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A COMPREHENSIVE VIEW ON THE FATE OF ^{15}N ISOTOPE-LABELED NITRITE IN MODEL SYSTEMS FOR CURED AND HEATED SAUSAGES

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I. INTRODUCTION

Nitrite is still a widely used component for food preservation leading to several desirable effects concerning taste, microbiological stability, antioxidative properties and stable red coloration. However, the chemistry of nitrite also involves the formation of toxic substances such as N-nitrosamines, which necessitates regulating the addition of nitrite [1]. If all sources of dietary nitrite are added together, the acceptable daily intake (ADI) of 0.07 mg nitrite per kg body weight can be exceeded for all age groups at maximum exposure and for children at medium exposure [2]. As a result, the new EU Regulation EC 2023/2108 was recently adopted, limiting the permitted amounts of nitrites and nitrates to a greater extent than before. To limit the direct intake of nitrite, its residual amount in processed food is a necessary parameter that needs to be determined [3]. However, certain processing treatments may lead to a noticeable reaction of nitrite with several different meat constituents (i.e. nitrite conversion), resulting in low residual amounts of nitrite, but possibly also in higher amounts of potentially harmful nitrosylated organic compounds. As a result, a clear understanding of the reactivity of nitrite in meat products including the reciprocal conversion of nitrite and nitrate as well as the formed nitrogen species is required. A noticeable formation of nitrous oxide (N_2O) in sausage curing model systems was reported as early as the 1970s [4], including isotope labeling with $Na^{15}NO_2$ [5]. These initial observations raised the question of how to quantify nitrite conversion, and in a first step the fate of nitrite in sausage curing model systems was investigated with ^{15}N-isotope labeling and isotope ratio mass spectrometry (IRMS) [6], but N_2O was not quantified. Further attempts to determine the fate of nitrite in sausage curing model systems remained controversial: Some researchers concluded that gas formation was of minor importance for the nitrogen balance [7]. Others assumed that considerable amounts were released into the gas phase after heating, or even most of the nitrite was present as nitric oxide radical (NO) [8]. Furthermore, an unidentified nitrogen species was described, which had been detected without, but to a greater extent with additional ascorbate [9, 10]. Most groups reported a common disappearance of varying amounts of nitrogen and some suggested that this loss occurred in the gas phase [11], but none of these investigations considered N_2O. Despite this unsolved problem, no further studies have been conducted since the 1970s as also stated in current reviews [12, 13], so that a systematic understanding of the fate of nitrite in processed meat products and the relationship between ingoing and residual nitrite amounts is still lacking, particularly in the presence of sodium ascorbate [14]. For this reason, we aimed to establish an IRMS-based method to comprehensively trace the fate of nitrite, focusing on its distribution between the gas phase and the sausage matrix in curing model systems.

II. MATERIALS AND METHODS

Model systems for sausage curing were prepared in pairs, with and without nitrite, each containing either 0 or 1000 mg/kg ascorbic acid (AS). Heating for 10 min at 70 °C and for 90 min at 100 °C was used to simulate half or full preserves, respectively. The gas phase was analyzed using headspace GC-MS. Accordingly, samples with ^{15}N-labeled nitrite were prepared and the gas phase was analyzed using headspace GC-IRMS. Both the gas phase and the sausage matrix were subjected to elemental analysis-(EA)-IRMS. Additionally, nitrite, nitrate and carbonyl content were quantified in the in the sausage matrix. To obtain a clear picture of the products formed, we initially used an increased nitrite content of 488 mg/kg, which was reduced to the usual content of 156 mg/kg in further experiments. Three different ratios of ^{15}N isotope labeled and unlabeled $NaNO_2$ were used: Unlabeled, fully labeled, and partially labeled, as we found that full labeling, as used in previous studies, results in the exclusion of most nitrite-derived nitrogen species from quantification in IRMS.

III. RESULTS AND DISCUSSION

The gas phase of heated sausage curing model systems contained relevant amounts of N_2O, which increased with stronger heating, and NO, which formed during mild heating and decreased as heating continued. A nitrogen balance describing the fate of the added nitrogen showed that about 16% in the absence and 28% in the presence of AS

degassed after 90 min boiling time in the form of N_2O with two labeled nitrogen atoms (Fig.1). The N_2O content in the gas phase increased significantly after the addition of AS, confirming that it originated from nitrite, which had been reduced by AS via NO. Partially labeled $^{29}N_2$, originating from the degassing of unstable primary nitrosamines, was detected at a level of 13% in the absence of AS and of 5% in the presence of AS. Addition of AS led to a decrease of $^{29}N_2$ content in the gas phase and residual nitrite, thereby confirming its desired inhibitory activity against nitrosylation.

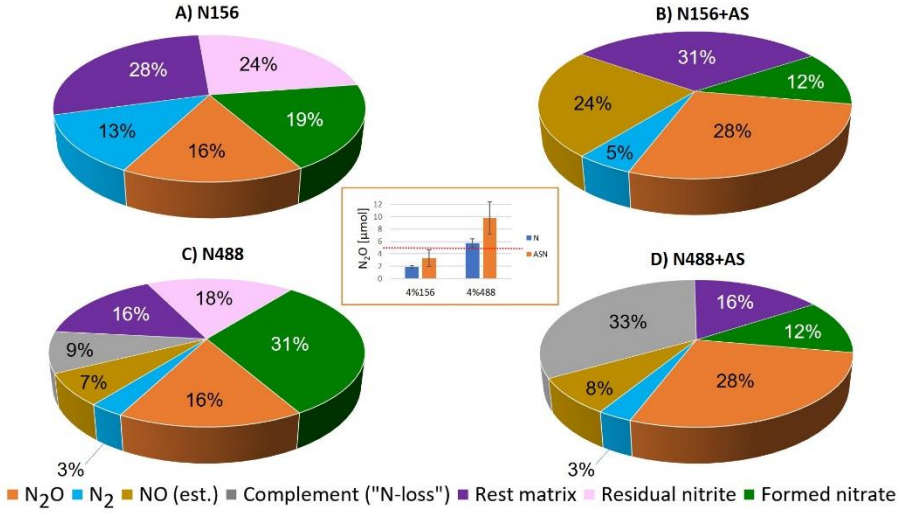


Fig. 1: Distribution of recovered ingoing nitrite depending on treatment of the model sausages with 156 mg/kg of A) nitrite (N156), or B) nitrite + ascorbic acid (N156+AS) and 488 mg/kg of C) nitrite (N488), or D) nitrite + ascorbic acid (N488+AS). The complement to 100% in D) was used as estimated calibration for absolute quantification of NO, which was only possible in relative fashion without NO analyzer. Insert: absolute amount of N_2O detected in the curing gas; the observed solubility in the matrix is depicted with a dotted line. It is assumed that the amount exceeding the solubility is released into the atmosphere before the measurement in the EA. N = nitrite, N+AS = nitrite + ascorbic acid.

IV. CONCLUSION

This is the first systematic explanation of nitrogen loss observed in the curing balance. Several improvements in the applied analytical procedures allowed a complete recovery of labeled nitrogen under food-relevant nitrite concentrations: (I) inclusion of N_2O in the nitrogen balance, (II) partial labeling with 4% ^{15}N to avoid the formation of unquantifiable $^{30}N_2$ in IRMS, (III) consideration of a major proportion of the curing gas (mainly N_2O) dissolved in the aqueous sausage matrix, and (IV) its release during homogenization of the matrix. The heat-induced reduction of nitrite to N_2O simultaneously causes undesirable oxidation of the sausage matrix leading to increased carbonyl formation, cross-linking and a decrease in protein solubility. N_2O was also detected in the gas phase of commercially available canned emulsion-type sausages containing nitrite with and without AS, but not in corresponding commercial samples prepared without nitrite.

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