Biogenic amines in commercial dry fermented sausages as possible precursors of *N*-nitrosamines

De Mey E. [1], Dewulf L. [1], Drabik-Markiewicz G. [1,2], Derdelinckx G. [3], Peeters M.-C. [3] and Paelinck H. [1]

[1] Research Group for Technology and Quality of Animal Products, Catholic University College Ghent, Belgium
[2] Institute of Chemistry, University of Silesia, Katowice, Poland
[3] Department Microbial and Molecular Systems, Catholic University Leuven, Belgium

Abstract— **Regarding food safety, the accumulation of biogenic amines must be avoided in all kind of food products, especially in dry fermented sausages. In addition, it is assumed that the biogenic amines can function as precursors for the formation of carcinogenic *N*-nitrosamines when sodium nitrite and/or nitrate is used in the sausage formulation. To estimate the food safety of the dry fermented products available on the Belgian market, a screening of the residual sodium nitrite and nitrate content, biogenic amine and volatile *N*-nitrosamine concentrations at the end of shelf life was performed. The median concentrations of residual sodium nitrite and nitrate were lower than 20 mg/kg. In general the biogenic amine concentrations remained low, but in a few products the amount of cadaverine and putrescine could reach intoxicating levels. Although it is assumed that cadaverine, putrescine, spermidine and spermine are precursors of *N*-nitrosamines, no correlation was observed. Only the relationship between the use of sodium nitrite and *N*-nitrosamine formation was confirmed. However, mostly all the detected *N*-nitrosamine concentrations remained under the limit of quantitation.**

Keywords— **Dry fermented sausages, *N*-nitrosamines, biogenic amines.**

I. INTRODUCTION

The occurrence of biogenic amines in dry fermented products is mainly related to the action of decarboxylase-positive bacteria and meat-enzymes. But also other intrinsic and extrinsic factors such as salt content, drying parameters,.. during the sausage processing are influencing the decarboxylation rate of free amino acids [1]. Hereby the wide range in product formulations and its manufacturing is translated into a great variety in accumulation of biogenic amines.

Concerning food safety, elevated concentrations of biogenic amines must be avoided. For instance, histamine and tyramine may induce migraines and hypertensive crises in sensitive individuals [2]. Moreover, putrescine (PUT) and cadaverine (CAD) are known to intensify the adverse effects of histamine and tyramine as they compete for some of the mechanisms involved on its detoxification [3].

In addition to the direct toxicological effect, the biogenic amines (PUT and CAD) and the natural polyamines spermine (SPM) and spermidine (SPD) can react with the added nitrite to form carcinogenic *N*-nitrosamines [4].

The aim of this study was to examine the safety, regarding the occurrence of biogenic amines and volatile *N*-nitrosamines, in dry fermented sausages available on the Belgian market. In addition, correlations between the accumulated biogenic amines, residual sodium nitrite and nitrate concentration and the prevalence of *N*-nitrosamines was investigated.

II. MATERIALS AND METHODS

A. Sampling

Modified atmosphere packed dry fermented sausages (n=101) from several brands were purchased in 4 different supermarkets and kept refrigerated at 7°C. At the end of shelf life, the samples were minced and homogenized for analysis.

B. Nitrite and nitrate determination

The dry fermented sausage samples were subsequently extracted with hot water and

deproteinized with acetonitrile. The filtered extracts were injected onto the HPLC (Hitachi La ChromElite, VWR International). Nitrite and nitrate were separated by ion chromatography using a Hamilton PRP-X100 column and isocratic elution with $NaCl/NaH_2PO_4$ (40mM:2mM). The nitrite and nitrate levels were expressed as residual $NaNO_2$, resp. $NaNO_3$. The limits of detection (LOD) and limit of quantitation (LOQ) are given in table 1.

Table 1 The limits of detection (LODs) and the limits of quantitation (LOQs) of the analytes

Nitrosating reagens	*LOD (mg/kg)*	*LOQ (mg/kg)*
$NaNO_2$	1.0	5.0
$NaNO_3$	1.0	5.0
Biogenic amines	*LOD (mg/kg)*	*LOQ (mg/kg)*
PUT	0.2	0.4
CAD	0.1	0.3
SPM	0.9	2.9
SPD	0.2	0.6
N-nitrosamines	*LOD (µg/kg)*	*LOQ (µg/kg)*
NDMA	0.2	0.8
NDEA	0.3	0.9
NDBA	0.6	2.0
NPIP	0.8	2.5
NPYR	0.5	1.6
NMOR	0.5	1.5

C. Biogenic amine determination

The biogenic amines were analysed by extracting the meat samples with 0.4 M $HClO_4$ and subsequently derivatisation with dabsyl-chloride. After solid phase extraction on a C18 cartridge (Grace Davison Discovery Sciences) the biogenic amines, i.e. putrescine (PUT), cadaverine (CAD), spermine (SPM) and spermidine (SPD) were separated and detected by RP-HPLC-UV. Therefore two Chromolith C18e columns (VWR International) were coupled and a gradient elution was performed starting with a water-methanol-acetonitrile (50:12.5:37.5, v/v/v) blend to a 100% organic mixture (methanol – acetonitrile, 25:75, v/v) [5]. In table 1 the LOD and LOQ-values of the investigated biogenic amines are listed.

D. N-nitrosamines determination

The volatile *N*-nitrosamines were, according the method of Drabik-Markiewicz et al. [6], extracted from the meat samples by means of vacuum distillation. Subsequently the distillates were extracted with dichloromethane and concentrated. In the course of detection and quantification of *N*-nitrosamines, i.e. *N*-nitrosodimethylamine (NDMA), *N*-nitrosodiethylamine (NDEA), *N*-nitrosodibutylamine (NDBA), *N*-nitrosopiperidine (NPIP), *N*-nitrosopyrrolidine (NPYR) and *N*-nitrosomorpholine (NMOR), the samples were injected onto a gas chromatograph coupled to a Thermal Energy Analyzer (GC-TEA, Thermo Electron Cooperation). The LOD and LOQ-values of the analyzed *N*-nitrosamines are included in table 1.

E. Statistical analysis

Data were statistically analysed by PASW Statistics 18.0.0 (SPSS Inc.), and to determine relationships among nitrite, nitrate, biogenic amines and *N*-nitrosamines levels, the Spearman's correlation coefficients were calculated.

III. RESULTS

A. Residual sodium nitrite and nitrate levels

As can be seen in table 2, the screening of the commercial sausages at the end of shelf life revealed a residual sodium nitrite and nitrate level around 6.3 mg/kg, resp. 11.8 mg/kg with a strongly negatively skewed distribution and maximum levels of resp. 147.5 mg/kg and 167.8 mg/kg.

Table 2 The residual sodium nitrite and nitrate levels, the biogenic amine levels and the *N*-nitrosamine levels in commercial dry fermented sausages (n=101)

Nitrosating reagens (mg/kg)	*Median (min – max)*
$NaNO_2$	6.3 (nd – 147.5)
$NaNO_3$	11.8 (nd – 167.8)
Biogenic amines (mg/kg)	*Median (min – max)*
PUT	9.3 (0.3 – 316.4)
CAD	1.1 (nd – 641.4)
SPM	6.1 (nd – 21.1)
SPD	2.3 (nd – 13.3)
N-nitrosamines (µg/kg)	*Median (min – max)*
NDMA	nd (nd – 1.0)
NDEA	nd
NDBA	nd (nd – 1.4)
NPIP	nd (nd – 12.3)
NPYR	nd (nd – 1.5)
NMOR	nd (nd – 1.6)

B. Biogenic amine accumulation

In general the accumulation of biogenic amines at the end of shelf life was very low. There were a few exceptions where the amount of PUT and CAD rised to a maximum of 316.4 mg/kg, resp. 641.4 mg/kg. The concentrations of the natural polyamines SPM and SPD were situated in a limited range with maximum levels of resp. 21.1 mg/kg and 13.3 mg/kg (table 2).

C. Occurrence of volatile N-nitrosamines

None of the commercial products contained NDEA. And mostly, no other *N*-nitrosamines could be detected in these products and thus they are considered to be safe during the whole shelf life. In a few samples *N*-nitrosamines were detected, but the amounts remained under the limit of quantitation. As can be seen in table 2, quantifiable levels (max. 12.3 µg/kg) were only observed for NPIP.

D. Correlation between the precursors and N-nitrosamines

In table 3 a summary of the significant correlation coefficients between the precursors sodium nitrite and nitrate and the biogenic amines and the occurrence of *N*-nitrosamines is given.

Regarding the risk of *N*-nitrosamine formation in dry fermented sausages, the residual level of sodium nitrite is positively correlated to the occurrence of NDMA ($p<0.05$), NDBA ($p<0.01$), NPYR ($p<0.05$) and NMOR ($p<0.05$). However no significant correlation between the biogenic amines and the *N*-nitrosamines could be observed.

IV. DISCUSSION

Following the European Parliament and Council Directive 95/2/EC, it can be decided that 87% of the analysed commercial dry fermented sausage samples showed a residual level of sodium nitrite below the limit of 50 mg/kg. However, the most recent directive 2006/52/EC restricted the addition of sodium nitrite (150 mg/kg) and nitrate (150 mg/kg) in non-heated meat products, and only for a few exceptions (traditionally cured meat products) limits for residual amounts were mentioned. In this way, it is not possible to verify the correct use of nitrite and nitrate by analysing the end products. Similar to a study of German meat products [7], the median concentration of the residual sodium nitrite remained under 20 mg/kg.

Table 3 Spearman's correlation coefficient (ρ) between residual sodium nitrite and nitrate levels, the biogenic amines and the *N*-nitrosamines concentrations in commercial dry fermented sausages (n=101)

	$NaNO_2$	$NaNO_3$	PUT	CAD	SPM	SPD
$NaNO_2$	-		0.330**	0.209*	-0.220*	
$NaNO_3$		-				0.205*
PUT			-	0.732**	-0.337**	-0.318**
CAD				-	-0.303**	-0.334**
SPM					-	0.499**
SPD						-
		NDMA	NDBA	NPIP	NPYR	NMOR
$NaNO_2$		0.231*	0.235**		0.199*	0.222*
$NaNO_3$		-0.180*				
PUT						
CAD						
SPM						
SPD						
NDMA		-	0.184*		0.295**	0.278*
NDBA			-		0.398**	0.285**
NPIP				-	0.304**	
NPYR					-	
NMOR						-

Only significant correlation coefficients are shown: $p \leq 0.05$ (*), $p \leq 0.01$ (**)

As can be found in literature [2], PUT is the omnipresent biogenic amine in dry fermented sausages. Also CAD is a dominant biogenic amines. Due to the complex interaction of microflora and processing parameters, there is a great variation in concentrations between the different analysed products. Only in a few exceptions, there can be a risk for human health since both compounds may aggravate histamine and tyramine food poisoning.

As a consequence of the high *N*-nitrosamine values found in bacon in the 70s, good manufacturing practices such as lowering the ingoing nitrite and the use of ascorbate were acquired in the meat industry. As a result, several reviews of the last decades [8] reported only very low amounts of these carcinogenic compounds, which was in accordance with the results of this study. Moreover, in our research the relationship between the residual nitrite level and the occurrence of NDMA, NDBA, NPYR, NMOR was confirmed. However no correlation was found with NPIP, which was the most detected one and was present at even some quantifiable level.

Despite the generally accepted assumption, no relationship between the accumulation of the investigated biogenic amines and the occurrence of the carcinogenic *N*-nitrosamines could be ascertained in the commercial products.

V. CONCLUSIONS

Only in a few cases the amount of PUT and CAD in dry fermented sausages accumulated to intoxicating levels. Although it is assumed that biogenic amines such as CAD, PUT and the natural polyamines SPD and SPM are potential precursors of *N*-nitrosamine formation, no relationship between both was observed. On the other hand, the influence of the residual sodium nitrite concentration on the formation of NDMA, NDBA, NPYR and NMOR was confirmed. Nevertheless the detected amounts of these carcinogenic compounds in the commercial meat products remained very low.

ACKNOWLEDGEMENT

This work was supported by the grant IOF/BAM KaHoSL/08-02 of the Industrial Research Found of the Catholic University Leuven, Belgium.

REFERENCES

1. Suzzi G, Gardini F (2003) Biogenic amines in dry fermented sausages: a review. Int J Food Microbiol 88: 41-54
2. Latorre-Moratella M L et al. (2008) Biogenic amines in traditional fermented sausages produced in selected European countries. Food Chem 107: 912-921
3. Bardocz S (1995) Polyamines in food and their consequences for food quality and human health. Trends Food Sci Tech 6: 341-346
4. Drabik-Markiewicz G et al. (2011) Influence of putrescine, cadaverine, spermidine or spermine on the formation of N-nitrosamine in heated cured pork meat. Food Chem 126: 1539-1545
5. De Mey E et al. (2011) Dabsyl derivatization as an alternative for dansylation in the detection of biogenic amines in fermented meat products by reversed phase high performance liquid chromatography. Food Chem (submitted)
6. Drabik-Markiewicz G et al. (2009) Role of proline and hydroxyproline in *N*-nitrosamine formation when heating cured meat. Meat Sci 81: 479-486
7. Honikel K (2008) The use and control of nitrate and nitrite for the processing of meat products. Meat Sci 78: 68-76
8. Demeyer D et al. (2000) Control of bioflavor and safety in fermented sausages: First results of a European project. Food Res Inter 33: 171-180