Levels of Nine Volatile N-Nitrosamines in Chinese-Style Sausages as Determined by QuECHERS-Based Gas Chromatography-Tandem Mass Spectrometry

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Abstract

Volatile N-nitrosamines (VNAs) are class 2 carcinogens (by International Agency for Research on Cancer (IARC), and they have been detected in various food samples, including meat products. Chinese style sausages are produced with some unique procedures, however, the conditions of contamination of Chinese sausages with VNAs are poorly known. In the present study, nine VNAs were analyzed in 94 sampled Chinese sausages from six provinces of China, using QuECHERS-based gas chromatography-tandem mass spectrometry. The most frequently detected VNAs included N-nitrosodimethylamine, N-nitrosodibutylamine, N-nitrosopyrrolidine (NPYR), N-nitrosomorpholine (NMOR), and N-nitrosodiphenylamine. The sum of concentrations of the nine VNAs detected in each sample ranged from 0.5 to 100.7 (median of 7.5) μg/kg. The levels of either total VNAs or particular components (including N-nitrosomethylethylamine, NPYR, and NMOR) in home-made sausages were statistically higher than those in commercial sausages, and this may be attributable to the varied ingredients and modes of package applied at home and commercial sausage production.

INTRODUCTION

Many studies suggest that consumption of smoked or processed meats and other nitrite-related foods is associated with increased risk of gastrointestinal (especially colorectal), nasopharyngeal, and pancreatic tumors [1-4]. In 2015, consumption of processed meat has been classified by International Agency for Research on Cancer (IARC) as carcinogenic to human (group 1 carcinogen). The statement by IARC also specifies that each 50 gram portion of processed meat eaten daily may increase the risk of colorectal cancer by 18% [5]. N-nitrosamines (NAs) are considered as one of the causative agents for the carcinogenicity of processed meat. Most frequently detected NAs in foods are N-nitrosodimethylamine (NDMA), N-nitrosodiethylamine (NDEA), N-nitrosopyrrolidine (NPYR), N-nitrosomorpholine (NMOR), and N-nitrosodiphenylamine. The sum of concentrations of the nine VNAs detected in each sample ranged from 0.5 to 100.7 (median of 7.5) μg/kg. The levels of either total NAs or particular components (including N-nitrosomethylethylamine, NPYR, and NMOR) in home-made sausages were statistically higher than those in commercial sausages, and this may be attributable to the varied ingredients and modes of package applied at home and commercial sausage production.

ABBREVIATIONS

NAs: N-nitrosamines; VNAs: Volatile N-nitrosamines; NDMA: N-nitrosodimethylamine; NDEA: N-nitrosodiethylamine; NDBA: N-nitrosodibutylamine; NPYR: N-nitrosopyrrolidine; NPIP: N-nitrosopiperidine; NMEA: N-nitrosomethylethylamine; NDPA: N-nitrosodipropylamine; NMOR: N-nitrosomorpholine; NDPhA: N-nitrosodiphenylamine; NDMA-d₆; N-nitrosodimethylamine-d₆; NDPA-d₁₄; N-nitrosodipropylamine-d₁₄

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Citation
Several NAs have been repeatedly detected in various processed meat products. Late in 1960s and early in 1970s high concentrations of NDMA and NPYR have been detected in fried bacon [12]. From 1980 to 1986, a survey showed that all of the four analyzed salami samples on the Italian market were contaminated with various amounts of NAs, e.g. NDMA (0.59-7.76 μg/kg), NDEA (n.d.-4.04 μg/kg), NDPA (0.73-50.12 μg/kg) and NPIP (n.d.-0.38 μg/kg) [13]. Based on a recent survey carried out in European countries, NAs occurs in commercially available processed meat regularly at low levels (< 5μg/kg), with occasionally increased values (up to 20 μg/kg) [14]. Recently, a decrease in the levels of NAs in meat products has been reported [15,16], and this may be caused by a reduction of the amounts of nitrite and nitrate added to processed meat products, subsequent to the implementation of a regulation limiting the use of these preservatives and the application of reagents inhibiting nitrosation reactions [such as ascorbic acid/erythorbic acid and α-tocopherol] [17,18].

Chinese sausage is one of the most popular traditional meat products in China. It is prepared by mixing chopped pork meat with lard, salt, sugar, garlic, rice wine, typical Chinese spices, additives (nitrate, nitrite, and antioxidants) and/or starter cultures, then the mixture is stuffed in pork casing and in cubated at 2-5 for 1-2 days, and finally sun or electric oven dried at 50-55 for 8-24 h. These sausages are 'semi-dry' or 'short-time fermented sausages', which differs from Western-style dry fermented sausages in terms of manufacturing process, ingredients, bacterial ecology and flavor [19,20]. Some Chinese families also make sausages at home by themselves during winter time. The processing procedure and curing ingredients applied in home-made sausages are different from that in commercial sausages; for example, nitrite or nitrate is never added in home-made sausages, and unlike in some food industries where nitration-inhibiting agents are used, in home-made sausages higher levels of salt is usually added for minimizing fermentation. Therefore, we were interested in the levels of the several NAs, which are highly carcinogenic, in Chinese sausages, particularly for a comparison between those in commercial and home-made sausages. In this study, the levels of nine volatile N-nitrosamines (VNAs) [NDMA, NDEA, NPYR, NPIP, NDBA, N-nitrosomethylamine (NMEA), NDPA, NMOR, and NDPhA] in Chinese sausages, sampled from six provinces of China (Guangdong, Anhui, Guizhou, Hunan, Shandong, Sichuan), were determined by using QuEChERS-based gas chromatography-tandem mass spectrometry. Particularly, a comparison between VNAs in commercial and home-made sausages was made.

MATERIALS AND METHODS

Sample material

Sampling of Chinese-style sausages was conducted in the period from December 2014 to February 2015, representing the current market supply. A total of 94 sausages were collected from supermarkets or farmers' markets (where home-made sausages were on sales) from six provinces of China, including Guangdong (n = 40), Anhui (n = 10), Guizhou (n = 7), Hunan (n = 14), Shandong (n = 10), and Sichuan (n = 13). These provinces were chosen for their relatively high levels of production and consumption of Chinese-style sausages. Guangdong Province, one of the main regions where production and consumption of Chinese-style sausage is popular and the incidence of nasopharyngeal cancer is high (probably associated with an heavy intake of salted fish and sausages) [21], was selected as the main sampling region. Sampling of sausages in each province was conducted in two cities with relatively high incidences of gastrointestinal tumors and common consumption of sausages.

The samples were categorized by label information and mode of production (commercial or home-made). After the storage period, the whole sample was cut and fast-cooled in liquid nitrogen, then minced into powder in a metal mincing machine and divided into portions. Then the samples were stored in a Ziploc bag at -80 until use for analysis.

Standards, reagents and materials

The mixed solutions of nine VNAs (NDMA, NDEA, NMEA, NDPA, NPYR, NPIP, NDBA, NMOR and NDPhA) containing 2000 μg/mL of each compound were purchased from Sigma-Aldrich (St. Louis, MO, USA). The internal standards, i.e., N-nitrosodimethylamine-d₆ (NDMA-d₆) and N-nitrosodipropylamine-d₁₄ (NDPA-d₁₄), were purchased as standard solutions (1000 μg/mL) from ANPEL Laboratory Technologies (Shanghai, China).

Bond Elut QuEChERS-buffered extraction kits (Part No.5982-5550) and Bond Elut QuEChERS d-SPE kits (Part No.5982-4950) were purchased from Agilent Technologies (Santa Clara, USA). The extraction kit contained salt mixtures (4 g of MgSO₄ and 1 g of NaCl) and the d-SPE kit contained 50 mg of primary and secondary amine extraction material (PSA), 150 mg of C18EC (end capped) and 900 mg of Na₂SO₄. Acetonitrile of HPLC grade was purchased from Auto Science (Tianjin, China).

Sample preparation

The extraction and cleanup of VNAs was performed according to the method of QuEChERS [18,22] with minor modifications. The QuEChERS (quick, easy, cheap, effective, rugged, and safe) method, which is widely used for sample preparation in recent years, was adopted in the pre-treatment of samples, with a procedure as followed: subsequent to mincing and homogenization, about 10g of processed sample was transferred into a 50-ml centrifuge tube...
tube, which was then added with two internal standards (30 ng NDMA-d14 and 30 ng NDPA-d14) and 10 ml of acetonitrile. As an internal reference, NDMA-d14 was used for NDMA, NMEA, NDEA, and NDPA-d14 for the rest six NAs. Afterwards the tubes were vortexed for 3 min and stored at -20 for 30 min. Two Agilent Bond Elut ceramic homogenizers and one QuEChERS buffered extraction kit were then added into the sample tube. Each tube was vortexed for 30s and then centrifuged for 10 min at 3800 g at 0. Six mL of the supernatant was transferred into a 15-ml centrifuge tube which contained QuEChERS d-SPE kit. This mixture was shaken vigorously for 1 min and centrifuged again at 3800 g for 10 min at 0. An aliquot of 5 mL of the supernatant was evaporated to 1 mL under nitrogen using a nitrogen evaporator (MTN-2800W, Auto Science, Tianjin, China) at room temperature. The concentrated extracts were filtered through 0.22 μm Nylon syringe filters (Agilent Technologies, Santa Clara, USA) and transferred to auto-sampler vials.

**Analytical conditions for gas chromatography-tandem mass spectrometry (GC-MS/MS)**

The sample extract was analyzed with a gas chromatography-tandem mass spectrometry (GC-MS/MS) using the Agilent 7890A Triple Quadruple (QqQ) GC/MS instrument. The chromatographic separation was performed with a DB-WAXETR capillary column (30 m × 0.25 mm, 0.25 μm of film thickness) (Agilent Technologies, Santa Clara, USA). Chromatographic conditions were as follows: inlet temperature, 250; inlet mode, split-less; column flow, 1.2 mL/min, with helium gas (purity ≥ 99.999%) as carrier gas; injection volume, 2μL. The oven temperature was programmed as follows: start temperature of 50 for 1min, then increased to 110 at a rate of 10/ min, followed by a further increase to 200 at a rate of 15/min, then increased to 280 at a rate of 4/min, and finally reaching isothermality at 280 for 13.75 min. The total GC run time was 46.75 min.

The QqQ-MS/MS was operated in electron ionization (EI) mode with two mass transitions for each VNA, in the multiple reaction monitoring (MRM) conditions. The energy was set at 70eV. The temperature of the ion source and transfer line to tandem MS were set at 230 and 150, respectively. The time for solvent delay was set to 2 min.

**Validation of the method**

For quantitative analysis, the peak areas in the chromatograms of the sample extracts were compared with the area of the respective peaks in the chromatogram of a standard solution containing the following nine NAs: NDMA, NDEA, NMEA, NDPA, NPYR, NPIP, NDBA, NMOR and NDPhA. Suitable transitions from precursor to product ions (MRM transitions) were identified for each compound. Each N-nitrosamine was also identified by comparing the retention time with the corresponding standard. The retention time, precursor and product ions, collision energy were indicated in Table (1).

To calibrate the GC-MS chromatogram, the standard solutions of seven different NAs in the concentrations ranging from 0.05 to 200 μg/L were prepared. The detection value against concentration was linear, with correlation coefficients (R2) between 0.9995 and 0.9999 for different VNAs. Based on a signal-to-noise ratio (S/N) equal to 3, the limit of detection (LOD) was calculated. The LOD values ranged from 0.01 to 0.10 μg/kg, as indicated in Table 2. When the signal-to-noise ratio (S/N) was 10, a limit of quantification (LOQ) ranging from 0.03 to 0.33 μg/kg was resulted (Table 2). As a test for recovery, a sample with low content of NAs was chosen and fortified with three different levels of NA concentration (1.5 μg/kg, 3 μg/kg, and 10 μg/kg). The mean recoveries for VNAs in six replicates at each fortification level were between 90.7% and 116.0% (Table 2). The precision, expressed as relative standard deviation (RSD), was in the range of 1.8-10.5%.

**Statistical analysis**

In this study, each sample was determined in triplicates. All data were expressed as medians (min-max). Data in various groups were compared using Mann-Whitney U test, with p values lower than 0.05 as statistically significant. Statistical analysis was performed using the SPSS 20.0 software.

**RESULTS AND DISCUSSION**

As indicated in Table (3), all of the (94) samples contained detectable amounts of two or more NAs. The total concentration

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**Table 1:** Analyte MS/MS transitions, retention time and instrument conditions of nine VNAs and two internal standards determined by GC-MS/MS.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Retention time (min)</th>
<th>Quantitative transition mass (m/z)</th>
<th>CE (eV)</th>
<th>Qualitative transition mass (m/z)</th>
<th>CE (eV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NDMA</td>
<td>7.31</td>
<td>74 &gt; 44</td>
<td>5</td>
<td>74 &gt; 42</td>
<td>18</td>
</tr>
<tr>
<td>NMEA</td>
<td>7.85</td>
<td>88 &gt; 42</td>
<td>18</td>
<td>88 &gt; 71</td>
<td>2</td>
</tr>
<tr>
<td>NDEA</td>
<td>8.20</td>
<td>102 &gt; 56</td>
<td>2</td>
<td>102 &gt; 85</td>
<td>18</td>
</tr>
<tr>
<td>NDPA</td>
<td>9.65</td>
<td>130 &gt; 58</td>
<td>2</td>
<td>130 &gt; 113</td>
<td>6</td>
</tr>
<tr>
<td>NDBA</td>
<td>11.33</td>
<td>116 &gt; 99</td>
<td>2</td>
<td>158 &gt; 141</td>
<td>6</td>
</tr>
<tr>
<td>NPIP</td>
<td>11.54</td>
<td>114 &gt; 84</td>
<td>6</td>
<td>114 &gt; 97</td>
<td>6</td>
</tr>
<tr>
<td>NPYR</td>
<td>11.79</td>
<td>100 &gt; 55</td>
<td>6</td>
<td>100 &gt; 70</td>
<td>5</td>
</tr>
<tr>
<td>NMOR</td>
<td>12.20</td>
<td>116 &gt; 86</td>
<td>2</td>
<td>116 &gt; 56</td>
<td>15</td>
</tr>
<tr>
<td>NDPhA</td>
<td>15.88</td>
<td>169 &gt; 168</td>
<td>18</td>
<td>168 &gt; 167</td>
<td>30</td>
</tr>
<tr>
<td>NDMA-d14</td>
<td>7.30</td>
<td>80 &gt; 46</td>
<td>5</td>
<td>80 &gt; 50</td>
<td>18</td>
</tr>
<tr>
<td>NDPA-d14</td>
<td>9.58</td>
<td>144 &gt; 126</td>
<td>2</td>
<td>144 &gt; 50</td>
<td>8</td>
</tr>
</tbody>
</table>

*Collision energy
Central processing procedures in China, especially the production styles in western countries; this may reflect the multiplicity of sausage study (about 20 fold difference) was greater than those detected the total amounts of VNAs in the sausage samples of the present (LOQs 0.3-0.4 μg/kg) [10]. It seems that the range of variation in μg/kg) with the other seven VNAs below the limit of detection to contain NDMA (averaged 0.6 μg/kg) and NDPA (averaged 0.6 as 14.0 μg/kg [12]. The Frankfurt sausage samples were found of the nine VNAs in the sausage samples of this study ranged from 0.5 to 100.7 μg/kg, with a median value of 7.5 μg/kg. This median value of VNAs was slightly higher than that detected in sausages produced in some western countries, in which the same VNAs or 2-4 fewer VNAs were determined. Many studies in other countries indicate that NAs occurs generally at levels lower than 5 μg/kg. For example, the sum of six VNAs (NDMA, NDEA, NDPA, NPYR, NPIP, NDBA) in sausage samples produced in Turkey ranged from 0.45 to 2.93 μg/kg [23]. Similarly, the total amounts of six VNAs (NDMA, NDEA, NDBA, NPIP, NPYR, NMOR) in commercial dry fermented sausages produced in Belgium were below 5.5 μg/kg except for one sample showing extremely high level of VNAs as 14.0 μg/kg [12]. The Frankfurt sausage samples were found to contain NDMA (averaged 0.6 μg/kg) and NDPA (averaged 0.6 μg/kg) with the other seven VNAs below the limit of detection (LOQs 0.3-0.4 μg/kg) [10]. It seems that the range of variation in the total amounts of VNAs in the sausage samples of the present study (about 20 fold difference) was greater than those detected in western countries; this may reflect the multiplicity of sausage processing procedures in China, especially the production styles for home-made sausages may be determined by both local traditions and cultures and individual family hobbies.

NDMA and NPYR have been identified as the most commonly detected VNAs in processed meat products in different countries [17,24]; in the meantime, NDMA is found to be the most mutagenic compound among various VNA chemicals, and NPYR is generally detected with relatively high levels. Therefore, NDMA and NPYR have been used as indicators of VNAs contaminating processed foods worldwide. Likewise, in the present study NDMA and NPYR were detected very frequently (98.9% and 97.9% respectively) in the sausage samples. Moreover, these two NAs were present in the samples at concentrations of 0.8 μg/kg and 3.5 μg/kg, respectively, apparently higher than the other VNAs. The variations of the level of NPYR were particularly large, i.e., from undetectable to 96.1 μg/kg, thus it may heavily contribute to the high concentration of total VNAs in some samples.

Of the detected VNAs, NDBA and NMOR were present in all (100.0%) samples, though their concentrations were relatively low. The median concentration of NDBA and NMOR was 0.1 μg/kg and 0.7 μg/kg, respectively. The presence of NDBA in meat products was related to the rubber netting and plastics used for packaging sausages; and the presumed precursor of NMOR was morpholine, produced primarily through the use of waxes in packaging materials or the use of anticorrosion agents in the meat products [12]. The low concentrations of NDBA and NMOR in the samples indicate that the use of packaging material in the market of Chinese sausages may be of quality and in accordance to related safety guidelines. NDEA was at the lowest concentration among the VNAs for detection, ranging from below the limit of detection to 0.5 μg/kg, with a detectable rate of 40.4%. In this study, NMEA was the least detectable VNA, with only nine samples detected with NMEA, at quite low concentrations.

A limit of 10 μg/kg total VNAs has been set for cured meat products in the United States (USDA, 2005), and a limit of 4 and 7 μg/kg NDMA in fish and related products was made for products imported from China (USDA, 2005) [25]. Indeed, a limit of 10 μg/kg for NPYR in retail products has been set in the U.S., and 10 μg/kg for NDMA in Canada [17]. A limit of 4 μg/kg NDMA in seafoods and 3 μg/kg NMOR in processed meat have been set in China. In this study, the concentrations of total VNAs in 28 (29.8%) samples exceeded 10 μg/kg; the limit value (USDA, 2005). The concentrations of NDMA in 12 samples (12.8%) passed 3 μg/
kg (the limit of NDMA in processed meat, the only limit of VNAs in processed meat already established in China), however, no samples contained NDMA at concentrations over 10 µg/kg (the limit in Canada). Nine (9.6%) samples were detected with NPYR at levels above its limit (10 µg/kg). The rates of sausages with total VNAs, or NDMA and NPYR as single compounds, exceeding the safety limits were fairly high, and with the vast population of sausage consumers in China considered, it is highly necessary to set up safety limits for total VNAs and single VNA compounds in this country.

In this study, commercial sausages are generally products manufactured by large-scale meat processing factories, which have obtained production licenses and other qualifications. The ingredients of commercial sausages are clear and described on the package labels, including additives (e.g. nitrate, nitrite, and antioxidants) and spices (e.g. chili, pepper, five spicy powder). Most commercial sausages are in vacuum packing. However, the home-made sausages are produced by individual residents or private workshops, and the processing environments are often irregular which may promote the growth and reproduction of microbes. The procedures and ingredients applied for sausage production at home are free of any guidelines and depend largely on personal preferences. For example, all home-made sausages in the samples from Guizhou province are smoked while those from Sichuan and Hunan provinces are usually added with chili and pepper in high amounts. Almost none additives are added to home-made sausages, but a large amount of salt, as an alternative of antiseptic agents, is added. The raw material and processing methods may have significant influence on the formation of VNAs, likewise, there have been reports indicating varied degrees of VNA contamination in the two categories of Chinese-style sausages [26].

Nitrite is one of the important precursors for the formation of NAs, therefore, its level in the meat being processed is influential on the concentration of VNAs. Besides of the amount of nitrite added to sausages during production, many other factors may also influence the formation of NAs in sausages, which include the quality of raw meat used (e.g. microbial activity), use of additional additives (e.g., antioxidants), use of spices (e.g., paprika and black pepper) and the temperature during smoking processes, storage conditions, etc. These factors are sometimes so influential that the amount of nitrite added contributing to formation of NAs might be masked, as indicated by an absence of correlation between amount of nitrite added and degree of NA formation in the processing of meat products [27].

In this study, while the levels of some VNAs detected in commercial sausages were not different from that in the home-made sausages, however, the median values of the total VNAs and three single chemicals (NMEA, NPYR, NMOR) in the home-made sausages were statistically higher than that in commercial sausages, as indicated in Table (4). This difference is probably related to several factors associated with the processing of meat for sausage production in food industries, in comparison with those for home-made sausages. For the production of commercial sausages, the addition of nitrite is legally restricted to a maximum of 150 mg/kg according to the limit established in China. In addition, the common use of nitrite-scavenging additives, such as ascorbic acid/α-tocopherol, for the production of commercial sausages may further reduce the residual nitrite levels, thus these factors may lower the level of NAs generation [28]. Moreover, commercial sausages are mostly in vacuum packing, which may contribute to lower levels of NA production than sausages without vacuum packaging, as commonly practiced for home-made sausages [29]. However, in home-made sausages nitrite-scavenging additives are not usually added. Home-made sausages are usually in bulk (without packaging) and therefore exposed to the air, which may promote the growth of microbes. Biogenic amines (BAs), as a precursor of NAs, are primarily produced from decarboxylation of amino acids by microbes, such as Enterobacteriaceae and Micrococcaceae, which commonly contaminate foods [30]. Accordingly, a positive correlation between the level of biogenic amines and microbial count has been observed [31].

The higher concentration of NPYR in home-made sausages could be due to the use of large amounts of spices (such as paprika and pepper), which contain pyrrolidine. Putrescine, a BA compound formed from protein degradation, can be transformed to NPYR via a reaction with nitrosating agents. The production of putrescine in processed meat may be enhanced by active bacterial growth, as a result of unhygienic storage and processing [32]. Moreover, the smoking treatment applied generally for home-made sausage can also result in higher levels of NPYR formation, since incomplete combustion and the resulting high temperature are favorable for efficient formation of NPYR from its precursors, such as asparaginolide, proline, and spermidine [12].

**Table 4: Levels of VNAs in commercial and home-made sausages.**

<table>
<thead>
<tr>
<th>Compounds</th>
<th>commercial sausages (µg/kg) (n=44)</th>
<th>home-made sausages (µg/kg) (n=50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NDMA</td>
<td>1.1 (n.d.-7.4) *</td>
<td>0.6 (0.2-6.7) ^</td>
</tr>
<tr>
<td>NMEA *</td>
<td>n.d. (n.d.-1.5)</td>
<td>n.d. (n.d.-2.8)</td>
</tr>
<tr>
<td>NDEA</td>
<td>n.d. (n.d.-0.3)</td>
<td>n.d. (n.d.-0.5)</td>
</tr>
<tr>
<td>NDBA</td>
<td>0.1 (0.0-1.2)</td>
<td>0.1 (0.0-0.7)</td>
</tr>
<tr>
<td>NPIP</td>
<td>0.2 (n.d.-9.5)</td>
<td>n.d. (n.d.-4.3)</td>
</tr>
<tr>
<td>NPYR</td>
<td>2.8 (n.d.-96.1)</td>
<td>3.9 (0.5-96.1)</td>
</tr>
<tr>
<td>NDPA</td>
<td>0.5 (0.1-2.3)</td>
<td>0.9 (0.1-7.5)</td>
</tr>
<tr>
<td>NDEA</td>
<td>0.2 (n.d.-4.5)</td>
<td>0.2 (n.d.-12.3)</td>
</tr>
<tr>
<td><strong>Total VNAs (µg/kg)</strong></td>
<td>7.4 (0.5-99.9) *</td>
<td>7.8 (1.8-100.7) ^</td>
</tr>
</tbody>
</table>

Data are medians of values in different samples [µg/kg].

* p<0.05, commercial sausages versus home-made sausages.

n.d.: not detected.

**Compounds**

- Concentrations of NDMA in 9 (18.2%) commercial sausages exceeded the tolerant limit of 3 µg/kg, as set by the Ministry of Health of China.
- Concentrations of NPYR in 4 (8.0%) home-made sausages exceeded the tolerant limit 3 µg/kg (by the Ministry of Health of China).
- Concentrations of NDMA in 4 (8.0%) commercial sausages exceeded the tolerant limit 10 µg/kg (by the USDA).
- Concentrations of total VNAs in 14 (31.8%) commercial sausages exceeded the tolerant limit 10 µg/kg (by the USDA).


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each VNA compound in different Chinese sausage samples varied in a wide range (from 9.6% to 100.0%). The levels of individual VNAs were relatively low; however, the total levels of the nine VNAs were generally higher than those reported in other countries. The levels of NMEA, NPYR, NMOR and total VNAs in home-made sausages were higher than that in commercial sausages, which is probably attributed to several differences in the procedures for commercial and home-made sausages.

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