Nitrite-cured cooked pork products – Characterisation of antioxidative and antimicrobial activities - DTU Orbit (02/11/2019)

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Nitrite is a multifaceted additive contributing to colour and flavour formation as well as extending shelf-life of processed meat products by ensuring oxidative and microbiological stability. It is generally agreed to be a necessary additive especially for its anti-botulinum effect and despite massive research efforts, no true alternative has been found. Application of nitrite in meat curing is however still receiving immense attention for its role in formation of carcinogenic N-nitrosamines. The resulting public scepticism toward nitrite and an industrial desire to lower nitrite addition has created a need for investigations of the existence, formation and functional importance of antioxidative and antimicrobial compounds in nitrite-cured cooked meat products in order to ultimately reduce nitrite addition. Consequently, the focus of this PhD has been on characterising antioxidative and antimicrobial activities in a ≤10kDa aqueous fraction of nitrite-cured cooked pork products (NCCPPs) and investigating the impact of processing, including the effects of amount of added curing agents – nitrite and ascorbate. Three different in vitro antioxidant activity assays were applied to ≤10kDa aqueous extracts of a selection of nitrite-cured cooked commercial hams (Paper I) as well as model hams (Paper III) and sausages of varying nitrite/ascorbate addition. A clear effect of curing on reducing power and 2,2′-azino-bis(3ethylbenzthiazoline-6-sulfonic acid) (ABTS) radical scavenging activity were evident for all samples. The results showed these two types of in vitro antioxidant activity to be strongly connected with ascorbate, however, whether the correlation was with added or residual ascorbate varied with sample categories. Furthermore, the interplay between added ascorbate and added nitrite seemed to greatly affect the detected in vitro antioxidant activity. This could be due to mutual reactions, leading to formation of reaction products of increased or decreased antioxidative properties, compared to the individual reactants. Great attention should also be paid to the added nitrite:ascorbate ratio (<1:2.3), in order to avoid conversion to pro-oxidant activities when surpassing an unknown threshold concentration. A storage experiment comprising the model hams were also conducted (Paper III). Reducing power activity increased with extract concentrations at lower levels of nitrite/ascorbate addition but at higher addition levels reducing power increased with extract concentration, only to decrease once extract concentration had reached a certain level. Interestingly this changed during storage to reducing power activity increasing with extract concentrations for all nitrite/ascorbate addition levels. The same development was also shown for residual ascorbic acid. Normalised ABTS radical scavenging activity increased throughout storage, while iron chelating activity tended to increase with storage time in samples of higher nitrite/ascorbate addition. Thus, nitrite/ascorbate addition, beyond a certain threshold concentration, could be affecting a time-dependent formation of active iron chelating component(s). No other connections between iron chelation and curing were observed. Overall the results showed that addition of ≤150ppm nitrite and ≤600ppm ascorbate constituted the tested levels, at which the best overall antioxidative response was obtained. In the attempt to characterise the very complex ≤10kDa aqueous extracts the samples were subjected to further fractionation using size exclusion chromatography (among others Paper II). It was clear from this characterisation that processing had an impact on chromatographic peaks that coincides with fractions displaying antioxidant activity. In addition to containing residual amounts of additives and active species hereof, the extracts were generally found to constitute a very complex mixture of peptides. Through the characterisation of the extracts, it became clear that the in vitro antioxidant activities had to originate from a highly diverse selection of compounds, and that small peptides and certain amino acids e.g. tyrosine, tryptophan, histidine, proline and cysteine may have been of great importance for the in vitro antioxidative properties measured in the tested NCCPPs. Other methods including liquid chromatography-mass spectrometry were also employed but it was not possible to obtain a full molecular characterisation of the antioxidative origin. Yet, as part of the extract characterisation the samples were examined for content of S-nitrosated and C-nitrated peptides, yet such peptides were not found. It was speculated that the lack of detectable 3-nitrotyrosine (3NT) might be due to the presence of strong antioxidants or degradation during sample preparation, however, spiking experiments indicated that 3NT might have been degraded by compounds not transferring to the aqueous ≤10kDa fraction during dialysis. Regardless of differences in product type for its anti-oxidising condition, including type and amount of additives, of the tested NCCPPs no growth inhibitory activities were detected. A potential explanation could be that antimicrobial activities in NCCPPs are in fact related to the curing process, but that the active compounds might somehow be associated with the meat matrix and thus, could not be measured in the aqueous extracts. Alternatively, the tested NCCPP fractions could contain antimicrobial compounds but they were merely tested in too low concentrations to generate a response. This study has emphasized the importance of the established curing agents – nitrite and ascorbate – for the oxidative stability of cured meat products but has also pointed out, that other compounds such as yet unidentified reaction products of curing agents and meat constituents, as well as (modified) peptides and amino acids may also contribute to this property. This does, however, need further investigation and a full molecular characterisation for future utilisation in the processed meat industry.

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