N-nitrosamines in processed meat products – analysis, occurrence, formation, mitigation and exposure - DTU Orbit (05/12/2018)

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N-nitrosamines (NA) occur in sodium nitrite (nitrite) preserved meat products as bacon, sausages, ham and several types of luncheon meats. Several of these NA are carcinogenic and high intake of processed meat products has been associated with increased risk of cancer and other adverse health effects in some epidemiologic studies. Exposure to NA via meat products may be the underlying reason for this association. The levels of NA in processed meat products ought therefore to be as low as possible. There is a large amount of literature on the occurrence, formation and mitigation of NA in meat products already available, though several areas especially regarding non-volatile NA (NVNA) are relatively unexplored. Studies performed in actual meat products are also scarce. The more that is understood about which factors affects the formation of both volatile NA (VNA) and NVNA the more likely it is to identify strategies for the prevention of NA formation in general and not only for a few NA. The aim of the present thesis was therefore to study the role of ingoing amount of nitrite, factors relevant for industrial processing of meat, fat content and the effect of heat treatment on the formation of VNA and NVNA in meat. Secondly data on the occurrence of VNA and NVNA in processed meat products on the Danish market were to be generated and used for an evaluation of the exposure level resulting from consumption of processed meat products. A method allowing for the simultaneous determination of both VNA and NVNA has not been described in the literature. In order to meet the defined aims, a method based on acetonitrile extraction and liquid chromatography tandem mass spectrometry using both atmospheric pressure chemical ionisation and electrospray ionisation was developed and validated. Data on the occurrence of NA in processed meat products was obtained by analysing products taken from the Danish market. The mean levels of the individual VNA were generally found to be low (<0.8 μg kg⁻¹), whereas the mean levels of the NVNA were considerably higher (~1000 μg kg⁻¹). The most frequently detected NVNA were N-nitrosomethylamine (NDMA) and N-nitrosopyrrolidine (NPYR) and the most frequently detected VNA were N-nitrosodimethylamine (NDMA) and N-nitrosopyrrolidine (NPyR). The levels of the individual NA were reduced with up to 20 to 75%. No additional protection against NA formation was obtained by also adding ascorbyl palmitate, a fat soluble antioxidant. Sodium chloride was found to have minor effects on the NA levels compared to nitrite and erythorbic acid. The NA formation happened rapidly and was relatively unaffected by storage for up to 13 days. Black pepper significantly increased the levels of NPyR. Fe(III) increased the levels of NHPRO, NMTCA and NTCA, whereas haem had no effect on the NA levels.

A clear positive effect of heat treatment on the levels of NPIP was demonstrated in all the heat treatment experiments performed. Depending on the temperature obtained in the meat different effects were found for the other NA. If the sausages produced with different levels of nitrite were fried until a centre temperature of 100°C also the levels of NSAR, NTCA and NMTCA increased. Depending on the temperature and duration of drying and storage than on the ingoing amount of nitrite. A range of studies were performed using both minced pork meat and sausages in order to evaluate the effects of sodium chloride, antioxidants (erythorbic acid and ascorbyl palmitate), sodium tripolyphosphate, dextrose, fat content, black pepper and time on the NA formation and their interactions with nitrite and each other. Factorial experiments were employed in order to gain as much information with a reasonable number of samples. The ingoing amount of nitrite and the presence of erythorbic acid affected the levels of NA most. The levels of NHPRO, NPRO, NPIP, NTCA and NMTCA were inversely related to the amount of erythorbic acid (<396-1104 mg kg⁻¹), whereas the levels of the volatile N-nitrosamines were considerably higher (~1000 mg kg⁻¹). The levels of the NVNA were generally reduced with up to 20 to 75% against NaCl in general. The levels of NPIP was obtained by also adding ascorbyl palmitate, a fat soluble antioxidant. Sodium chloride was found to have minor effects on the NA levels compared to nitrite and erythorbic acid. The NA formation happened rapidly and was relatively unaffected by storage for up to 13 days. Black pepper significantly increased the levels of NPIP. Fe(III) increased the levels of NHPRO, NMTCA and NTCA, whereas haem had no effect on the NA levels.

A clear positive effect of heat treatment on the levels of NPIP was demonstrated in all the heat treatment experiments performed. Depending on the temperature obtained in the meat different effects were found for the other NA. If the sausages produced with different levels of nitrite were fried until a centre temperature of 100°C also the levels of NSAR, NTCA and NMTCA increased. Though when products purchased at the local supermarkets and butcher stores were heated to a higher temperature (~250°C), the levels of NTCA and NMTCA decreased. Depending on the product and heat treatment the levels of NPRO, NPyR, Nnitrosomethylamine (NDEA) and N-nitrosomethyline (NMA) either increased or decreased. From the data acquired on the occurrence of NA in meat products on the Danish market it was estimated that consumption at the 95th percentile of these products resulted in an exposure to VNA of 0.5 ng kg⁻¹ day⁻¹ and 1.6 ng kg⁻¹ day⁻¹ for Danish adults and children, respectively. The calculated Margin Of Exposure (MOE) was well above 10,000 indicating that the exposure is of low concern. Though, it cannot be ruled out that the exposure to these VNA is accountable for the stronger association between adverse health effects and consumption of processed meat than for consumption of red meat. The MOE is not possible to risk assess because data concerning the toxicological relevance of these compounds are lacking. Overall the present thesis show that if nitrite is used for meat preservation and/or colouration the levels of NA generally increase. Because of the possible adverse health effects of NA the exposure level ought to be kept at a minimum. Based on the present knowledge it is evaluated that low levels of NA in processed meat products are best achieved by using as little nitrite as possible and use it in combination with erythorbic acid (~1000 mg kg⁻¹) or another C-vitamin compound. Furthermore by storing the processed meat products protected from oxygen, depletion of the erythorbic acid is prevented. The European Food Safety Authority has concluded that microbiological safe meat products generally may be produced by the addition of 50 mg kg⁻¹ of nitrite. Other means besides nitrite addition can insure the microbiological safety. However, the occurrence of the carcinogenic NDMA and perhaps NPYR seems neither to be related to the levels of nitrite or to the levels of erythorbic acid.