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# The occurrence of volatile *N*-nitrosamines in Estonian meat products

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**Abstract**

*N*-Nitrosamines (NAs) are a group of carcinogens, which have been detected in various meat products. The level of five NAs, namely *N*-nitrosodimethylamine, *N*-nitrosodiethylamine, *N*-nitrosodibutylamine, *N*-nitrosopiperidine, and *N*-nitrosopyrrolidine was determined in 386 various samples of meat during 2001–2005. Raw, fried, grilled, smoked, pickled, and canned meat products were analyzed. For a sample cleaning the two-step solid-phase extraction with Extrelut and Florisil sorbents was used. NAs were separated by gas chromatography and detected by positive-ion chemical ionization using ammonia as reagent gas. The HP 6890 Plus GC/HP 5973 MSD was used in the selected ion-monitoring mode with pulsed splitless injection. In this work, the limit of detection and the limit of quantitation of NA were approximately 0.09 and 0.29 μg/kg, respectively, with about 85% recovery. NDMA was noted in above 88% of samples, NDEA in 27%, NPYR in 90%, NPIP in 65%, and NDBA in 33% at the mean levels of 0.85, 0.36, 4.14, 0.98, and 0.37 μg/kg, respectively. The level of total volatile NAs with the mean of 3.97 μg/kg was calculated.


*Keywords:* *N*-Nitrosamines; Positive-ion chemical ionization; Gas chromatography; Mass spectrometry; Meat products

## 1. Introduction

Volatile *N*-nitrosamines (NAs) are reasonably anticipated to be human carcinogens based on sufficient evidence of carcinogenicity on experimental animals (IARC, 1978). Humans are exposed to NAs from diet and other environmental sources as well as from endogenous synthesis within the body (Rubenchik, 1990).

The formation of NAs in meat is a complex process and a large diversity of substances could influence nitrosation reaction. Starting materials for NA formation in meat products are nitrate, nitrite, primary, secondary, and tertiary amines, amides, proteins, peptides, and amino acids or precursors of these, which are transformed into NA precursors by microbial action.

Microorganisms could take part in NAs formation by nitrates reduction to nitrites, degradation of proteins to amines and amino acids (Tricker & Preussmann, 1991). NAs are formed after cooking, by an oxygen-dependent mechanism, the key step being the oxidation of nitric oxide (NO) and the formation of higher nitrogen oxides, which could act as direct nitrosating agents. The nitrosating agent responsible for the formation of NAs in fried meat might be $N_2O_3$, formed during heating of nitrite in meat, or NO radical formed by dissociation of $N_2O_3$ at high temperature (Sen, 1986). The structure of the nitrosating agent is not known, but evidence suggests that it is a reaction product of nitrite and lipids in the meat (Liu, Conboy, & Hotchkiss, 1988).

The concentration of NAs in meat products depends on the method of cooking, cooking temperature and time, residual and added nitrite concentration, concentration of NA precursor, presence of nitrosation catalysts and inhibitors, and storage conditions (Fiddler et al., 1978; Gray, Reddy, Price, Mandaguere, & Wilkens, 1982; Sen, Donaldson, Charbonneau, & Miles, 1974a, Sen, Iyengar, Donaldson, & Panalaks, 1974b; Sen, Donaldson, Seaman, Iyengar, & Miles, 1976).

NAs occur as contaminants in different food categories and beverages including vegetable oil (Fiddler, Pensabene,

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& Kimoto, 1981), cheese (Dellisanti, Cerutti, & Airoldi, 1996), drinking water (Jenkins et al., 1995), beer (Sen, Seaman, Begeson, & Brousseau, 1996; Yurchenko & Mölder, 2005), and fish products (Howard, Fazio, & Watts, 1970; Iyengar, Panalaks, Miles, & Sen, 1976; Yurchenko & Mölder, 2006; Zou, Lu, & Liu, 1994). Also the meat products have been studied and NAs detected in preserved sausages (Eerola, Otegui, Saari, & Rizzo, 1998; Karmysheva, Zhukova, Safronova, Somina, & Petrakova, 1985; Sanches Filho, Rios, Valcarcel, Zanin, & Caramao, 2003), smoked lamb meat (Thorkelsson, 1989), cured meat (Biaudet, Mavelle, & Debry, 1994; Groenen, Jonk, van Ingen, & ten Noever de Brauw, 1976; Sen, Seaman, & Miles, 1979; Sen, Baddoo, & Seaman, 1987), ham (Fiddler, Doerr, Ertel, & Wasserman, 1971; Rywotycki, 2002), and in bacon (Gray, Skrypec, Mandagere, Booren, & Pearson, 1984; Hotchkiss & Vecchio, 1985; Skrypec, Gray, Mandagere, Booren, & Cuppet, 1985; Tricker, Perkins, Massey, & McWeeny, 1985).

In the literature, methods for sample preparation are based on vacuum- (Telling, Bryce, & Althorpe, 1971) and steam distillation (Stephany, Freudenthal, & Schuller, 1978), mineral oil distillation (Greenfield, Smith, & Malanovski, 1982; Liu et al., 1988), supercritical fluid extraction (Fiddler & Pensabene, 1996; Maxwell, Pensabene, & Fiddler, 1993), celite column extraction method (Österdahl & Alriksson, 1990), and solid-phase extraction (Andrade, Reyes, & Rath, 2005; Raoul, Gremaud, Biaudet, & Tureski, 1997; Sanches Filho et al., 2003).

The determination of volatile NAs in meat and meat products has been carried out by different analytical methods, including gas–liquid chromatography with thermal energy analyzer (Fiddler et al., 1971; Zhukova, Torskaia, Rodin, & Khotimchenko, 1999) or with a Coulson electrolytic conductivity detector (Mottram, Patterson, Edwards, & Gough, 1977), fluorometric method (Pokrovskii, Kostiukovskii, Melamed, & Medvedev, 1978), micellar electrokinetic chromatography (Sanches Filho et al., 2003), and gas chromatography high resolution mass spectrometry (Stephany et al., 1978).

The purpose of the present study was to measure the content of volatile NAs in Estonian meat products using the method of sample preparation performed in our previous work (Jurtchenko, Tenno, Mölder, & Reinik, 2002), applying mass spectrometry for determination of NAs, and to investigate the influence of cooking temperature and time, storage conditions, and addition of sodium nitrite to the volatile NAs concentration in meat products.

## 2. Method

### 2.1. Samples

All samples of meat products and the oil used for the cooking of meat were purchased from supermarkets in Estonia. Information on the smoking conditions, the species and kind of the meat was collected for each sample and is presented in Table 1. Approximately 50 g of each meat product was crushed, mixed, and analyzed during one month. The samples of meat products and vegetable oil were stored before analysis in closed box at $-20 \pm 2$ °C, and in plastic bottle at $5 \pm 1$ °C, respectively.

### 2.2. Chemicals

Methanol from J.T. Baker (Holland), dichloromethane from Sigma–Aldrich (USA), hexane from Rathburn (Scotland), 0.1 N NaOH solution from Chemapo (Czechoslovakia), Extrelut from Merck (Germany), and Florisil 100/200 from Alltech (Belgium) were purchased for the sample preparation. NDMA, NDEA, *N*-nitrosodibutylamine (NDBA), *N*-nitrosopiperidine (NPIP), and *N*-nitrosopyrrolidine (NPYR) in methanol are commercial products from Aldrich. Mixtures were stored at $-20 \pm 2$ °C and analyzed at room temperature. Helium (99.9996%) was used for gas chromatographic (GC) analysis. Sodium nitrite from Sigma–Aldrich was used for the addition experiment.

### 2.3. Sample preparation

The method of preparation of meat and oil sample was described in our previous work (Jurtchenko et al., 2002). Two-step solid-phase extraction with Extrelut and Florisil sorbent was used for the sample cleaning. The meat sample ($6.0 \pm 1.0$ g) and the oil sample ($10 \pm 1.0$ mL) were mixed with 0.1 N NaOH (6 mL).

As the first step, about 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). After that, the sample was eluted with two 20 mL portions of hexane/dichloromethane solution. The eluate was collected in a 50 mL concentrator flask and evaporated in water bath at 60 °C. As the second step, about 1 g Florisil was placed at the bottom of the Florisil cartridge (6.5 cm × 1.3 cm), wetted with 6 mL dichloromethane/methanol 95:5 (v:v), and eluted with 6 mL dichloromethane/methanol solution. The solution was evaporated at 60 °C to 1 mL. The prepared solution was transferred to the GC injector vial. Extractions were performed in duplicate.

### 2.4. Gas chromatography with mass selective detector (MSD)

GC analysis was carried out using Hewlett–Packard Model 6890 gas chromatograph equipped with a split/splitless injector. Five microlitre of the sample solution was injected into the gas chromatograph using pulsed splitless injection in the selected ion-monitoring mode. Detection was done by a Hewlett–Packard MSD 5973 MSD mass spectrometer using a positive-ion chemical ionization with ammonia as reagent gas.

Sample portions were injected into a chromatograph column (30 m HP-1701 MS; 0.25 mm i.d., 0.25 μm film thickness) containing 14% cyanopropylphenyl and 86%

Table 1
The level of NAs in various meat products analyzed during 2001–2005

| Product | No. of samples | Mean concentration ($n = 3$) of NAs (μg/kg) | | | | | Sum of five NAs (μg/kg) |
|---|---|---|---|---|---|---|---|
| | | NDMA | NDEA | NPYR | NPIP | NDBA | |
| *Raw meat* | | | | | | | |
| Pork | 8 | n.d.[a] | n.d. | n.d. | n.d. | n.d. | n.d. |
| Beef | 10 | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| Mutton | 9 | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| Poultry | 8 | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| *Smoked meat* | | | | | | | |
| Half-smoked sausage | 32 | 1.44 | 0.48 | 3.10 | 2.33 | 0.60 | 7.95 |
| Smoked sausage | 25 | 1.01 | 0.33 | 2.43 | 0.89 | 0.44 | 5.10 |
| Cooked sausage | 10 | 0.34 | 0.29 | 0.81 | 0.42 | n.d. | 1.86 |
| Frankfurters | 10 | 0.16 | n.d. | 0.48 | n.d. | n.d. | 0.64 |
| Salami | 10 | 0.84 | 0.67 | 0.93 | 0.64 | 0.84 | 3.92 |
| Ham | 28 | 1.00 | 0.37 | 3.73 | 1.79 | 0.44 | 7.33 |
| Bacon | 12 | 1.12 | 0.65 | 2.04 | 1.23 | 0.94 | 5.98 |
| Pork | 33 | 1.16 | 0.40 | 7.48 | 1.61 | 0.72 | 11.37 |
| *Grilled meat* | | | | | | | |
| Sausage | 10 | 0.81 | 0.42 | 2.28 | 1.77 | 0.26 | 5.54 |
| Poultry | 8 | 1.23 | 0.45 | 8.38 | 1.69 | 0.22 | 11.97 |
| Poultry with spices | 10 | 1.41 | 0.64 | 14.60 | 1.96 | 0.26 | 18.87 |
| Pork | 8 | 1.14 | 0.34 | 6.53 | 1.51 | 0.19 | 9.71 |
| Pork with spices | 10 | 1.32 | 0.52 | 11.34 | 1.57 | 0.21 | 14.96 |
| *Fried meat* | | | | | | | |
| Poultry | 8 | 1.16 | 0.71 | 15.24 | 1.09 | 0.32 | 18.52 |
| Poultry with paprika | 15 | 1.30 | 0.88 | 20.67 | 1.14 | 0.43 | 24.42 |
| Pork | 8 | 1.02 | 0.66 | 10.23 | 1.13 | 0.29 | 13.33 |
| Pork (lean only) | 5 | 0.41 | 0.22 | 2.38 | 0.33 | 0.21 | 3.55 |
| Pork (fat only) | 5 | 3.23 | 0.51 | 14.11 | 1.02 | 0.44 | 19.31 |
| Pork with paprika | 20 | 1.20 | 0.74 | 14.65 | 1.28 | 0.36 | 18.23 |
| Pork with paprika (lean only) | 5 | 0.61 | 0.31 | 3.63 | 0.51 | 0.37 | 5.43 |
| Pork with paprika (fat only) | 5 | 4.92 | 0.79 | 24.42 | 1.62 | 0.71 | 32.46 |
| Mutton | 10 | 1.04 | 0.64 | 2.58 | 0.97 | 0.28 | 5.51 |
| *Canned meat* | | | | | | | |
| Pork | 19 | 1.05 | n.d. | 2.24 | 1.15 | 0.65 | 5.09 |
| Beef | 15 | 0.95 | n.d. | 2.12 | 1.21 | 0.52 | 4.80 |
| Poultry | 12 | 0.93 | n.d. | 2.02 | 1.13 | 0.51 | 4.59 |
| *Pickled meat* | | | | | | | |
| Pork | 9 | 0.83 | n.d. | 0.41 | 0.31 | 0.20 | 1.75 |
| Poultry | 9 | 0.67 | n.d. | 0.44 | 0.28 | 0.19 | 1.58 |

[a] n.d., not detected.

methyl polysiloxane. For the gas chromatography separation of NAs oven programme started at 35 °C (held 1 min), set at 50 °C/min from 35 to 240 °C and held isothermally at 240 °C for 1 min; the velocity of He carrier gas (99.9996%) was 1 mL/min.

To calibrate the GC–MSD spectra six different standard solutions were prepared which cover the concentration range 0.1–50 ng/mL. In this study, the squared correlation coefficient for different calibration curves of NAs was found to be 0.9991–0.9998. This calibration curve enables to calculate NA concentration using the GC–MS peak area measurement.

### 2.5. Validation of the method

To demonstrate the method was under analytical control the limit of detection (LOD), the limit of quantitation (LOQ), and recovery experiments were performed. The LOD and the LOQ have been established using spiked samples. The sample of raw mutton was fortified with appropriate volumes of standard solutions in methanol to get recovery at the level 0.50 μg/kg. The value of the LOD was calculated as follows:

$$\mathrm{LOD} = X_{\mathrm{bl}} + K \times \mathrm{SD}_{\mathrm{bl}},$$

where $X_{\mathrm{bl}}$ is the mean of the blank measures, $\mathrm{SD}_{\mathrm{bl}}$ is the standard deviation of the blank measures, and $K$ is a numerical factor chosen according to the confidence level desired. If confidence level is 95%, the $K$ is 3.36. The LOQ is then 3.3 times the LOD (The Nordic Committee of Food Analysis, 1996). The values of the LOD and the LOQ for this method are presented in Table 2.

For the recovery experiment, a sample with a low content (raw mutton) of NAs was chosen and fortified with

Table 2
The LOD and the LOQ data (μg/kg) of volatile NAs in raw mutton ($n = 6$)

| Compound | Unfortified sample | Fortified samples[a] | | | | | |
|---|---|---|---|---|---|---|---|
| | | Average concentration of NA (μg/kg) | Rec (%)[b] | SD[c] | RSD (%)[d] | LOD | LOQ |
| NDMA | n.d. | 0.40 | 79 | 0.023 | 5.75 | 0.08 | 0.26 |
| NDEA | n.d. | 0.42 | 82 | 0.027 | 6.43 | 0.09 | 0.30 |
| NPYR | n.d. | 0.44 | 88 | 0.032 | 7.27 | 0.11 | 0.35 |
| NPIP | n.d. | 0.40 | 86 | 0.022 | 5.50 | 0.07 | 0.24 |
| NDBA | n.d. | 0.41 | 88 | 0.027 | 6.59 | 0.09 | 0.30 |

[a] 0.50 μg/kg of NA added.
[b] Average recovery of NA in percent.
[c] Sample standard deviation.
[d] Relative standard deviation in percent.

Table 3
Validation data of five NAs spiked at two levels in raw mutton ($n = 6$)

| Compound | Unfortified sample | Fortified samples | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Level I[a] | | | | Level II[b] | | | |
| | | Average concentration of NA (μg/kg) | Rec (%) | SD | RSD (%) | Average concentration of NA (μg/kg) | Rec (%) | SD | RSD (%) |
| NDMA | n.d. | 1.13 | 75 | 0.092 | 8.14 | 2.90 | 73 | 0.223 | 7.69 |
| NDEA | n.d. | 1.18 | 79 | 0.085 | 7.20 | 3.01 | 75 | 0.206 | 6.84 |
| NPYR | n.d. | 1.21 | 81 | 0.113 | 9.34 | 3.12 | 78 | 0.323 | 10.35 |
| NPIP | n.d. | 1.20 | 80 | 0.131 | 10.92 | 3.08 | 77 | 0.311 | 10.10 |
| NDBA | n.d. | 1.23 | 82 | 0.124 | 10.08 | 3.15 | 79 | 0.324 | 10.29 |

[a] 1.5 μg/kg of NA added.
[b] 4 μg/kg of NA added.

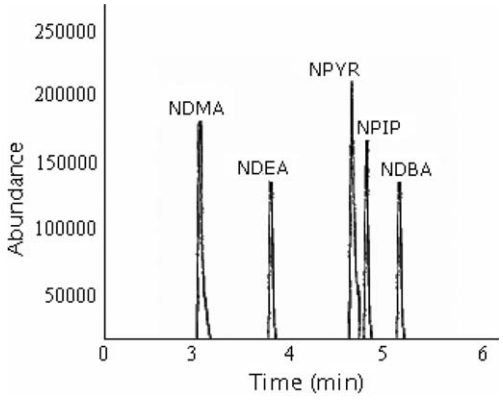


Fig. 1. Total-ion chromatogram of five volatile NAs extracted from smoked sausage sample after analytical cleanup (spiked with 5 μg/kg of each single NA).

Table 4
Analytical repeatability of the proposed method (sample of canned pork)

| Compounds | Mean concentrations ($n = 6$) of NAs (μg/kg) | SD | RSD (%) |
|---|---|---|---|
| NDMA | 1.01 | 0.071 | 7.03 |
| NDEA | n.d. | – | – |
| NPYR | 2.20 | 0.192 | 8.75 |
| NPIP | 1.08 | 0.091 | 8.43 |
| NDBA | 0.52 | 0.045 | 8.65 |

two different levels of NAs standard solutions. The sample was analyzed by GC–MS and recovery of NAs was calculated as follows (Eurachem Guide, 1998):

$$\text{Rec } (\%) = [(C_1 - C_2)/C_3] \times 100,$$

where $C_1$ is the concentration determined in the fortified sample, $C_2$ is the concentration determined in the unfortified sample, and $C_3$ is the concentration of fortification. Table 3 lists for the two fortification levels, the NA amount (μg/kg) found in the sample of mutton after fortification, the percentage recovery (mean of six replicates), the standard- and relative standard deviation of the replicates. Fig. 1 shows the chromatogram of five NAs extracted from smoked sausage spiked with 5 μg/kg of each single NA. The repeatability data resulting from six replicate analyses of the same sample of canned pork are reported in Table 4. The results characterize the suitability of the method for determination the NAs in various samples of meat products.

## 3. Results and discussion

In present study we concern with various meat products, which are a very popular dish in Estonia. The levels of volatile NAs, namely NDMA, NDEA, NDBA, NPYR, and NPIP in 386 samples of meat products purchased from Estonian market were determined. The samples with high NA content were repeatedly analyzed.

Table 1 shows the concentrations of NAs found in studied samples of meat products. The results reported for NAs levels were not corrected for recovery. In samples of raw meat, NAs were not detected. In 189 of 386 samples of various meat products, the concentration of NDMA exceeded 1 μg/kg. The concentration of all NAs observed in smoked sausages was lower than in half-smoked sausages and fried meat products. This may be due to the variation in nitrite level in various meat products. One may anticipate that in smoked sausages the nitrite concentration is lower than in half-smoked sausages. Among the analysed assortments, the highest level of NDMA was recorded in half-smoked sausage, in grilled poultry and pork, and in fried pork. The highest level of NDEA with the mean concentration of 0.88 μg/kg was found in fried poultry. The very high level of NPYR with the mean concentration of 20.67 μg/kg was detected in fried poultry with paprika. The paprika may contain precursors of NPYR (Huxel, Scanlan, & Libbey, 1974). The level of NAs in meat without paprika is lower than in meat with paprika. Differences in the NPIP content may be the results of applied spices and different degree of meat crumbling, thus different penetration of spices into the whole capacity of meat pieces (Domanska & Kowalski, 2003). The black pepper, which contains piperidine, could be the main source of NPIP. We may anticipate that the concentration of NA in grilled meat without spices is lower than in grilled meat with spices. Cadaverin, the product of lysine decarboxylation during thermal processing of meat or its maturing could be also the precursor of NPIP (Sen et al., 1974a, 1974b). As pyrolysis of protein by cooking can be a source of secondary amines, the formation of significant amounts of volatile NAs in the meat may be also caused by the interaction of nitrites and amines in the meat.

Sodium nitrite is normally added to meat products such as canned sausages, ham, and salamis to prevent the formation of toxins produced by *Clostridium botulinium*. Nitrite is also responsible for the development iron reaction with some meat pigments to produce the desirable red colour, and flavour characteristic for these products. Nitrite, however, is converted to nitrosating agents that may react with amines and amino acids in meat to produce carcinogenic NAs (Gloria, Barbour, & Scanlan, 1997; Sanches Filho et al., 2003; Woods & Woods, 1982). Since nitrite is highly reactive, its concentration in meat products gradually decreases with storage and depends on many factors such as processing conditions, duration and condition of storage, and cooking methods (Sen, 1986). Frouin has hypothesized that much of nitrite added to meat is rapidly converted to various forms of bound nitric oxide, i.e., by reaction with proteins, thiols, hydroxyls, carboxylas, and reducing agents (Frouin, 1976). NA formation from bound nitric oxide may occur either by direct transnitrosation of the secondary amines or by an indirect process involving the initial release of the nitric oxide moiety (Dennis, Massey, & McWeeny, 1982).

The raw mutton contains no detectable amounts of NAs. To investigate the influence of addition of sodium nitrite on the growth of volatile NAs in meat products, sodium nitrite solutions with 50, 100, 150, and 200 mg/kg concentrations were added to raw mutton. After adding, the samples of mutton were stored during 24 h at $5 \pm 1$ °C before analysis. Since, part of the samples was fried in a frying pan during 30 min using natural gas. The samples of raw and fried mutton prepared were analyzed for NAs. All samples with added nitrite, fried or not, contained detectable levels of NAs. Data of NA concentration in fried and raw mutton samples with different concentrations of sodium nitrite are presented in Table 5. In general, the sample of mutton processed with high levels of added nitrite and fried in a conventional frying pan contains the highest levels of NAs. From the results, it may be concluded that meat curing with sodium nitrite increases remarkably the level of short chain NAs. The process of frying causes increase of slope, i.e. the sensitivity of all NAs to nitrosation about 10 times compared to raw mutton samples. The dependence of concentration of NAs produced in fried mutton with added sodium nitrite is roughly linear in selected range of concentrations. Most sensitive for sodium nitrite addition is NPYR (about 0.16 μg pro 1 mg $NaNO_2$). The other NAs were produced in less amounts, the concentration increase pro 1 mg added $NaNO_2$ was about 0.020 μg for NDMA, 0.014 for NPIP, 0.005 for NDEA and NDBA, respectively. The results shows, that the concentration of NA is in direct dependence from the nitrite concentration.

Increased level of NAs was observed after baking and frying indicating the formation of these compounds during cooking. In 81 samples of fried meat from market, the average of NDMA concentration was 1.26 μg/kg, NDEA was

Table 5
Level of NAs in fried and raw mutton samples prepared with different level of sodium nitrite

| Sodium nitrite (mg/kg) | Mean concentrations ($n = 3$) of NAs (μg/kg) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Fried mutton | | | | | Raw mutton | | | | |
| | NDMA | NDEA | NPYR | NPIP | NDBA | NDMA | NDEA | NPYR | NPIP | NDBA |
| 0 | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| 50 | 1.25 | 0.21 | 5.24 | 0.63 | 0.24 | 0.32 | 0.14 | 0.83 | 0.26 | 0.14 |
| 100 | 2.03 | 0.32 | 14.85 | 1.20 | 0.43 | 0.47 | 0.22 | 1.91 | 0.31 | 0.22 |
| 150 | 2.83 | 0.64 | 20.71 | 1.92 | 0.61 | 0.63 | 0.26 | 2.66 | 0.43 | 0.30 |
| 200 | 4.22 | 1.17 | 32.81 | 2.75 | 0.88 | 0.79 | 0.36 | 3.83 | 0.64 | 0.42 |

0.85 μg/kg, NPYR was 13.63 μg/kg, NPIP was 1.33 μg/kg, and NDBA – 0.44 μg/kg. The effect of baking in an electrical oven on the volatile NA level was investigated as following. Approximately 80 g of mutton was divided into six roughly equal parts. One portion was reserved for analysis in the raw state. The other five portions (3 mm mutton slices) were baked separately in pre-heated electrical oven in open oven-proof glass dishes with 50 mL olive oil at regulo 50, 100, 150, 200 and 250 °C, during 30 min. The cooked portions were allowed to cool but before becoming cold the excess fat and oil were separated from the sample and analysed. Neat olive oil has also been analysed after baking in electrical oven at different temperatures. The level of NAs was determined also in baked mutton without oil. All these data are presented in Table 6. There was significant increase in NAs level in the meat and in the oil after baking with electrical oven at temperature around 150 °C. The concentration of NA in baked mutton parts with olive oil is equal to the sum of the concentration of NA in baked mutton without oil and in neat olive oil. Among them, the concentration of NDMA and NDEA at all used temperatures in neat olive oil prevails remarkable the concentration in mutton. Particularly important is the role of fat in baked mutton. It appears, that about 73% of NAs are concentrated in fat of baked mutton. Thereby, at baking temperatures lower than 200 °C about 90% of NDMA is concentrated in fat whereas NPYR abundance in fat reaches but 30% by these temperatures. At 250 °C baking temperature, the abundance of NDMA in fat is 73% and NPYR 64%, respectively.

Table 6
Influence of baking temperature on the content of volatile NAs in samples of olive oil and mutton

| Temperature (°C) | Mean concentrations ($n = 3$) of NAs (μg/kg) | | | | | Sum of NAs (μg/kg) |
|---|---|---|---|---|---|---|
| | NDMA | NDEA | NPYR | NPIP | NDBA | |
| *Baked mutton with olive oil* | | | | | | |
| 50 | 0.68 | 0.51 | 0.60 | 0.24 | n.d. | 2.03 |
| 100 | 0.70 | 0.52 | 0.62 | 0.24 | n.d. | 2.08 |
| 150 | 0.73 | 0.54 | 0.64 | 0.25 | n.d. | 2.16 |
| 200 | 1.35 | 0.95 | 1.96 | 1.29 | 0.14 | 5.69 |
| 250 | 1.37 | 0.95 | 1.96 | 1.30 | 0.14 | 5.72 |
| *Excess fat of baked mutton with olive oil* | | | | | | |
| 50 | 0.62 | 0.53 | 0.18 | 0.14 | n.d. | 1.47 |
| 100 | 0.63 | 0.53 | 0.18 | 0.15 | n.d. | 1.49 |
| 150 | 0.62 | 0.52 | 0.20 | 0.18 | n.d. | 1.52 |
| 200 | 0.99 | 0.84 | 1.26 | 0.87 | 0.21 | 4.17 |
| 250 | 1.00 | 0.85 | 1.27 | 0.87 | 0.21 | 4.20 |
| *Olive oil* | | | | | | |
| 50 | 0.52 | 0.46 | 0.12 | 0.11 | n.d. | 1.21 |
| 100 | 0.52 | 0.48 | 0.13 | 0.11 | n.d. | 1.24 |
| 150 | 0.53 | 0.48 | 0.12 | 0.12 | n.d. | 1.25 |
| 200 | 0.90 | 0.71 | 1.10 | 0.75 | 0.11 | 3.57 |
| 250 | 0.91 | 0.71 | 1.09 | 0.76 | 0.11 | 3.58 |
| *Baked mutton without oil* | | | | | | |
| 50 | 0.15 | n.d. | 0.50 | 0.10 | n.d. | 0.75 |
| 100 | 0.16 | n.d. | 0.50 | 0.11 | n.d. | 0.77 |
| 150 | 0.15 | n.d. | 0.52 | 0.11 | n.d. | 0.78 |
| 200 | 0.41 | 0.21 | 0.81 | 0.49 | n.d. | 1.92 |
| 250 | 0.41 | 0.22 | 0.81 | 0.50 | n.d. | 1.94 |

Table 7
The effect of cooking (30 min) in an electrical oven, in a microwave oven, and in a frying pan on the level of volatile NAs in mutton

| Compound | Mean concentrations ($n = 4$) of NAs (μg/kg) | | |
|---|---|---|---|
| | Microwave oven (120 °C) | Electrical oven (150 °C) | Frying pan (150 °C) |
| NDMA | 0.48 | 0.71 | 1.16 |
| NDEA | 0.36 | 0.55 | 0.72 |
| NPYR | 0.51 | 0.62 | 3.17 |
| NPIP | 0.19 | 0.22 | 1.14 |
| NDBA | n.d. | n.d. | 0.35 |

To determine the influence of cooking method on the growth of volatile NAs in meat products the sample of mutton (thin slices) was baked in an electrical oven at 150 °C, cooked in a microwave oven without oil at 120 °C, and fried in a frying pan with olive oil at 150 °C. The results are given in Table 7. The level of NA in gas-frying sample of mutton exceeded the level in the electric-oven- and in microwave oven-baking mutton. The formation of NAs increases with time and temperature of frying. For this experiment, the sample of mutton was fried in a frying pan using natural gas. Mutton slices were fried during 30 min at $150 \pm 1$ °C. The temperatures of meat were determined by thermocouples inserted into the slices. After frying for 10 min, the temperature of mutton maximized to 102 °C. As shown in Fig. 2, the concentration of NAs increases rapidly in range from 40 to 100 °C and then stays stabile. Hence, the formation of NAs takes place in temperatures below 100 °C.

In the next experiment, the pork was fried in a frying pan with olive oil using natural gas. The total amount of

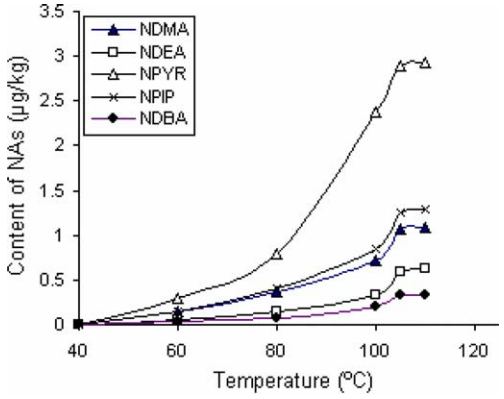


Fig. 2. Influence of frying temperature on the content of volatile NAs in mutton.

Table 8
Mean concentrations of five volatile NAs in studied samples of fried mutton and canned pork stored in different conditions

| Time of storing (h) | Mean concentrations ($n = 3$) of volatile NAs (μg/kg) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Fried mutton | | | | | Canned pork | | | | |
| | NDMA | NDEA | NPYR | NPIP | NDBA | NDMA | NDEA | NPYR | NPIP | NDBA |
| Purchase day | 1.31 | 0.76 | 4.15 | 2.07 | 0.41 | 0.97 | n.d. | 2.15 | 1.18 | 0.60 |
| *At 5 ± 1 °C* | | | | | | | | | | |
| 24 | 1.30 | 0.75 | 4.20 | 2.07 | 0.43 | 1.10 | n.d. | 2.20 | 1.20 | 0.60 |
| 48 | 1.33 | 0.77 | 4.18 | 2.08 | 0.42 | 2.05 | n.d. | 3.65 | 2.10 | 0.78 |
| 72 | 1.31 | 0.76 | 4.19 | 2.08 | 0.42 | 2.12 | 0.21 | 4.53 | 3.20 | 0.88 |
| 96 | 1.32 | 0.77 | 4.20 | 2.06 | 0.44 | 2.21 | 0.32 | 4.54 | 3.32 | 0.90 |
| 120 | 1.30 | 0.78 | 4.19 | 2.07 | 0.42 | 2.24 | 0.31 | 4.60 | 3.35 | 0.93 |
| *At 20 ± 2 °C* | | | | | | | | | | |
| 24 | 1.28 | 0.73 | 4.16 | 2.02 | 0.38 | 0.96 | n.d. | 2.04 | 1.10 | 0.57 |
| 48 | 1.22 | 0.68 | 4.11 | 1.96 | 0.33 | 0.84 | n.d. | 1.92 | 0.95 | 0.48 |
| 72 | 1.17 | 0.63 | 4.07 | 1.91 | 0.28 | 0.75 | n.d. | 1.83 | 0.74 | 0.38 |
| 96 | 1.14 | 0.59 | 4.01 | 1.86 | 0.23 | 0.68 | 0.18 | 1.65 | 0.59 | 0.35 |
| 120 | 1.14 | 0.58 | 4.00 | 1.86 | 0.22 | 0.62 | 0.20 | 1.64 | 0.56 | 0.34 |

NAs obtained from the frying of whole rashers, separated lean and fat component is given in Table 1. After frying for 30 min per side, NAs were found in 5 of 5 samples of pork. The total yield of NAs from the fat of pork was much greater than that from either the lean or even whole rasher. In fried pork with paprika, the concentration of NAs in fat exceeds the concentration in lean 6 times (among them NDMA 8 times, NDEA 2.5 times, NPYR 6.7 times, NPIP 3.2 times, and NDBA 1.9 times). In fried pork without paprika, the concentration of NAs in fat exceeds the concentration in lean 5.4 times. Hence, one can conclude that the effect of paprika in meat does not prevail in the formation of NAs in frying process.

The effect of different storage conditions on NA content of fried meat products was investigated. Thirty three samples of fried mutton was stored in different conditions, e.g., to 120 h at 5 ± 1 °C and at 20 ± 2 °C. After storage period, the NA content of fried mutton was determined. Mean concentrations of five volatile NAs studied in mutton after storage under different conditions are given in Table 8. It was observed that temperature and time of storage had a significant effect on the formation of NAs. The changes of NAs concentration observed in our experiments after applied different storage conditions seem to be the result of chemical reactions between precursors of NAs present or formed in meat products. No significant storage effects are observed for samples stored at 5 °C for 120 h. Higher storage temperature (20 °C) is optimal for bacterial growth and their metabolism, thus the decrease in NA levels after storage could be caused by bacterial actions (Domanska & Kowalski, 2003) or vaporization as well. A different effect appeared as we stored canned pork purchased from market. As shown in Table 8, we observe the surprising increase of NAs concentration with time of storing at 5 ± 1 °C, and the decrease at 20 °C. As the latter effect can be understood as before, the increase of NAs amount in time at low temperatures must be due to different reactions paths of precursors of NAs introduced in samples (sodium nitrite, spices). The changes of NAs concentrations observed in experiments after applied storage conditions seem to be the result of chemical reactions between precursors of NAs present or formed in meat products.

For the comparison, the content of NAs found in various meat products was confronted with data from the literature. The levels of NAs in Estonian meat products in the present study show good agreement with results from France (Biaudet et al., 1994), Russia (Pokrovskii et al., 1978; Zhukova et al., 1999), Germany (Kuehne & Mirna, 1981;Spiegelhalder, Eisenbrand, & Preussmann, 1980; Tricker & Preussmann, 1991), Poland (Domanska & Kowalski, 2003), Sweden (Österdahl, 1988), UK (Gough, Webb, & Coleman, 1978), and Japan (Maki, Tamura, Shimamura, & Navi, 1980; Yamamoto, Iwata, Ishiwata, Yamada, & Tanimura, 1984).

## 4. Conclusion

In the present study, five NAs were separated by gas chromatography and differentiated by positive-ion chemical ionization using ammonia as a reagent gas. The limit of detection and the limit of quantitation for this method were approximately 0.09 and 0.29 μg/kg, respectively. The recovery of NAs in meat products varied from 79% to 88%.

Total concentrations of NAs in 386 studied samples of meat ranged from non-detectable to 30 μg/kg. The highest levels of NAs were found in samples of fried meat. Relatively high level was found in grilled meat, in smoked pork, in half-smoked sausage, and in ham. With the addition of sodium nitrite, one can observe roughly linear increase in concentration of NAs in fried and raw meat. About 73% of NAs are concentrated in fat of baked mutton. In fried pork, the concentration of NAs in fat exceeds the concentration in lean 6 times. Apparently, the temperature and

time of cooking, nitrite concentration, and storage conditions of meat have a significant effect on the concentration of NAs.

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