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MASS SPECTROMETRIC IDENTIFICATION OF NITRITE-INDUCED MODIFICATIONS IN HEATED MODEL PEPTIDES AND THE CURED PORK MATRIX

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I. INTRODUCTION

Nitrite has been used in meat products for many centuries to ensure their microbiological safety by inhibiting the growth of human pathogenic bacteria, such as *Escherichia coli*, *Salmonella sp.*, *Listeria sp.*, and especially *Clostridium botulinum* [1]. In addition, nitrite imparts other positive characteristics to the product, such as its curing aroma and the formation of a stable red coloration caused by nitroso-myoglobin (FeII), which is developed after reaction of NO with myoglobin [2]. However, nitrite can also lead to the formation of carcinogenic *N*-nitrosamines (*N*-NAs), especially upon heating [3, 4]. Nitrosylation of primary amino groups most commonly found in meat as amino acids, immediately causes degradation to the corresponding alcohols and nitrogen. Only secondary amino groups are forming stable *N*-NAs. Until now, all described *N*-NAs are small molecules derived from secondary amino acids or degradation products of the meat matrix [3], with their levels increasing particularly after storage or heating [5]. The vast majority of amino acids in fresh meat are bound in proteins [6], meaning that proteins are more abundant than the nitrosatable amines resulting from the breakdown of amino acids, which are the known precursors of *N*-NAs. From this perspective, the question arises of whether proteins could also be modified by nitrite and which products would form from such reactions, especially upon heating. The primary challenge lies in the extremely low abundance of these modifications. So far, nitrite-induced modifications have been identified by mass spectrometry only in peptide and single protein model studies describing tyrosine nitration [7] and deamination of the N-terminal amino group by its elimination or addition of water to the α-carbon atom [8]. The aim of the present study was to identify nitrite-induced modifications with model reactions of selected tryptic meat peptides which can be used for the targeted detection of protein modifications in meat products.

II. MATERIALS AND METHODS

A combined mass spectrometry-based approach involving synthetic tryptic meat peptides and the analysis of pork products was used to identify these modifications. Digested samples of cured sausages were prepared using a mild protocol to minimize degradation or artificial modifications. Based on these results, model peptides were selected according to their abundance, tyrosine content, or position at the protein N-terminus and synthesized. The model peptides were treated in canning simulations with and without nitrite to analyze the products formed with an untargeted proteomics approach by ESI-Tof LC-HRMS/MS. The nitrite-induced modified peptides were then subjected to a targeted search in the sausage samples by Triple-quadrupole LC-LRMS/MS.

III. RESULTS AND DISCUSSION

The peptide model studies enabled the identification of previously undescribed nitrite-induced deamination products, which were formed by the addition of chloride and phosphate to the α-carbon atom of the peptide N-terminus, as well as α,β-unsaturated elimination products of N-terminal methionine residues (Fig.1).

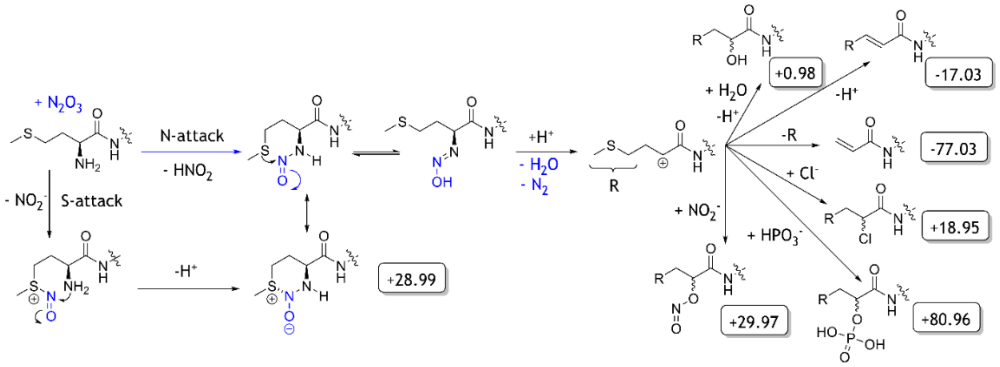


Fig. 1: Proposed molecular mechanisms leading to the formation of observed nitrite-induced products indicated by the corresponding mass shifts relative to the unmodified peptide.

The most abundant modification of peptide side chains was the nitrite-induced nitration of tyrosine residues. Furthermore, nitrosoproline was described for the first time as part of a peptide. This nitrosoprolyl peptide was also detected in nitrite-treated sausages after tryptic digestion (Fig.2).

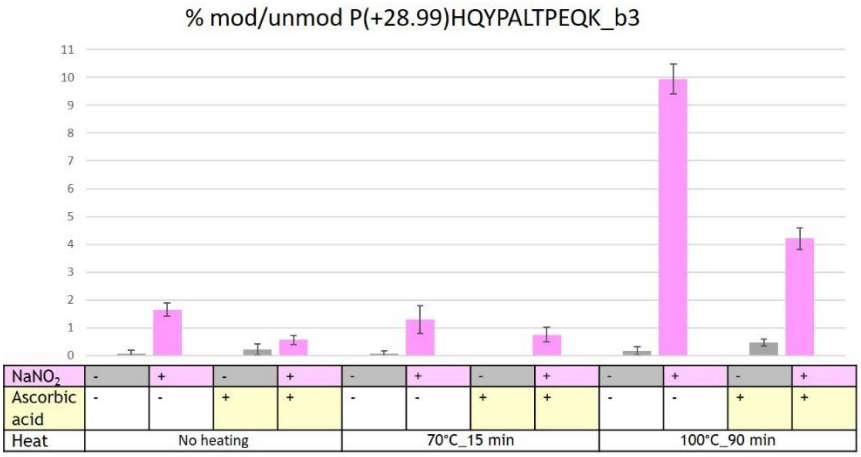


Fig. 2: Mean values and standard deviations (N = 3) of the ratio of peak areas (b3-ions) from the modified to the unmodified form of the peptide PHQYPALTPEQK in sausages prepared with (pink) and without nitrite (grey) at different processing conditions.

IV. CONCLUSION

The search for nitrite-induced protein modifications is motivated by the need to identify the products formed for toxicological evaluation and the demand for marker peptides that can facilitate the optimization of processing conditions for curing meat products. We employed a combined approach involving meat analysis and peptide model studies to address this challenge. In summary, the combined strategy presented here was used to identify hitherto undescribed nitrite-induced peptide modifications and a potential marker peptide for nitrite-induced protein modifications.

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REFERENCES

[1] Majou, D., Christieans, S., Mechanisms of the bactericidal effects of nitrate and nitrite in cured meats. *Meat science* 2018, *145*, 273–284.

[2] Honikel, K.-O., The use and control of nitrate and nitrite for the processing of meat products. *Meat science* 2008, *78*, 68–76.

[3] Schrenk, D., Bignami, M., Bodin, L., Chipman, J. K. et al., Risk assessment of N-nitrosamines in food. *EFSA journal. European Food Safety Authority* 2023, *21*, e07884.

[4] Herrmann, S. S., Granby, K., Duedahl-Olesen, L., Formation and mitigation of N-nitrosamines in nitrite preserved cooked sausages. *Food chemistry* 2015, *174*, 516–526.

[5] Bellatti, M., Parolari, G., Aliphatic secondary amines in meat and fish products and their analysis by high pressure liquid chromatography. *Meat science* 1982, *7*, 59–65.

[6] Wu, G., Cross, H. R., Gehring, K. B., Savell, J. W. et al., Composition of free and peptide-bound amino acids in beef chuck, loin, and round cuts. *Journal of Animal Science* 2016, *94*, 2603–2613.

[7] Thao, M. T., Karumanchi, D. K., Yacout, S. M., Gaillard, E. R., Nitrite ion modifies tyrosine and lysine residues of extracellular matrix proteins. *Nitric oxide : biology and chemistry* 2018, *79*, 51–56.

[8] Deng, H., Nitrite anions induce nitrosative deamination of peptides and proteins. *Rapid communications in mass spectrometry : RCM* 2006, *20*, 3634–3638.