OCCURRENCE OF VOLATILE N-NITROSAMINES IN POLISH PROCESSED MEAT PRODUCTS

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N-nitrosoamine content was determined in 170 samples of processed meat products collected from meat factories located in different areas of Poland in 1998-1999. These products included cooked hams and the like, sausages and edible offals processed meat products. The analyses were performed by gas chromatography-thermal energy analyser (GC-TEA). Detectable levels of N-nitrosamines were found in above 83% of the individual samples. About 75% of samples contained NDMA at the mean concentration of 2.55 g/kg. NPIP was noted in above 20% of samples, NDBA in 13% and NDEA in 17% at the mean levels of 0.05 µg/kg, 0.35 µg/kg and 0.08 µg/kg, respectively. Sporadically, NMOR and NPYR were detected. The level of total volatile N-nitrosamines with the mean of 3.15 µg/kg was calculated. The average daily intake of NDMA with processed meat products in Poland was found to be at the level of about 0.2 µg/person.

Key words: processed meat products, N-nitrosamines, contamination.

N-nitroso compounds have mutagenic, teratogenic and carcinogenic activity. The first evidence of N-nitrosamine occurrence possibility in food or animal feed came from Norway as the result of the analysis of nitrite-preserved herring meal used for animal feeding (10, 19). The fact that N-nitrosodimethylamine (NDMA) could be formed in food products and cause acute hepatotoxic and sometimes lethal effect in the animal consuming contaminated fish meal influenced a great deal of concern (11). In many countries a monitoring program was applied to follow the level of volatile N-nitrosamines in food. Moreover, USA set the level of 10.0 µg/kg of N-nitrosopyrrolidine (NPYR) as the limit permitting to introduce products to market (7). Besides, Russia set the maximum level of the sum of NDMA and N-nitrosodiethylamine (NDEA) at 2.0-4.0 µg/kg, above this limit meat products are not permitted to market (15).

NDMA is the most commonly encountered volatile nitrosamine in food samples and it occurs in the highest concentrations. NDMA is also one of the most toxic nitrosamines: LD$_{50}$ in rats, after single administration of this compound, is 30 mg/kg body weight; the lowest effective carcinogenic doses of NDMA in chronic feeding studies on mice are about 10 µg/kg food (1, 12). Nevertheless, NDMA levels
found in some human food and beverage samples can be greater than 10 μg/kg. The monitoring studies undertaken to determine the level of N-nitrosamines in French food revealed that 427 of 556 analysed food samples (68%) contained NDMA (2). This N-nitroso compound was found in meat products in trace quantity (0.04-0.46 μg/kg) and the highest level was observed in processed fish (13.4 μg/kg). In a food survey conducted in Germany, NDMA was detected in 31.5% of analysed samples. The major dietary sources of this compound included cooked meat products (0.2-2.5 μg/kg), cooked fish (0.5-8.0 μg/kg) and spices (0.1-1.4 μg/kg) (24). The food survey conducted in Poland revealed that 71% cured and 61% pasteurized pork hams contained NDMA in the range of 0.1-0.5 μg/kg; also N-nitrosodimethylamine (NDEA) (0.3-1.6 μg/kg), N-nitrosodibutylamine (NDBA) (1.7-7.5 μg/kg), and N-nitrosopiperydine (NPIP) (1.4-2.2 μg/kg) were found occasionally (26). Kowalski et al. reported NDMA in 63.3% of 330 analysed processed poultry products with the mean concentration of 1.57 μg/kg and also sporadically NDBA and N-nitrosomorpholine (NMOR). Similar situation was observed in the survey of edible offals processed meat products: 67.6% samples contained NDMA with the mean concentration of 1.39 μg/kg. Analysis of 280 samples of tinned food revealed that 46.1% contained NDMA and/or NPIP in the range of 0.07-4.5 μg/kg (13).

The present survey was performed to evaluate the occurrence of volatile N-nitrosamines in Polish processed meat products and to estimate daily dietary intake of NDMA in Poland in 1998-1999.

**Material and Methods**

**Samples.** One hundred and seventy samples of different processed meat products such as cooked hams and the like, sausages, and edible offals processed meat products were collected in 1998-1999 by veterinary inspectors from meat processing plants located in different parts of Poland and transported in refrigerated containers to the laboratory for analysis (18). Samples were analysed directly after transportation or frozen at –35°C and the N-nitrosamine levels were determined later.

**Volatile nitrosamines analysis.** Volatile nitrosamines were extracted by low temperature vacuum distillation according to the method recommended by Food Safety and Inspection Service (FSIS) (6). The distilled extracts were quantitatively analysed on a gas chromatograph (GC, Varian, model 1440) interfaced with a thermal energy analyser (TEA, model 502A, Thermo Electron Corporation, Waltham, MA). Identification and quantification of the nitrosamines were carried out by analysis of known amounts of nitrosamine standard mixture containing N-nitrosodimethylamine (NDMA), N-nitrosodimethylamine (NDEA), N-nitrosodipropylamine (NDPA), N-nitrosodibutylamine (NDBA), N-nitrosopiperidine (NPIP), N-nitrosopyrrolidine (NPYR), N-nitrosomorpholine (NMOR) (certified standards from Chem-Services, which is also supplier of N-nitrosamine standards for Food and Drug Administration - FDA), N-nitrosodisopropylamine (NDiPA) added to the samples before extraction was used as the internal standard. GC-TEA conditions were as follows: column: 2.7 m x 3 mm i.d. packed with 15% Carbowax 20 M-TPA on a Chrom W-HP 80/100 mesh; column temperature: 170°C; injection port temperature: 200°C; carrier gas: He at 25-30ml/min.; TEA furnace temperature: 475°C; vacuum: 0.3 Torr, velocity of oxygen: 15-20 ml/min. The method enables the identification and simultaneous quantification
of seven nitrosamines at the level above of 0.05-0.2 μg/kg depending on analysed material and compound. Recoveries were at the level of 76-98%. Direct confirmation of analysed compounds was performed in a few selected extracts by gas chromatography coupled with mass spectrometry (GC: model 5970B, Hewlett-Packard; MSD: Electron Impact, EI, 70eV) according to the method recommended by FSIS (6).

**Statistical calculations.** Results are expressed as mean ± SD of N-nitrosamine concentrations (μg/kg). The Anova Kruskal-Wallis test was used to conduct statistical analysis. Differences between mean values were considered significant at P < 0.05.

**Results**

The number of studied processed meat products and number of the positive ones are given in Table 1. These products were grouped into three assortments: cooked hams and the like, sausages and edible offals processed meat products. In a great majority (above 83%) of 170 analyzed samples one or more volatile N-nitrosamines were detected, in all assortments the percentage of positive samples were more or less the same. Mean concentrations of seven volatile N-nitrosamines in studied processed meat products are given in Fig. 1. The number of positive samples and the minimum and maximum concentration of the studied volatile N-nitrosamines are presented in Table 2. About 75% of samples contained NDMA at the mean levels of 2.55 μg/kg (the range 0.08-30.10 μg/kg). NPIP was noted in above 20% of samples at the mean concentration of 0.05 μg/kg. NDBA and NDEA were identified in 13% and about 17% of samples at mean levels of 0.35 μg/kg and 0.08 μg/kg, respectively. NMOR and NPYR were found in 9 and 3 samples, respectively. No sample contained NDPA. The level of total volatile N-nitrosamines (TN) with the mean of 3.15 μg/kg was calculated.

Among the analysed assortments the highest level of NDMA was recorded in edible offals processed meat products with the mean concentration of 5.07 μg/kg; in cooked hams and the like and sausages the mean concentrations of this compound were significantly lower, 0.62 and 0.95 μg/kg, respectively. The highest level of NDEA with the mean concentration of 0.18 μg/kg in cooked hams and the like was found. The highest level of NPIP with the mean concentration of 0.07 μg/kg was detected in edible offals processed meat products. NDBA was present in cooked hams and the like and edible offals processed meat products with similar concentration of 0.48 μg/kg-0.50 μg/kg and with similar frequency. The highest concentration of TN at the level of 5.90 μg/kg was in edible offals processed meat products. Detected difference in TN concentration in processed meat products in comparison with the other assortments was statistically significant at P < 0.05. In sausages and cooked hams and the like TN were found at the concentration of 1.03 μg/kg and 1.34 μg/kg, respectively.
Table 1
Results of volatile N-nitrosamine analysis in processed meat products

<table>
<thead>
<tr>
<th>Assortment</th>
<th>Number of samples studied</th>
<th>Number of positive samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>hams and the like</td>
<td>50</td>
<td>40</td>
</tr>
<tr>
<td>sausages</td>
<td>50</td>
<td>42</td>
</tr>
<tr>
<td>edible offals processed meat products</td>
<td>70</td>
<td>59</td>
</tr>
<tr>
<td>processed meat products</td>
<td>170</td>
<td>141</td>
</tr>
</tbody>
</table>

Table 2
Number of positive samples and the minimum and maximum concentration (μg/kg) of individual volatile N-nitrosamines in analyzed processed meat products

<table>
<thead>
<tr>
<th>NDMA</th>
<th>NDEA</th>
<th>NDBA</th>
<th>NPIP</th>
<th>NPYR</th>
<th>NMOR</th>
</tr>
</thead>
<tbody>
<tr>
<td>n min.</td>
<td>n min.</td>
<td>n min.</td>
<td>n min.</td>
<td>n min.</td>
<td>n min.</td>
</tr>
<tr>
<td>max.</td>
<td>max.</td>
<td>max.</td>
<td>max.</td>
<td>max.</td>
<td>max.</td>
</tr>
<tr>
<td>cooked ham and the like</td>
<td>32 0.08</td>
<td>13 4.80</td>
<td>11 1.50</td>
<td>11 0.50</td>
<td>2 0.15</td>
</tr>
<tr>
<td>sausage</td>
<td>39 0.30</td>
<td>11 4.60</td>
<td>11 0.23</td>
<td>- -</td>
<td>47 0.10</td>
</tr>
<tr>
<td>edible offals processed meat product</td>
<td>57 0.30</td>
<td>6 30.10</td>
<td>11 0.80</td>
<td>11 2.00</td>
<td>16 4.30</td>
</tr>
<tr>
<td>processed meat product</td>
<td>128 0.08</td>
<td>30 30.10</td>
<td>22 1.50</td>
<td>22 0.50</td>
<td>65 6.30</td>
</tr>
</tbody>
</table>

Fig. 1. Mean concentrations (µg/kg) of volatile N-nitrosamines in analysed processed meat products.


1 - significantly different at P < 0.05 in comparison with the cooked ham and the like
2 - significantly different at P < 0.05 in comparison with the sausages

Discussion

The present study has concerned processed meat products which are a very popular meat dish in Poland. NDMA was the most frequently occurring volatile nitrosamine, found in 75.3% of samples (Table 2). The most contaminated samples were edible offals processed meat products. Above 27% of these products contained NDMA at the concentration exceeding the level of 5.0 µg/kg. Very limited reportson the occurrence of N-nitrosamines in such kind of meat products are available from other countries because such products are not produced or produced on a small scale. This fact made difficult comparison of the obtained results with data obtained in other countries. However, it seems that high concentration of NDMA in some samples of edible offals processed meat products could be caused by improper sampling, transport or storage of these products. Results of our previous studies on N-nitrosamines occurrence in Polish edible offals processed meat products revealed that different storage conditions very markedly influence the appearance of N-nitrosamines. The changes of N-nitrosamine concentrations observed during storage seem to be the result of chemical reactions between precursors of nitrosamines present or formed in edible offals processed meat products as well as the result of microbial action (4).
NDMA concentrations observed in cooked hams and the like and sausages were lower in comparison to those in edible offals processed meat products (Fig. 1). No samples contained NDMA above the level of 5.0 μg/kg. Spiegelhalder et al. (22) revealed in the food survey conducted in West Germany in 1980 that 30% of analysed samples were contaminated by NDMA, and in 6% of these samples NDMA concentration exceed the level of 5.0 μg/kg. Tricker et al. (25) showed in similar studies carried out in 1991 that 91% of meat products contained NDMA at the range from 0.2 μg/kg to 0.25 μg/kg. In sausages, the mean concentration of this compound was 0.84 μg/kg. The food survey conducted in France revealed that 50-100% of meat products, depending on the assortment, contained NDMA at the mean concentration of 2.15 μg/kg. NDMA was noted in 30% of examined salamis, in 0% of potted delicacies, and in 55% of non-smoked hams at the mean concentration of 6.6 μg/kg, 1.0 μg/kg, and 1.0 μg/kg, respectively (3). Biaudet et al. (2) detected NDMA in 83% of studied sausages and 93% of hams at the mean level of 0.45 μg/kg and 0.31 μg/kg, respectively.

NPIP at the range of 0.02-2.30 μg/kg was detected in about 20% of 170 analysed samples. Most frequently in sausages and edible offals processed meat products, rarely in cooked ham and the like. Differences in the NPIP content may be the result of applied spices and different degree of meat crumbling, thus different penetration of spices into the whole capacity of meat pieces. Cooked hams and the like in contrast to sausages and edible offals meat products are produced from one or a few anatomical parts of a carcass. The black pepper which contains the precursor of NPIP — piperidine could be the main source of this compound. Cadaverin, the product of lysine decarboxylation during thermal processing of meat or its maturing could be also the precursor of NPIP (21).

NDEA and NDBA occurrence in the analysed processed meat products could originate from elastic netting and plastic cover applied to the production and storage of meat products. Sen et al. (20) detected NDEA and NDBA in elastic rubber nettings and cured meats packaged in such nettings. They noted that most unused nettings contained only traces of nitrosamines but the used nettings contained high levels of the same nitrosamines. The homogenized meats from such packages contained NDBA up to 29 μg/kg and traces of NDEA. From rubber packaging material nitrosamines as well as different precursors of nitrosation (i.e. dialkyldithiocarbamates used as vulcanization accelerator) could migrate and react with nitrites in meat (17). In Poland no studies were conducted on the presence of N-nitrosamines in elastic nettings and in cured meats packaged in such nettings.

More than 5% of the studied samples, mainly edible offals processed meat products contained NMOR at the range of 0.9-4.2 μg/kg. Some authors detected this compound in various packaging materials containing wax (8, 17). The other source of NMOR could not be ruled out. In meat industry anticorrosion agents are used which could contain morpholine as one of the components (23).

NPyR at the concentration of 0.06 μg/kg was detected in only 3% of the examined samples. Precursors of NPyR formation could be the amino acids proline and ornithine produced from collagen and also aliphatic poliamines such as spermine and spermidine. These compounds occur in plant and animal tissues. Some spices also may contain precursors of NPyR e.g. paprika (9).

Considering the level of NDMA in the tested processed meat products the mean daily intake of NDMA from these products can be proposed. Assuming that the mean daily intake of the meat and meat products in Poland is about 0.012 kg/person,
the calculated average daily intake of NDMA was about 0.2 \( \mu g/\text{person} \) (14). This estimate agrees with those published in several European countries: 0.19 \( \mu g/\text{person} \) in France, 0.10 \( \mu g/\text{person} \) in the Netherlands, and 0.22 \( \mu g/\text{person/day} \) in Germany (5, 16, 24).

In conclusion, the results of the present studies on volatile N-nitrosamine occurrence in Polish processed meat products indicate comparatively low contamination of these food products with carcinogenic N-nitroso compounds. The daily exposure to NDMA is about 0.2 \( \mu g/\text{person} \) and this value is comparable with values noted in other countries.

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References