OCCURRENCE OF VOLATILE N-NITROSAMINES IN POLISH TINNED FOODS

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Abstract

N-nitrosamine content was determined in 150 samples of tinned foods collected from meat factories, located in different areas of Poland, in 2000-2001. These products included tinned fish, meat and offals. The analyses were performed by gas chromatography-thermal energy analyser (GC-TEA). Detectable levels of N-nitrosamines were found in about 61% of the individual samples. About 58% of samples contained NDMA at the mean concentration of 3.01 µg/kg. NPIP was noted in about 6% of samples. No other volatile N-nitrosamines were detected. The most contaminated products were tinned fish (total N-nitrosamines at the concentration of 8.20 µg/kg). Tinned meat and tinned offals contained much lower concentrations of the compounds (0.55 µg/kg and 0.39 µg/kg, respectively).

Key words: N-nitrosamines, analysis, tinned foods, contamination.

Many epidemiological studies have showed connections between diet and various forms of cancer (18). There is considerable evidence from analytic studies that diet plays a substantial role in the etiology of many tumours, and it has been estimated that as much as 40-60% of cancer cases may be attributed to factors associated with diet (10). In this specific attention has been focused on the possible role of N-nitrosamines. These compounds are strong mutagens and powerful animal carcinogens and there is epidemiologic evidence as to their importance for cancer in certain body organs and tissues, e.g. stomach, urinary bladder and others (9, 12). Nitrosamines may be ingested directly with food or may be formed in vivo in the stomach by interaction of nitrosating agents and ingested amine compounds (5). Ingested nitrates can be reduced to nitrite in the saliva and thus these compounds are considered too as potential precursors of endogenously formed nitrosamines (16).

Many studies in several countries have been carried out to determine levels of volatile nitrosamines in food and beverages. Moreover, USA set the level of 10.0 µg/kg of N-nitrosopyrrolidine (NPYR) in bacon as the limit permitting to introduce this product to market (4). Besides, Russia set the maximum level of the sum of N-nitrosodimethylamine (NDMA) and N-nitrosodiethylamine (NDEA) at 2.0-4.0 µg/kg, above this limit meat products are not permitted to market (11).

Our previous study showed that detectable levels of N-nitrosamines were present in above 83% of Polish processed meat products (2). About 75% of samples contained NDMA at the mean concentration of 2.55 µg/kg. N-nitrosopiperidine (NPIP) was noted in above 20% of samples, N-nitrosodibuthylamine (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, N-nitrosomorpholine (NMOR) and NPYR were detected. The level of total volatile N-nitrosamines (TN) with the mean of 3.15 µg/kg was calculated. The average daily intake of NDMA with processed meat products by man in Poland was calculated as 0.2 µg/d/person.

Although the tinned foods do not present significant contribution in general food consumption, there are evidences that these kind of food products could contain considerable level of N-nitrosamines (1, 17). On the other hand, very limited reports on the occurrence of N-nitrosamines in such kind of meat products are available from other countries. The present survey was performed to evaluate the occurrence of volatile N-nitrosamines in Polish tinned foods including tinned fish, meat, and offals.

Material and Methods

Samples. One hundred and fifty samples of different tinned foods such as tinned fish, meat and offals were collected in 2000-2001 by veterinary inspectors from processing plants located in different parts of Poland and transported to the laboratory for
Samples were analysed directly after transportation or stored 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 the Food Safety and Inspection Service (FSIS) (3). 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-nitrosodiethylamine (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 25-30 ml/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, 70 eV) according to the method recommended by FSIS (3).

**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 the studied tinned foods and number of the positive ones are given in Table 1. These products were grouped into three assortments: tinned fish, tinned meat, and tinned offals. In a majority (above 61%) of 150 analysed samples one or more volatile N-nitrosamines were detected, the highest percentage of positive samples were in tinned fish (about 78%) and the lowest one in tinned offals (about 40%). Mean concentrations of the detected volatile N-nitrosamines in processed meat products are given in Fig. 1. The number of positive samples and the minimum and maximum concentrations of volatile N-nitrosamines are presented in Table 2. In tinned foods only two N-nitrosamines, NDMA and NPIP, were noted. About 58% of the samples contained NDMA at the mean level of 3.01 µg/kg (the range 0.10-33.00 µg/kg). NPIP was noted in only few samples of tinned meat and tinned offals (about 6%) at the mean concentration of 0.04 µg/kg. In a few samples trace concentrations NDEA and NPYR were found (not presented). In no sample NDPA, NDBA, and NMOR were present.

### Table 1
Results of volatile N-nitrosamine analysis in tinned food of animal origin

<table>
<thead>
<tr>
<th>Assortment</th>
<th>Number of samples</th>
<th>Number of positive samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>tinned fish</td>
<td>50</td>
<td>39</td>
</tr>
<tr>
<td>tinned meat</td>
<td>50</td>
<td>33</td>
</tr>
<tr>
<td>tinned offals</td>
<td>50</td>
<td>20</td>
</tr>
<tr>
<td><strong>tinned foods</strong></td>
<td><strong>150</strong></td>
<td><strong>92</strong></td>
</tr>
</tbody>
</table>

### Table 2
Number of positive samples, the minimum and maximum concentrations (µg/kg) of individual volatile N-nitrosamines in analysed products

<table>
<thead>
<tr>
<th></th>
<th>NDMA</th>
<th>NPIP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>range</td>
</tr>
<tr>
<td>tinned fish</td>
<td>39</td>
<td>0.05 – 33.00</td>
</tr>
<tr>
<td>tinned meat</td>
<td>30</td>
<td>0.30 – 2.70</td>
</tr>
<tr>
<td>tinned offals</td>
<td>18</td>
<td>0.10 – 3.14</td>
</tr>
<tr>
<td>tinned food</td>
<td>87</td>
<td>0.10 – 33.00</td>
</tr>
</tbody>
</table>

NDMA - N-nitrosodimethylamine, NPIP – N-nitrosopiperidine
Among the analysed assortments the highest level of NDMA was recorded in tinned fish with the mean concentration of 8.21 µg/kg; in tinned meat and tinned offals the mean concentrations of this compound were similar, 0.47 and 0.36 µg/kg, respectively. No sample of tinned fish contained NPIP and in tinned meat the mean concentration of this compound was higher (0.08 µg/kg) than in tinned offals (0.04 µg/kg). The highest concentration of TN at the level of 8.20 µg/kg was found in tinned fish. In tinned meat and tinned offals TN were found at the concentration of 0.55 µg/kg and 0.39 µg/kg, respectively. The detected difference in TN concentration in tinned fish in comparison with the other assortments was statistically significant at P<0.05.

Discussion

The present study has concerned tinned foods which are products rather rarely consumed but could contain substantial level of N-nitrosamines. Very limited reports on the occurrence of N-nitrosamines in such kind of meat products are available from Poland and other countries. NDMA was the most frequently occurring volatile nitrosamine, found in 87 of the samples tested (58%) (Table 2). The most contaminated samples were tinned fish. Above 78% of these products (39 of the samples) contained NDMA and the mean concentration of this compound exceeded the level of 8.0 µg/kg. These values in tinned fish are higher than values given in other publications. Biaudet et al. (1) in the food survey conducted in France in 1987-92 found NDMA at the mean concentration of 0.49 µg/kg (range 0.04-3.50 µg/kg) in tinned fish. Somewhat higher levels of NDMA observed Tricker et al. (17). The mean concentration of this nitrosamine in tinned fish sold in the German market was at the level of 2.10 µg/kg (range 0.70-5.30 µg/kg). Karłowski et al. (7) in 49% of studied Polish tinned fish observed NDMA at the concentrations from 0.10 µg/kg to 1.80 µg/kg and NDEA in 27% of this product at the concentrations from 0.10 µg/kg to 1.20 µg/kg. In one sample NPYR (4.40 µg/kg) and NMOR (2.40 µg/kg) were detected. The authors stressed that NDMA was present in these tinned foods which contain smoked fish. Kann et al. (6) revealed that N-nitrosamines levels in fresh fish did not exceed 5.00 µg/kg; however, their concentrations in tinned fish increased to 13.00 µg/kg. Most fish products from Western Europe contain rather low NDMA concentration (15). The levels of volatile nitrosamines found in preserved fish samples collected in China or Greenland were higher than those reported in Western European foods. In Chinese fish products levels of NDMA ranged from 12.64 µg/kg to 322.92 µg/kg and NDEA from 7.65 µg/kg to 50.27 µg/kg (20). In other study the same authors reported total volatile N-nitrosamines in salted fish at the concentration of 0.028 mg/kg to 4.54 mg/kg (21). In Greenland in dried and unsalted fish, levels of NDMA varied from 8.6 µg/kg to 38.0 µg/kg (mean 25.0 µg/kg) (13).

The presence of high concentrations of N-nitrosamines could be caused by numerous various factors. Hygienic conditions of fish used for tinned food productions are of great importance. The great amounts of amines are formed during fish storage dependently on applied conditions. Even during fish storage in minus temperature dimethylamine could accumulate in their tissues; however, during storage in higher temperature, in the presence of microorganisms, trimethylamine could accumulate and could be nitrosated even under alkaline condition (8). Both compounds arose from trimethylamine oxide commonly found in fish organism serving essential part in osmoregulation (19). Technological processes – smoking, salting, drying could deliver other N-nitrosamine precursors such as nitrogen oxides, nitrates or nitrites and create favourable conditions for nitrosation. The smoking has a great influence on nitrosation. Nitrogen oxides included in the smoke could cause even a dozen or so-fold increase of nitrite in fish tissues. The salt used for fish preserving could contain high amount of nitrates as contaminant. It could not be ruled out the possibilities of fish
contamination by chemicals present in water used by local fisheries.

In many technological processes of individual kinds of tinned fish as „Szpyt Mereantil w oleju“, „Filety śledziowe Harengo“, „Filety śledziowe w oleju po rybacku“ the salting in 15% salt solution during 2-30 min and smoking at 50-120°C during 40-110 min are included. These processes create very favourable conditions for nitrosation. Many added different spices as pepper, paprika, mushrooms, cucumber etc. could deliver chemical precursors of N-nitrosamines.

NDMA content in other assortments of tinned foods, both tinned meat and tinned offals, were considerably lower, its concentrations were 0.47 µg/kg and 0.36 µg/kg, respectively. These values are comparable with NDMA contents in these type of products in other countries. In Germany NDMA was observed in tinned foods at the level of 0.51 µg/kg (range 0.20 µg/kg – 1.20 µg/kg) (17). Biaudet et al. (1) revealed NDMA at the mean concentration 0.07 µg/kg (range 0.0µg/kg - 0.95 µg/kg) in French tinned foods.

NPIP occurrence in the studied tinned foods was sporadic. This compound was noted only in 5 samples of tinned meat and 4 samples of tinned offals and its concentrations were between 0.46 µg/kg and 1.07 µg/kg.

Considering the results of volatile N-nitrosamine estimation in samples of tinned meat and tinned offals it should be stated that detected amounts of nitrosamines are relatively low and comparable to the values found in other countries. It should be also stressed that N-nitrosamines contents in tinned meat and tinned offals are 2-5-fold lower than in other meat products and do not pose a toxicological risk (2). On the other hand, higher NDMA concentrations detected in tinned fish could be of concern. Assuming that this kind of products contribute only small part in daily intake of meat products in Poland, N-nitrosamine consumption with tinned fish is rather low. But this assortment of tinned foods should be specially treated and N-nitrosamines level should be monitored.

In conclusion, the results of the present studies on volatile N-nitrosamines occurrence in Polish tinned foods indicate comparatively low contamination of these food products with carcinogenic N-nitroso compounds. However, some attention should be taken on tinned fish which may contain considerably higher concentrations of N-nitrosamines.

References