N-NITROSOPIPERIDINE FORMATION IN DRY FERMENTED SAUSAGES

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Abstract – The aim of this study was to determine the influence of sodium nitrite (0 and 150 mg/kg) and ascorbate (0 and 500 mg/kg) on the *N*-nitrosopiperidine (NPIP) formation during the production of dry fermented sausages. To provoke the NPIP formation, piperidine (PIP; 0, 10 and 100 mg/kg) was artificially added to the sausage model. Nitrite concentrations and PIP levels were determined by HPLC-UV. The NPIP formation was assessed by GC-TEA. When no PIP was added, no meaningful NPIP formation could be detected. The artificial addition of PIP resulted in higher NPIP concentrations. In the preparations with $NaNO_2$, but without sodium ascorbate, the highest NPIP levels could be measured. Nevertheless, the role of ascorbate as *N*-nitrosamine scavenger was only detectable at the beginning of the production. In the end products, NPIP was degraded and no influence of $NaNO_2$ or ascorbate could be seen any longer.

Key Words – Piperidine, Sodium ascorbate, Sodium nitrite

I. INTRODUCTION

Carcinogenic *N*-nitrosamines are formed by the nitrosation of secondary amines [1]. In fermented meat products, the most important source of secondary amines are biogenic amines. The accumulation of these amines are mainly attributed to the microbial decarboxylation of free amino acids. As a consequence, the main biogenic amines found in dry fermented sausages are tyramine, putrescine and cadaverine, derived from tyrosine, ornithine and lysine, respectively [2]. Cadaverine is considered to be the precursor of *N*-nitrosopiperidine (NPIP). However, firstly a deamination and cyclization at elevated temperatures should be assessed [3]. Therefore, the formation of NPIP from cadaverine in dry fermented sausages is most unlikely. However, besides the biogenic amines as potential precursors of *N*-nitrosamines, other amines can also be present in processed meat. The use of spices can be a source of piperidine (PIP). This alkaloid can be cleaved of the pungent compound piperine, which is mainly found in pepper [4]. Subsequently, can PIP can be nitrosated to NPIP.

The carcinogenicity of *N*-nitrosamines and the inevitable presence of secondary amines in meat products, forces the restriction of the nitrosation reaction. As a consequence the addition of nitrite is legally restricted to a maximum of 150 mg $NaNO_2$/kg [5]. Moreover, the use of nitrite scavenging additives like ascorbate and alfa-tocopherol are recommended [6].

The aim of this study was to investigate the influence of sodium nitrite and ascorbate on the formation of NPIP in dry fermented sausages. In first instance, the use of recommended concentrations of both additives was evaluated in a realistic dry fermented sausage model. Secondly, the sausages were enriched with PIP to evaluate the NPIP formation during the production process.

II. MATERIALS AND METHODS

Experimental set-up

Dry fermented sausage preparations were made, whereby different PIP concentrations were added (table 1). To evaluate the availability of nitrite as nitrosating agent, the addition of sodium nitrite and sodium ascorbate was varied. The sausages were analyzed after stuffing (day 0), fermentation (day 3), during the ripening (day 7 and 14) and at the end of production (day 21).

Table 1 Overview of the different dry fermented sausage preparations

Preparation	PIP (mg/kg)	$NaNO_2$ (mg/kg)	Sodium ascorbate (mg/kg)
A1	0	0	500
A2	0	150	500
A3	0	150	0
B1	10	0	500
B2	10	150	500
B3	10	150	0
C1	100	0	500
C2	100	150	500
C3	100	150	0

Dry fermented sausage production

The sausages were prepared by cutting the frozen meat (3.75 kg) and backfat (2.68 kg) into the bowl cutter. Subsequently, the starter culture and additives, e.g. dextrose (7 g/kg), sodium ascorbate (0 or 0.5 g/kg), white pepper (2 g/kg) and nutmeg (0.4 g/kg) and PIP (0, 10 or 100 mg/kg) were added. Finally, the binding of the meat batter was achieved by adding the fresh meat fraction (3.21 kg), salt (28g NaCl/kg) and sodium nitrite (0 or 150 mg/kg). The meat batter was stuffed into collagen casings (Ø 90 cm). The sausages were fermented for three days at 24°C and relative humidity of 94% RH. Finally, the sausages were dried (15°C/85% RH) until a weight loss of 20% was achieved.

Analyses

The PIP concentrations were measured by the method of De Mey et al. [7]. The dabsylated biogenic amines were separated and detected by RP-HPLC-UV. The method detection limit (MDL) and the limit of quantitation (LOQ) of PIP were 0.3 mg/kg dry matter (DM) and 0.7 mg/kg DM, respectively.

Nitrite was determined according to De Mey et al. [8]. Separation was assessed by ion chromatography The concentrations were expressed as residual $NaNO_2$ whereby the MDL and LOQ were 2 mg/kg DM and 10 mg/kg DM, respectively.

The NPIP concentrations were determined according to the method of Drabik-Markiewicz et al. [9]. Volatile *N*-nitrosamines were analyzed by Gas Chromatography (GC) coupled to a Thermal Energy Analyzer (TEA). The MDL and LOQ of NPIP were 0.9 µg/kg DM and 2.7 µg/kg DM, respectively.

Statistical evaluation

For all statistical comparisons PASW Statistics 19.0.0 (SPSS) was used. The evaluation of the residual sodium nitrite degradation and the formation of NPIP was assessed by one-way ANOVA for each sampling day. The Duncan's *post hoc* test was used to compare the influence of the nitrite and ascorbate addition at a significance level of $p < 0.05$.

III. RESULTS AND DISCUSSION

Piperidine concentrations

The sausages, prepared without the addition of PIP, contained initially a PIP concentration of 1.9±0.6 mg/kg DM and after production even an increase to 3.9±0.5 mg/kg DM was observed. Since fresh meat does not contain detectable amounts of PIP [10], the accumulation of PIP in the dry fermented sausage model was probably attributed to the cleavage of piperine, present in the used pepper.
When PIP was added to the sausage formulation, the measured concentrations represented the added amounts of 10 and 100 mg/kg, respectively.

Evolution of the residual nitrite concentration

As can be seen in figure 1, the availability of nitrite as nitrosating agent was influenced by the addition of sodium nitrite and ascorbate.

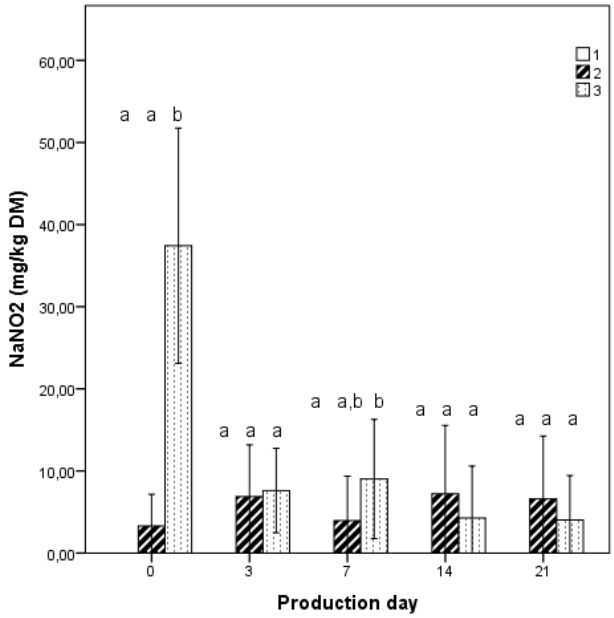

Figure 1. The mean residual sodium nitrite levels during the processing of the sausages, explanations of the numbers see table 1, bar charts with different superscript are significantly different at $p<0.05$ within production day.

As expected, only in the sausages prepared with 150 mg/kg $NaNO_2$, a residual amount can be detected. However, in the presence of sodium ascorbate, the $NaNO_2$ level dropped immediately after stuffing (day 0) below the LOQ of 10 mg/kg. If sodium ascorbate was omitted in the meat batter, the residual sodium nitrite level decreased significantly slower and at day 0 still 37±14 mg/kg was measured. Nevertheless, the difference in nitrite degradation was limited to the beginning of the production. After the fermentation (day 3) all the sausages contained $NaNO_2$ concentrations below the LOQ.

The formation of NPIP

When the sausages were not artificially enriched with PIP, the NPIP contamination was minute and no influence of the addition of nitrite or ascorbate could be detected (figure 2A).
As can be seen in figure 2B, the addition of 10 mg PIP per kg meat batter, comparable to an extra addition of 2g/kg black pepper to the sausages, resulted in an increased NPIP formation. However, almost all detected NPIP concentrations at this intermediate PIP level remained below the LOQ of 2.7 µg/kg DM, except at day 0, directly after stuffing, where significantly higher NPIP levels could be measured when nitrite was added. Nevertheless, no influence of ascorbate could be detected.

Only in the case of an excessive amount of PIP, namely 100 mg/kg, an important NPIP formation could be provoked (figure 2C). The highest concentrations were found at day 0 when nitrite was used in the ascorbate free sausages. However, the NPIP contamination in this particular preparation decreased during the production, possibly due to enzymatic breakdown attributed to the microbial activity [11]. By the time the sausages were ready for consumption, no influence of the nitrite and ascorbate formulations could be observed. Although the role of ascorbate as *N*-nitrosamine scavenger is clear, it's functionality is limited to the beginning of the production.

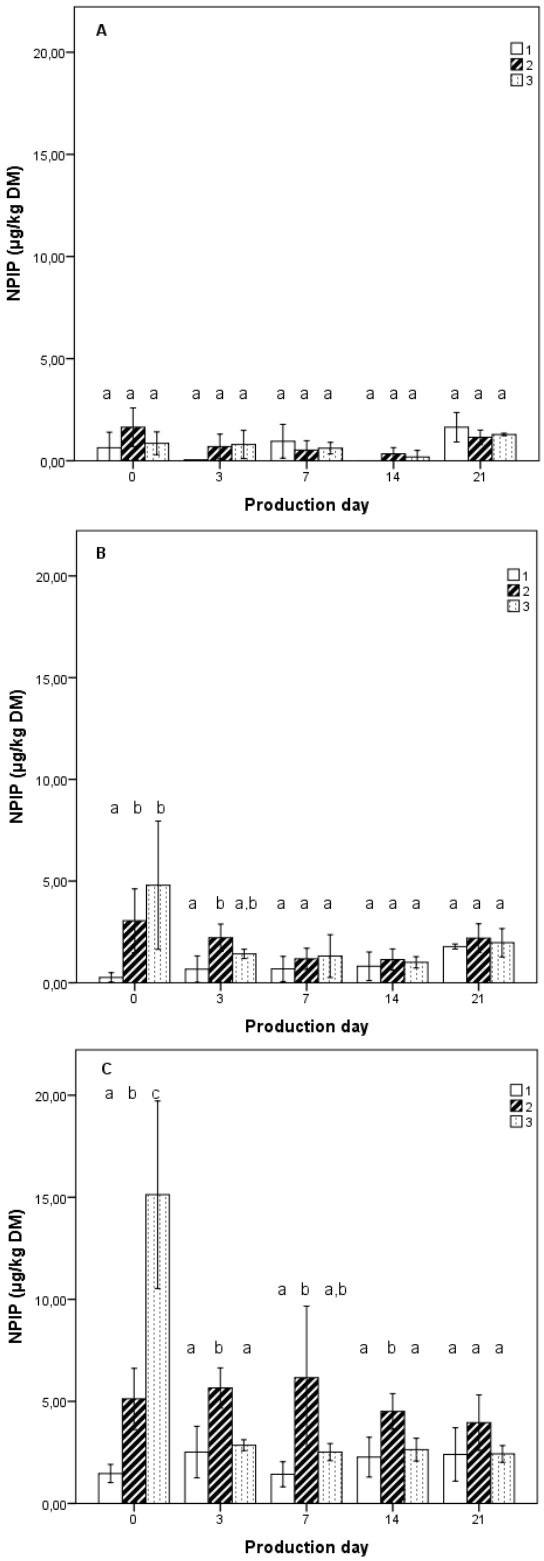

Figure 2. The NPIP concentrations during the processing of the sausages, explanations of the codes see table 1, bar charts with different superscript are significantly different at $p<0.05$ within production day

IV. CONCLUSION

Low concentrations of PIP can be present in dry fermented sausages due to the use of pepper. However, the accumulation is limited and the risk of NPIP formation remains low. In sausages, artificially fortified with PIP, increased NPIP levels were measured and the influence of nitrite and ascorbate can be seen immediately after stuffing. Nevertheless, the NPIP contamination decreased during the production. Consequently. the role of nitrite and ascorbate was minimal in the end products.

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