ROLE OF CADAVERINE AND PIPERIDINE IN THE FORMATION OF *N*-NITROSOPIPERIDINE IN HEATED CURED MEAT

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***Abstract*— *N*-nitrosamines are carcinogenic compounds, which formation in meat products depends from different factors e.g., temperature, storage time, precursors and/or added sodium nitrite. Sodium nitrite is important for meat processing as curing agent. The aim of this study was to determine the role of cadaverine and piperidine on the formation of *N*-nitrosamines in heated cured meat products. Such experimental products were processed with different amounts of sodium nitrite (0 mg kg^{-1}, 120 mg kg^{-1}, 480 mg kg^{-1}), 1000 mg kg^{-1} of cadaverine or 10 mg kg^{-1} of piperidine, and heated at 85°C, 120°C, 160°C or 220°C. Experimental evidence was produced using gas chromatography in combination with Thermal Energy Analyzer (GC-TEA). The obtained analytical results were statistically evaluated by means of the Univariate Analysis of Variance (ANOVA) approach. In the current study only *N*-nitrosodimethylamine (NDMA) and *N*-nitrosopiperidine (NPIP) were detected. Addition of cadaverine and piperidine did not affect the formation of NDMA. Otherwise the availability of these compounds had a significant effect on the NPIP yields. However the presents of piperidine has a major impact on the NPIP formation. The higher the temperature and nitrite concentration the higher the amounts of both *N*-nitrosamines.**

***Index Terms*—cured meat products, GC-TEA, *N*-nitrosamines, N-nitrosopiperidine**

I. INTRODUCTION

Occurrence of biogenic amines in meat products depends on technological processes, microbiological status of the raw materials and the presence of precursors such as biogenic amines (Saccani, Tanzi, Pastore, Cavalli & Rey, 2005; Halász, Baráth, Simon-Sarkadi & Holzapfel, 1994). Cadaverine is one of the most common biogenic amines in these materials. High amounts of cadaverine have been related to low quality of raw materials and relatively high counts of enterobacteria. In these circumstances amounts of cadaverine can raise to 120 mg kg^{-1} (Bover-Cid, Miguelez-Arrizado, Latorre Moratalla & Vidal Carou, 2006; Berthold & Nowosielska, 2008).

N-nitrosamines are formed by the reaction of secondary amines (such as cadaverine or putrescine) with a nitrosating agent during the curing process (Saccani et al., 2005). Their formation depends on various different parameters associated with preparation, storage and thermal processing of the cured raw materials. The concentration of volatile *N*-nitrosamines grows with elevated temperatures and time (Sen, Seaman & Miles, 1979; Drabik-Markiewicz, Van den Maagdenberg, De Mey, Deprez, Kowalska & Paelinck, 2009; Drabik-Markiewicz et al., 2010). A considerable role in the formation of carcinogenic *N*-nitrosamines plays sodium nitrite, which is added to meat for the colour and flavour formation and also as preservative (Domańska & Kowalski, 2002). Therefore the permitted amount of nitrite in cured meat products has been regulated by the European Commission (Directive 95/2/EC) which prescribes maximum addition of sodium nitrite at a level of 150 mg kg^{-1} processed meat.

Wei et al. (2009) suggested that *N*-nitrosopiperidine may be formed from piperidine or cadaverine, which can be formed by decarboxylation of lysine. Also Shalaby (1996) and Ciemniak (2006) pretend that cadaverine can be converted to piperidine from which *N*-nitrosopiperidine is formed by heating.

The aim of presented research was to determine the influence of the cadaverine and piperidine on the formation of *N*-nitrosoamines in cured meat products with or without the addition of sodium nitrite under different heating conditions.

II. MATERIALS AND METHODS

Preparation and processing of meat model: The samples were prepared according to a cooked ham model in which lean meat was mixed with brine containing 2% salt, 0.3% phosphate and different amounts of sodium nitrite (0, 120 or 480 mg kg^{-1}) and 0 or 1000 mg kg^{-1} cadaverine or 10 mg kg^{-1} piperidine. The samples were heated for 30 minutes using different temperatures to mimic the industrial processes: pasteurization (85°C), sterilization (120°C), baking (160°C) and roasting (220°C). *GC-TEA analysis*: Preparation of meat samples for chromatographic analysis embraced three principal steps: vacuum distillation, extraction according to the method of Gasarasi et al. (2003), and in the final phase-condensation in the Kuderna-Danish apparatus. The *N*-nitrosamines were analyzed and identified by thermal energy analyzer (TEA) which is a selective gas-chromatographic (GC) detector for *N*-nitroso compounds. The appearance of five volatile *N*-nitrosamines (*N*-nitrosodimethyalmine, *N*-nitrosodiethylamine, *N*-nitrosodibutylamine, *N*-nitrosopiperidine, *N*-nitrosopyrrolidine) was investigated in the processed meat samples. *Statistical evaluation:* For evaluation of an impact of the temperature and concentration of sodium nitrite on the *N*-nitrosamine formation Analysis of Variance (ANOVA) in two-factor mode was used. Models were built for each subsets: (1) blank meat samples processed as such, and samples fortified with (2) cadaverine or (3) piperidine. The *N*-nitrosamines models were visualized as 3D response surface plots.

III. RESULTS AND DISCUSSION

Although the analytical method is capable to determine five volatile nitrosamines, in the current study, only two nitrosamines were detected frequently (*N*-nitrosodimethylamine and *N*-nitrosopiperidine). At the higher meat processing temperatures and the higher amounts of sodium nitrite NDMA was detected in higher concentrations. However, the higher amounts of this *N*-nitrosamine were not due to the addition of the investigated precursors. The results obtained from the current study demonstrated a considerable impact of cadaverine and piperidine on the *N*-nitrosopiperidine formation. In the case of the blank subset only 2 positive results could be detected. Addition of piperidine has a major impact on the NPIP formation. Although that the meat samples were fortified with 1000 mg kg^{-1} cadaverine and only 10 mg kg^{-1} piperidine, the amounts of formed NPIP were much higher for piperidine (Fig. 1). Maximum concentration of NPIP reached the level of 0.97 µg kg^{-1} in the case of cadaverine and 8.21 µg kg^{-1} when piperidine was added. Additionally the meat processing temperature and the addition of sodium nitrite increased the NPIP amounts. It is shown in Table 1 that the temperature had the largest effect for cadaverine, while for piperidine this is the case for sodium nitrite.

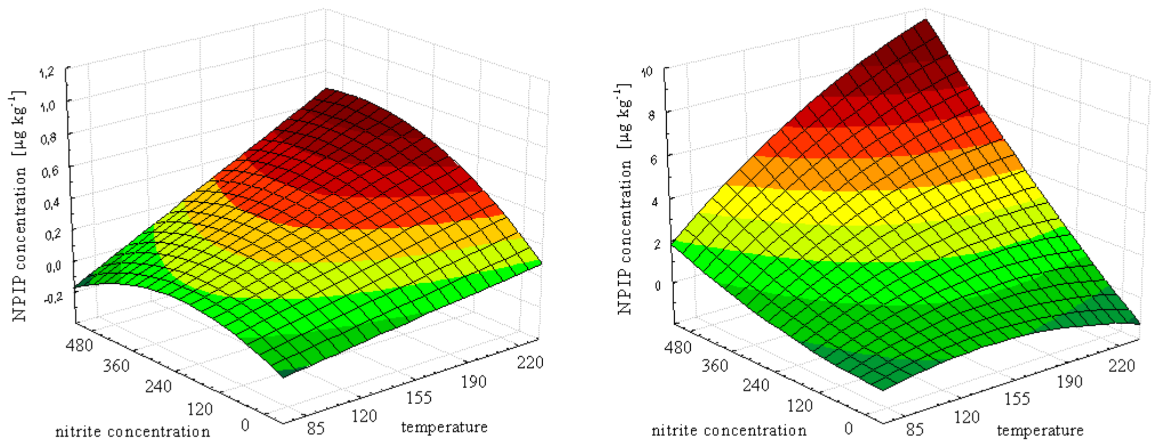
Table 1. Results of the ANOVA analyses (F and p values for the factors and interactions) on the NPIP concentrations observed in (A) individual data sets and (B) combined data sets; bold: significant terms at α=0.05 level.

(A)

Factor	Data set			
	Cadaverine (1000 mg kg^{-1})		Piperidine (10 mg kg^{-1})	
	F	p	F	p
temperature	**9.79**	**0.000**	**56.66**	**0.000**
[$NaNO_2$]	**5.64**	**0.008**	**519.96**	**0.000**
temperature×[$NaNO_2$]	**3.44**	**0.011**	**32.69**	**0.000**

(B)

Factor	Data set			
	Blank + Cadaverine		Blank + Piperidine	
	F	p	F	p
temperature	**10.86**	**0.000**	**84.98**	**0.000**
$[NaNO_2]$	**4.98**	**0.010**	**760.94**	**0.000**
precursor	**37.35**	**0.000**	**1399.76**	**0.000**
temperature×$[NaNO_2]$	**3.80**	**0.003**	**48.39**	**0.000**
temperature×precursor	**9.56**	**0.000**	**85.61**	**0.000**
$[NaNO_2]$×precursor	**6.32**	**0.003**	**773.22**	**0.000**



(A) (B)

Fig. 1. 3D response surface plots of the NPIP concentration as a function of temperature and nitrite concentration for samples fortified with (A) cadaverine and (B) piperidine.

IV. CONCLUSION

In the presented study only two *N*-nitrosamines were detected. From the experimental results, it could be concluded that higher meat processing temperatures and higher amounts of sodium nitrite yielded higher amounts of *N*-nitrosodimethylamine and *N*-nitrosopiperidine. Addition of cadaverine and piperidine did not affect the formation of NDMA. Otherwise addition of these compounds had a significant effect on the NPIP yields. The addition of piperidine has a major impact on the NPIP formation than cadaverine.

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