 μg L^{-1}). (B) GC–NCD chromatogram of six nitrosamine standards (each 50 μg L^{-1}).

of 0.1, 1.0, 3.0 and 5.0 μg kg^{-1} meat. LOD levels were determined according to EPA protocol 40CFR136 Appendix B, based on the standard deviations at low concentrations (Appendix B to Part 136, 1990). The experiments were repeated eight times ($N = 8$). The signal/noise ratio using 10 μg L^{-1} of stock solutions of the six NA standards was approximately 5.5, which is adequate for a GCxGC–NCD system. LOD and LOQ were calculated using the formulas below, based on the standard deviations at low concentration.

LOD = s · t (t-Student t-value for eight replicates, s: standard deviation).

LOQ = 10 · s.

3. Results and discussion

3.1. Optimization of NCD response to NAs

The main goal of this study was to demonstrate the feasibility of using GCxGC–NCD for analyzing NAs in meat samples. The optimization of the system in NA mode was carried out before the meat samples were analyzed. Two parameters were optimized: pyrolysis temperature and oxygen flow rate. The temperature of the NCD pyrolysis chamber controls the amount of energy available to break down NAs into nitric oxide, which is the basis of NCD response. Pyrolysis was carried out at temperatures of 350, 400, 450 and 500 °C. Increasing the pyrolysis temperature was seen to improve detector response until 450 °C. A temperature of 500 °C initiated baseline problems and also decreased the detector response. Thus, a temperature of 450 °C was selected for further NA studies. Grebel and Suffet (2007) used temperatures from 200 to 300 °C for the analysis of NAs in water and 300 °C gave the highest response. NCD detector technology has recently improved and now 350–500 °C is available to use as the pyrolysis temperature. Our optimized pyrolysis temperature was found to be higher than that found by Grebel and Suffet (2007). The use of oxygen is recommended at 5–10 mL min^{-1} for ozone production which reacts with NO to produce excited state NO_2. The NO_2 then undergoes relaxation emitting a photon which is the property used for measurement. Differing oxygen flow rates from 5 to 10 mL min^{-1} were tested, increasing by 1 mL min^{-1} increments. The yield of NDMA, NDEA and NDBA decreased with the increasing oxygen flow rate. The excess oxygen may have degraded those three NAs. Therefore, a 5 mL min^{-1} oxygen flow rate at a temperature of 450 °C was chosen as optimum for further studies.

3.2. Validation of the method

It was found that the efficiency of the extraction was in a range of 76% to 85% for the NAs investigated in this study. The final concentrations of the components were correlated according to their extraction efficiencies found from these preliminary studies.

The NCD is inherently capable of very high sensitivity, because the light emission is detected against a dark background. The following is a summary of the analytical work done to statistically establish the linearity, reproducibility, LOD and LOQ. To calibrate the GCxGC–NCD chromatogram, six different NA standard solutions, which cover the concentration range from 1 to 400 μg L^{-1}, were prepared in methanol. Correlation coefficients (R^2) for different calibration curves were found to between 0.9675 and 0.9936. These calibration curves were used to calculate NA concentrations from meat samples. Linear concentration ranges, LOD and LOQ results of the six NA standards are shown in Table 1.

LOD and LOQ were determined for each NA standard. This method is based on the standard deviation of replicated analyses. The lowest possible LOD using the optimized method was between 1.61 and 3.86 μg kg^{-1} for the six most common NAs in meat products. The LOQ value was between 6.96 and 16.71 μg kg^{-1}. Our results show that NCD is highly sensitive for the analysis of NAs.

Figs. 1 and 2 show the fast NCD response with 1- or 2-D separation. It can be seen in Fig. 1 that comprehensive GCxGC has advantages over GC in terms of both sensitivity and target analyte peak resolution. The highlighted region of Fig. 1A shows more detailed separation of NDPA and NPYR components using GCxGC. Sensitivity is approximately five times higher using the GCxGC method than the one-dimensional GC. The signal/noise ratios of six NA standards were found much better with using GCxGC–NCD. Adahchour et al. (2008) and Adam et al. (2007) showed LODs were typically three to five times higher using GCxGC compared with one-dimensional GC and the number of satisfactorily resolved peaks was up to ten times greater. NPYR and NPIP are more polar than N-alkyl NAs, and hence are displayed further alone the y-axis of the GCxGC chromatogram. As can be seen from Figs. 1 and 2, the selected four N-alkyl NAs are well separated in the 1st dimension and lie on a line of equivalent polarity, and the equal spacing shows this is an unbroken homologous series from C_1 to C_4. Table 1 and Figs. 1 and 2 show that GCxGC aided the separation of NDPA and NPYR, when they would have typically overlapped in the equivalent 1-D GC separation. NDPA and NPYR cannot be separated satisfactorily using a non-polar (such as DB1 or DB5). Twelve of meat samples contained both NDPA and NPYR in differing amounts according to the type of meat highlighting the need to fully resolve this species.

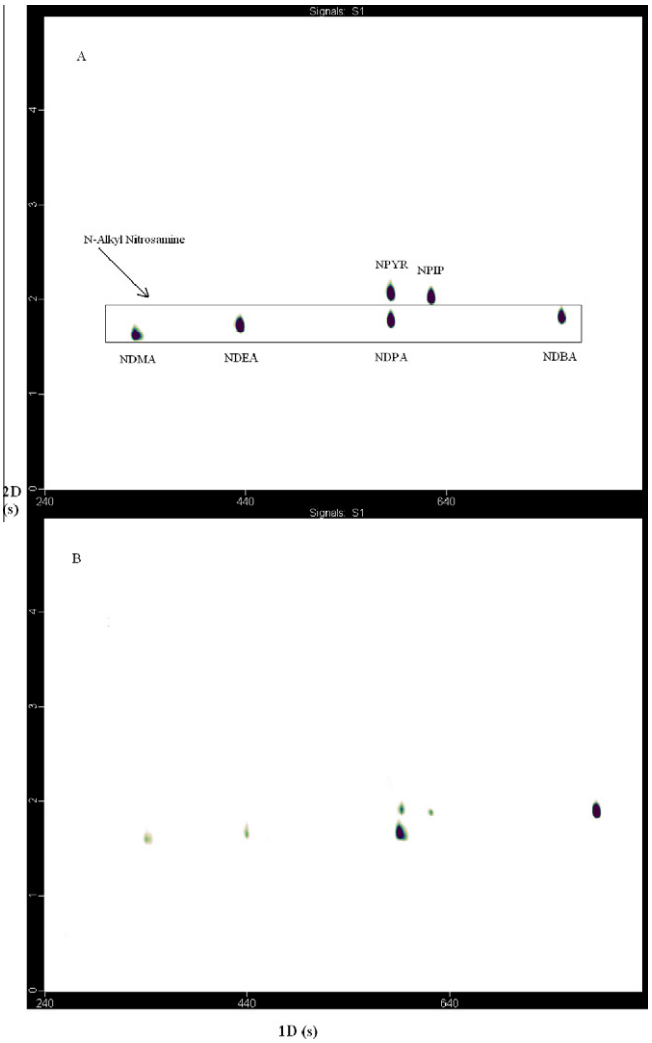

Fig. 2. (A) GCxGC–NCD contour plot of six nitrosamine standards (each 50 μg L^{-1}). (B) GCxGC–NCD contour plot of nitrosamines of meat product (Sucuk 6).

3.3. Pre-concentration and analysis of NAs from meat products

Comprehensive GCxGC–NCD was used to separate and identify volatile and semi-volatile NA components of different meat samples. A typical 2-D separation is shown in Fig. 2. The chromatograms are presented as a contour plot with the retention times on column 1 (indicating volatility) and 2 (indicating polarity) on the *x*- and *y*-axes, respectively and the NCD response as a greyscale contour. Each spot on the chromatogram represents an individual compound. Fig. 2A and B show the GCxGC chromatogram of six 50 μg L^{-1} NAs standards and the sucuk number six sample, respectively. Meat mainly contains six NAs. These compounds are identified in various meat samples and listed in Table 2.

After setting the optimized conditions, determination of concentrations of NAs in various meat products was carried out in triplicate. A standard curve equation was obtained and the concentration of each NA in every sample was calculated also taking into account the recovery from solid-phase extraction. The concentration of the sum of the six NAs found in each of the meat products ranged from 0.45 to 16.63 μg kg^{-1}.

These results support earlier studies showing that NDMA and NDEA are commonly found in sausages (Yurchenko and Molder, 2007). These two NAs were found in the highest concentrations in sausage samples even though these samples were seen to have the lowest sum of NAs overall. The other meat products containing nitrite and nitrate interestingly had lower NDMA and NDEA values. This might be explained by the differences in their processing conditions and compositions. Levels of NAs in food are not regulated directly by EU laws. However, a limit of 10 μg kg^{-1} is currently being observed for NDMA in meat products in Canada (CFIA, 2004). None of the meat products used in this study had a value of more than 10 μg kg^{-1} for any of the NAs.

It was found that NDBA was the least observed NA in meat products in this study. Only seven of the samples contained NDBA and their concentrations were quite low compared to those of other NAs.

The most interesting results were obtained for doner kebab samples. Although the sample did not contain any nitrite or nitrate, they were found to contain NAs. NDMA contamination has been reported before as being caused by the direct fire-kilning of barley malt in beer (Perez et al., 2008) and by natural gas grilling of fish (Key et al., 1982). We found strong NA content in doner kebabs roasted using a direct gas flame. However, NPYR and NPIP were the main components found instead of NDMA in this study. The concentrations reached up to 7.72 and 7.23 μg kg^{-1} for NPYR and NPIP, respectively, in one of doner kebab samples. Even though NPYR and NPIP were high in almost all the doner kebab samples, none of the samples had a level of NDMA of more than 10 μg kg^{-1}.

4. Conclusion

In this study, six NAs were separated and analysed successfully using GCxGC–NCD. GCxGC–NCD has an increased separation and identification power for the analysis of volatile nitrosamines of meat products, and the technique may have wider applicability in food analysis. The quality of data in terms of confidence in peak identification increased when GCxGC–NCD conditions were optimized. For NDMA, the LOD was 3.86 μg L^{-1} and LOQ was 16.71 μg L^{-1}. LODs and LOQs were lower for the other NAs tested.

The total concentrations of the six NAs in the studied meat samples ranged from 0.45 to 16.63 μg kg^{-1}. The highest levels of NAs were found in samples of gas flame cooked doner kebab despite it containing no nitrites or nitrates to begin with. NPYR and NPIP concentrations were highest in doner kebab samples. Most of the sausages had only two NAs, namely NDMA and NDEA and sausages were seen to have the lowest overall sum of NAs. Sucuk and salami contained a broader spectrum of the NAs studied and the overall sum of NAs was only moderate. It was observed that the direct gas flame cooking of meat products has a significant effect on the concentrations of NAs in addition to the content and availability of nitrites and nitrates in their composition. This study has been important in revealing the surprisingly high content of NAs in doner kebab, which has never been studied before, as doner kebab is being increasingly consumed throughout the world.

Conflict of Interest

The authors declared that there are no conflicts of interest.

Table 2
Mean concentrations with their standard deviations of six nitrosamines found in various meat products using a nitrogen chemiluminescence detector with GCxGC.

Product	Mean concentration of NAs (μg kg^{-1})						
	NDMA	NDEA	NDPA	NPYR	NPIP	NDBA	Sum of six NAs
Sucuk 1	0.19 ± 0.04[a]	0.95 ± 0.07	0.50 ± 0.06	0.54 ± 0.06	1.05 ± 0.13	n.d.[b]	3.23 ± 0.07
Sucuk 2	0.11 ± 0.02	0.10 ± 0.02	n.d.	0.11 ± 0.03	0.16 ± 0.05	0.15 ± 0.04	0.63 ± 0.03
Sucuk 3	0.30 ± 0.05	0.49 ± 0.05	0.59 ± 0.08	0.57 ± 0.07	1.02 ± 0.09	0.35 ± 0.07	3.32 ± 0.06
Sucuk 4	0.11 ± 0.02	n.d.	0.47 ± 0.05	0.82 ± 0.05	1.49 ± 0.17	0.19 ± 0.03	3.08 ± 0.06
Sucuk 5	n.d.	n.d.	0.27 ± 0.04	1.36 ± 0.12	2.71 ± 0.39	n.d.	4.34 ± 0.17
Sucuk 6	0.78 ± 0.08	0.47 ± 0.07	1.35 ± 0.21	0.18 ± 0.04	0.23 ± 0.06	1.68 ± 0.13	4.69 ± 0.09
Sausage 1	n.d.	n.d.	0.41 ± 0.03	0.28 ± 0.03	0.65 ± 0.09	n.d.	1.34 ± 0.05
Sausage 2	0.87 ± 0.09	0.15 ± 0.02	0.10 ± 0.02	n.d.	n.d.	n.d.	1.12 ± 0.04
Sausage 3	1.20 ± 0.19	1.73 ± 0.15	n.d.	n.d.	n.d.	n.d.	2.93 ± 0.16
Sausage 4	0.21 ± 0.03	0.34 ± 0.05	n.d.	n.d.	0.16 ± 0.03	n.d.	0.71 ± 0.04
Sausage 5	0.14 ± 0.02	0.10 ± 0.03	n.d.	0.11 ± 0.02	0.10 ± 0.02	n.d.	0.45 ± 0.02
Salami 1	n.d.	0.22 ± 0.02	n.d.	n.d.	1.44 ± 0.19	n.d.	1.66 ± 0.11
Salami 2	0.30 ± 0.03	0.28 ± 0.05	0.35 ± 0.04	0.47 ± 0.05	0.73 ± 0.20	0.10 ± 0.02	2.23 ± 0.07
Salami 3	0.10 ± 0.02	0.11 ± 0.02	0.26 ± 0.06	0.14 ± 0.03	0.19 ± 0.03	n.d.	0.80 ± 0.03
Salami 4	n.d.	n.d.	0.51 ± 0.05	0.37 ± 0.04	0.53 ± 0.10	0.21 ± 0.02	1.62 ± 0.05
Salami 5	n.d.	0.15 ± 0.02	n.d.	0.53 ± 0.08	0.82 ± 0.07	0.56 ± 0.07	2.06 ± 0.06
Doner Kebab 1	0.38 ± 0.05	0.88 ± 0.07	0.42 ± 0.07	7.72 ± 0.55	7.23 ± 0.89	n.d.	16.63 ± 0.32
Doner Kebab 2	n.d.	0.11 ± 0.01	n.d.	0.38 ± 0.05	0.80 ± 0.21	n.d.	1.29 ± 0.09
Doner Kebab 3	1.13 ± 0.12	0.98 ± 0.05	0.24 ± 0.05	3.07 ± 0.41	5.31 ± 0.90	n.d.	10.73 ± 0.30
Doner Kebab 4	0.11 ± 0.02	0.10 ± 0.02	n.d.	0.13 ± 0.05	0.17 ± 0.04	n.d.	0.51 ± 0.03
Doner Kebab 5	0.33 ± 0.04	0.52 ± 0.04	0.12 ± 0.04	1.09 ± 0.09	1.04 ± 0.08	n.d.	3.10 ± 0.06
Doner Kebab 6	0.41 ± 0.06	0.41 ± 0.05	n.d.	1.50 ± 0.21	1.59 ± 0.17	n.d.	3.91 ± 0.12

[a] The standard deviations for three ($n = 3$) experiments.
[b] Not detected.

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