

Role of proline and hydroxyproline in *N*-nitrosamine formation in roasted cured meat

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Introduction

Sodium nitrite and sodium nitrate are used as curing agents in meat and they are very important not only as preservatives (decrease the risk of botulism poisoning), but also for colour and flavour formation (for reviews, see Sebranek & Bacus, 2007; Honikel, 2008). On the other hand, sodium nitrite plays a considerable role in formation of the carcinogenic *N*-nitrosamines (IARC, 1978). Nitrosamines are formed in the reaction between a nitrosating agent and an amine, and their formation can be the result of a chemical and/or microbial reaction (Domańska & Kowalski, 2002). In this respect, the amount of nitrite in cured meat is an important factor because the rate of *N*-nitrosation of the secondary amines is directly proportional to the square of the nitrite concentration (Mirvish, 1975). Therefore, it is no surprise that the amount of nitrite permitted in cured meat products is regulated by legislation. The European Customs Inventory of Chemical Substances prescribes the sodium nitrite addition at the 120 mg/kg level.

Proline seems to be the most significant precursor for NPYR (*N*-nitrosopyrrolidine), which is formed from proline by nitrosation to nitrosoproline followed by decarboxylation at elevated temperatures, or by the decarboxylation of proline in the first step with the formation of pyrrolidine followed by nitrosation (Gray & Dugan, 1975; Coleman, 1978). Proline and hydroxyproline are natural components of many foods and they are particularly abundant in connective tissue. The aim of this study was to determine the role of proline and hydroxyproline in the *N*-nitrosamine formation during the heating of cured meat with or without the addition of sodium nitrite, in order to further refine and define the process conditions that can be responsible for an increased *N*-nitrosamine formation.

Material & methods

Preparation of meat samples: Lean meat originated from a pork m. Longissimus. The samples were prepared according to a cooked ham model: Minced meat was mixed with a brine containing 2% NaCl, 0.3% phosphate, different nitrite (NaNO₂) concentrations (0 ppm, 120 ppm, and 480 ppm) and the addition of proline or hydroxyproline (1000 ppm). After packaging, the cans were heated during 30 minutes at 220°C. Volatile nitrosamines were analyzed in 38 samples, almost equally distributed over the combinations.

Extraction and GC-TEA analysis: The extraction of the *N*-nitrosamines and GC-TEA analysis were done according to the method of Drabik-Markiewicz et al. (2008). The GC-TEA system was employed for detection and quantification of *N*-nitrosodimethylamine (NDMA), *N*-nitrosodiethylamine (NDEA), *N*-nitrosodibutylamine (NDBA), *N*-nitrosopiperidine (NPIP), *N*-nitrosopyrrolidine (NPYR), and *N*-nitrosodipropylamine (NDPA, as the internal standard, 0.25 µg/ml).

Statistical analysis: The majority of the analytical results in the current study were below the respective LOQ values (Table 1). Therefore, the statistics were done based on frequency of the detectable occurrence (<LOD, <LOQ, and >LOQ). To test for significant differences between treatments, a chi-square test was used. All data were analyzed using SPSS 16.0 for Windows (SPSS Inc., Chicago, USA).

Table 1: Limits of detection (LOD), limits of quantification (LOQ) and recovery for each investigated *N*-nitrosamine

<i>N</i> -nitrosamine	LOD (µg/kg)	LOQ (µg/kg)	Recovery (%)
NDMA	0.2	0.8	86.95
NDEA	0.3	0.9	101.88
NDBA	0.6	2.0	81.38
NPIP	0.8	2.5	112.49
NPYR	0.5	1.6	88.41

Results & Discussion

General detection level of N-nitrosamines: In the current study, only NDMA and NPYR were detected in respectively 60.5% and 31.6% of the cases and could be quantified respectively in only 7.9% and 13.2% of the observations with a maximum of respectively 1.40 µg/kg and 5.80 µg/kg. The nitrosamines NDEA, NDBA, or NPIP were not detected. The nitrosamine content in none of the experiments exceeded the level of 10 µg/kg. However, the concentration of N-nitroso compounds in foods are often tiny and thus, human exposure is small but should not be ignored because of the great carcinogenic potency of this group of carcinogens (Lijinsky, 1999).

Addition of proline and hydroxyproline: NDMA was not significantly influenced by the addition of proline or hydroxyproline compared to the blank (Figure 1). However, a higher NPYR frequency ($P < 0.05$) was obtained with the addition of 1000 mg/kg proline. In the group where the established yields of NPYR were above the LOQ, the mean NPYR concentration was 3.14 ± 1.56 µg/kg, with a maximum of 5.80 µg/kg (data not shown).

These results confirm that proline is a precursor for NPYR. Coleman (1978) established a linear relationship between the addition of free proline during cooking and the NPYR level in cooked-out fat. Other studies also demonstrated the importance of free proline as a precursor of NPYR formation (Gray & Randall, 1979). The high temperatures needed for the formation of NPYR are probably necessary for the decarboxylation step in the pathway (Gray & Dugan, 1975).

In contrast, the NPYR incidence did not differ when hydroxyproline was added. Moreover, in this study, hydroxyproline seemed to behave as an inhibitor for the NPYR formation, because no NPYR was detected when hydroxyproline was added to the meat model, and this in contrast with the blank. Chemical and structural differences in the proline and hydroxyproline molecule can explain the different results in NPYR occurrence.

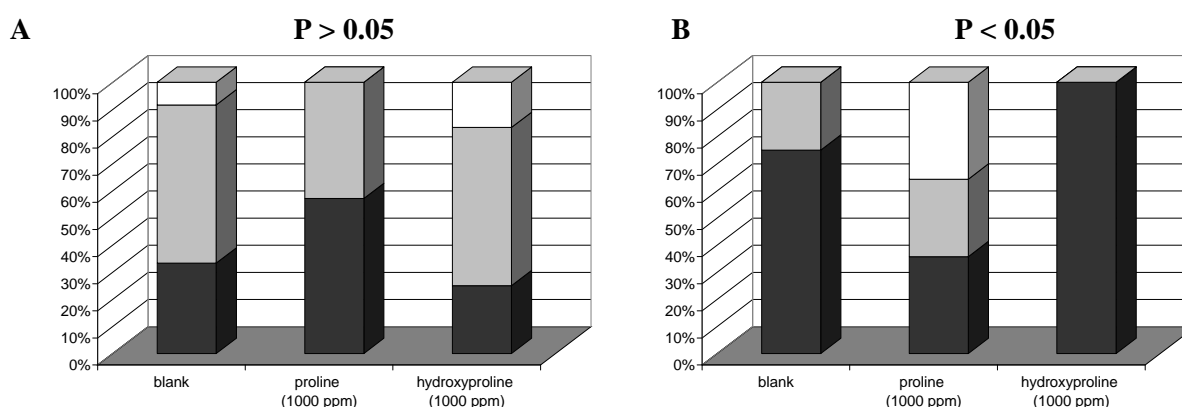


Figure 1. NDMA (A) and NPYR (B) detection in the blank, and after addition of proline (1000 ppm) and hydroxyproline (1000 ppm). Percentages of the samples were, respectively, below the limit of detection (black bars), below the limit of quantification (grey bars) and above the limit of quantification (white bars). For LOD and LOQ, see Table 1.

Concentration of nitrite: Nitrite and nitrate are both oxidation products and ready sources of nitric oxide. The latter acts as a nitrosating agents which easily reacts with many amines under acidic conditions to form N-nitrosamines, but the only stable nitrosamines are formed in the reaction with the secondary amines (Honikel, 2008).

NDMA is present in 15.4% of the samples without addition of nitrite, compared to 72.7% and 92.9% after the addition of 120 or 480 mg/kg, respectively (Figure 2, $P < 0.05$). The NPYR formation was only increased after addition of 480 mg/kg nitrite. Although traces (NDMA & NPYR < LOQ) of nitrosamines were found in the current study after addition of 120 mg/kg nitrite, the general conclusion can be that the amount of nitrosamines is very low in our meat model, as long as the prescribed by the legislation nitrite level of 120 mg/kg is not surpassed.

As a conclusion, the normally controlled use of nitrite in the processed meats represents no toxicity risk regarding the N-nitrosamine formation, especially after addition of the anti-oxidantia in the brine, which have an inhibiting effect on the N-nitrosamine formation (Sebranek & Bacus, 2007).

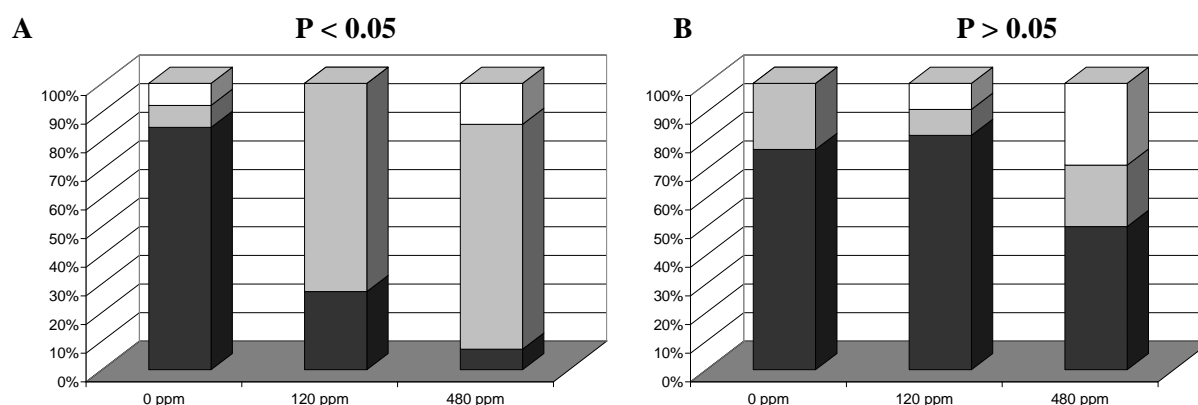


Figure 2. NDMA (A) and NPYR (B) detection after addition of 0 ppm, 120 ppm, and 480 ppm nitrite. Percentages of the samples were, respectively, below the limit of detection (black bars), below the limit of quantification (grey bars) and above the limit of quantification (white bars). For LOD and LOQ, see Table 1.

Conclusions

In this study, the importance of proline as a precursor for *N*-nitrosamine formation was confirmed. In contrast, hydroxyproline acted rather as an inhibitor of NPYR formation. The presence of NDMA almost doubled in the heated meat products due to the addition of nitrite compared with the samples with no nitrite added. NPYR formation was only increased after addition of 480 ppm nitrite.

To conclude, the nitrosamine content in none of the experiments exceeded the level of 10 µg/kg. Nevertheless, the nitrosamines levels in this study were always low (< LOQ) as long as the prescribed by the legislation nitrite level of 120 mg/kg is not surpassed. The experimental conditions ($T = 220^{\circ}\text{C}$ and 480 ppm nitrite) used in this study were artificial and were only employed to expand the platform for our comparisons.

Acknowledgement

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