Occurence of Nitroso- and Nitrophenols in Cured and Smoked Meat Products

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SUMMARY

A method was established to detect nitrosophenols as intermediates in cured and smoked meat products. The idea of the method was to stabilize a possible intermediate by a specific reagent. In the case of nitrosophenols, a Grignard reagent, methylmagnesiumbromide was applied to the extracts to give N-methyl-N-hydroxyphenylhydroxylamines, which would be detectable by massspectrometric procedures. With this method different substituted hydroxylamines could be detected in model reactions. This confirms, that the nitrophenols, detected in meat products are snythesized via a pathway which includes the nitrosophenol step. These substances, however, could not be detected in meat products. Thus the occurence of nitrosophenols in cured and smoked products as stable residues is rather unlikely.

The main reason for this result seems to be the numerous and fast side reactions between the intermediate nitrosophenols and on one hand the constitutents of the meat products and smoke and on the other hand the numerous different phenolic substances as reaction partners. Therefore the detection limits of the single nitrosophenols could not reach in real meat products.

INTRODUCTION

Smoke consists of various organic substance classes such as acids, esters, lactones, furanes, condensated polycyclic hydrocarbons and phenols. Some of the most important substances are the phenols. They are important for aroma and colour of the smoked food and have preserving properties. Phenols of smoke are mono-, di- and trihydroxibenzenes and their derivatives. They are mainly originated by pyrolitic reaction of lignins. Because the units of the lignin of hardwood contains more methoxigroups then lignin of soft wood, pyrolysis of hardwood creates phenols deriving from syringol while from softwood homologes from guajacol occur (FIDDLER et al., 1966; FUJIMAKI et al., 1974).

The amounts of phenols in smoke vary between 10 and 200 mg per m^3. The concentrations of phenols in smoked food detected vary between 6 ug and 4800 ug per kg (POTTHAST, 1976; MOEHLER, 1978; BORYS, 1976). Most of the chemical substances, which are carried over during smoking into the food remain concentrated in the outer layer and are able to react with constituents and additives of smoked food forming partially desirable substances which influence colour, smell and taste. But also unwanted substances can occur. In the case of meat products, the additive sodiumnitrite is of great importance. It has preserving properties and as reacting with myoglobin in a desired way influences colour and aroma of meat products. The reagent which leads to all these reactions is the nitrogenmonoxide (NO), which originates from nitrite in acid aqueous media (fig. 1). Because of its electrophilic properties it is able to react also with other electron rich substances. The products are often rather toxic substances like nitrosamines.

$$NaNO_2 + H^+ \longrightarrow HNO_2 + Na^+$$

$$HNO_2 + HNO_2 \longrightarrow N_2O_3 + H_2O$$

$$N_2O_3 \longrightarrow NO + NO_2$$

$$HNO_2 + H^+ \longrightarrow NO^+ + H_2O$$

Fig. 1: Behaviour of sodiumnitrite in acidic solution

Another possibility of reaction of nitrite leads to a nitrosyl cation (NO^+), which reacts with phenols (fig. 1). This reaction can occur within pH values of raw meat products and leads finally to nitrophenols. As intermediates nitrosophenols are supposed to occur.

Until now some nitrophenols could be detected in experimental sausages, but nitrosophenols were never detected in meat products.

Because of their toxicological properties and of catalytic effect on the synthesis of nitrosamines, the question had to be answered, whether nitrosophenols can occur as stable residues in cured and smoked meat products. The application of three "conventional" extraction- and clean up procedures together with massspectrometric analysis gave no hint about the occurence of nitrosophenols in meat products. Nitrophenols, however, the oxidation products of nitrosophenols were detected.

In preliminary experiments we found than in aqueus solutions of sodiumnitrite and different monophenols at pH values of raw sausages (pH 5.5) no nitrosophenols could be detected by applications of "conventional" analytical methods. But in all cases the theoretical possible nitrophenols were found.

There are three possible main reasons, why nitrosophenols were not detected in sausages nor in model reactions:

A. The oxidation of nitrosophenols to nitrophenols is so fast, that the concentrations of nitrosophenols in the equilibrium are so small, that their amounts are not detectable.

B. The sensitivity of nitrosophenols outside their matrices against oxigen is so high, that during extraction, clean up and derivatisation oxidation to nitrophenols occur very fast.

C. Nitrosophenols form very fast other substances - for example by tautomerisation to chinonmonoximes; thus they loose their acidic properties and are not present into the extract.

If these theoretical reasons are the cause, that nitrosophenols are not detectable, a specific intercepting reaction could stabilize the nitrosophenol step by forming substances which are more stable and therefore better detectable.

MATERIALS and METHODS

A method was elaborated to intercept and detect potential newly formed nitrosophenols by a specific reagent. As the intercepting agent, the Grignard-reagent methyl-magnesiumbromide was used, which is known to react in the first stage with nitrosophenols (fig. 2) to N-methyl-N-hydroxyphenyl-hydroxylamins (WIELAND and ROSEEN, 1912; GILMAN and McCRACKEN, 1927). These substances are more stable then nitrosophenols and we tried to detect them by massspectrometry of gaschromatogramms (GC/MS).

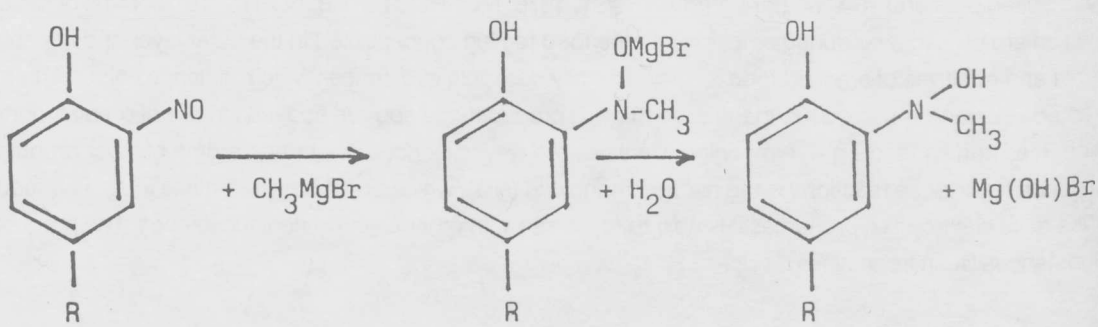

Fig. 2: Reaction of nitrosophenols with a Grignard reagent to substitued phenylhydroxylamines

This method was applied to reaction mixtures of monophenols and curing salt, to smoked "model sausages" and to smoked raw meat products from the market. There are three steps:

A. A Grignard reagent was prepared by the usual procedure from methylbromide in absolute ether with magnesium. During addition of the methylbromide the mixture was cooled with ice, stirred and kept under nitrogen. After addition the reaction mixture was heated under reflux for 30 min and cooled again.

B. The sample was extracted with ethyl-acetate, the extract was defatted and proteins were removed in the usual ways. The finally dried extract was evaporated and resolved with absolute diethylether. The filtered Grignard solution was added slowly under nitrogen protection, stirring and cooling. After the addition was complete, the mixture was heated for one hour under reflux to complete the reaction. The mixture was cooled down by addition of ice and acidified to slightly acidic pH values. The organic layer was separated, dried and evaporated and the residue derivatised using MBTFA in n-Pentan to get sufficient volatile derivatives for GC/MS analysis.

C. The gaschromatographic separation and the massspectrometric examination was done with a Finnigan MAT 4510 automated GC/MS system, consisting of a gaschromatograph Varian 3610, a quadrupole masspectrometer 4500 and a data system INCOS 2100.

GC-conditions: Capillary colomn CP Sil 19 CB, 0.33 internal diamter, length 25 m, mobile phase helium, temperature programm from 80 to 250°C, linear with 5 degrees per minute. Temperature of injector 250 C, injection one ul each, split 1:3.

MS-conditions: massfilter from 30 to 400 atomic mass units (amu), scantime 1.3 sec, electronimpact ionisation.

Because of the lack of standard substances of substituted hydroxylamines, massspectrometric analysis was done as follows:

The molecular weights of the phenols found in smoke are 94, 108, 122, 136, 150 and 206 for simple phenols, 110, 124, 138, 152, 164 and 166 for double phenols and 126, 140 154, 168, 178, 180, 192 and 194 for triple phenols (TOTH, 1981).

In the case of single nitrosation of these phenols the molecular weights increase with the entrance of one nitroso group by 29 amu. During the reaction with the Grignard reagent and derivatisation the molecular weights rise for another 88 amu. So, if e.g. a phenol with molecular weight of 108 is carried over into the reaction product, a molecular peak with 225 amu in the massspectrum must be present to prove the presence of the corresponding nitrosophenol. With this method, three different systems were examined:

A. Solutions of different phenol standards in water of pH values of 5.5 containing 5% of nitrite curing salt.

B. A "artificial smoked sausage". This was a solution of 5% curing salt in water of pH 5.5, filled in a collagen casing smoked for one hour.

C. Cured and smoked raw sausage and hams and hot smoked hams from the market.

RESULTS

A. The nitrosation of standard phenols in aqueus solution with curing salt followed by reaction with the Grignard reagent gave in two cases the corresponding hydroxylamin: 2,6-dimethylphenol gave 4-N-methyl-N-hydroxylamino-2,6-dimethylphenol and 3,5-dimethylphenol gave 2,4- and 2,6-bis(N-methyl-N-hydroxylamino)-3,5-dimethylphenol in small amounts (fig. 3). The additionally analysed amounts of nitrophenols were very small. This demonstrates that the detectable nitrophenols are originated from nitrosophenols. The equilibrium concentrations of the nitrosophenols are normaly very small so that only in the case of special configurations of the phenols detectable amounts of nitrosophenols can be found.

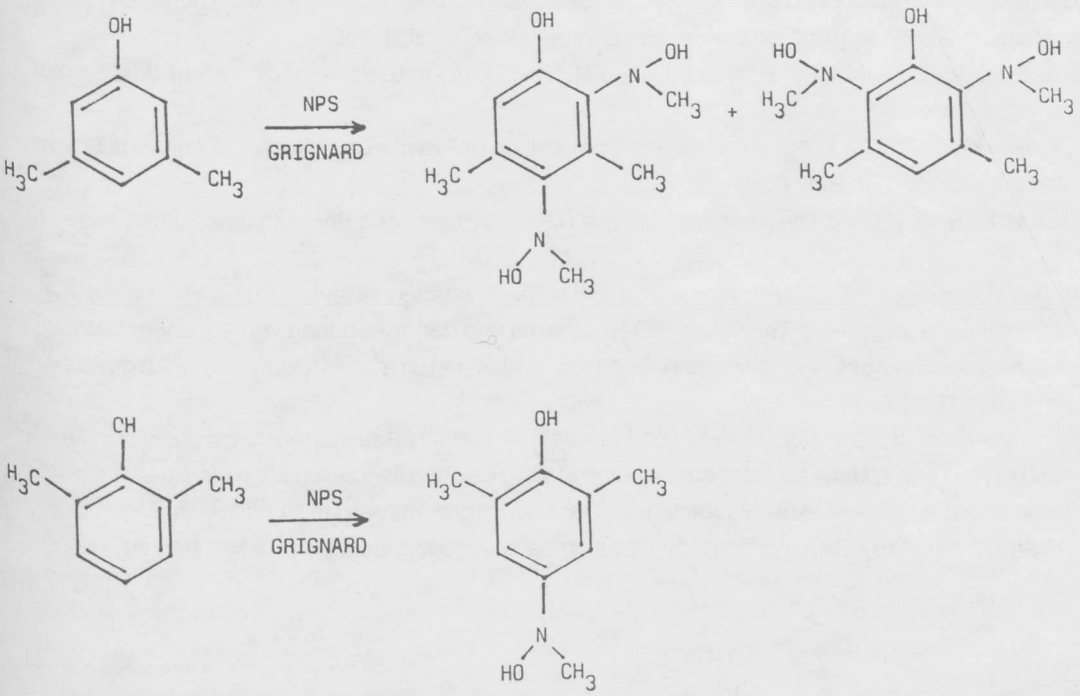

Fig. 3: In modelreactions detected substitued phenylhydroxylamines

B. In the case of the smoked "artificial sausage" no nitrosophenols were found. The reason seems to be, that the smoking procedure brings a lot of different phenols in very small amounts into the sample. Therefore many nitrosated phenols can occur in also very small concentrations; thus the limit never will be reached.

C. In smoked raw sausages and in smoked and hot smoked hams, no nitrosophenols were detectable. But some nitrophenols occured (fig. 4). The reason was the ripening period which oxidised nitrosophenols. It is interesting flat in smoked cooked hams only one nitrophenol was detected whereas in raw sausages most of these substances in fig. 4 were present. The reason is the lower pH value of a raw sausage compared with a hot smoked ham. This difference of half a pH unit leads to a higher concentration of nitrosyl cations in the raw sausage and to more nitro-products.

2-Nitro-4-methylphenol
4-Nitro-2-ethylphenol
2-Nitro-4-ethylphenol
2-Nitro-4-n-propylphenol
4-Nitro-2,6-dimethylphenol
6-Nitro-2,4-dimethylphenol
4-Nitroresorcin

Fig. 4: In raw meat products detected nitrophenols

CONCLUSION

With the described method, nitrosophenol were not detectable in raw meat products but in standard solutions of phenols. As a reason many and fast side reactions of these substances with constituents of meat products, smoke and oxigen occur. So the equilibration concentrations are no longer detectable. In no case of smoked meat products nitrosophenols as stable residues have to be expected.

REFERENCES

BORYS, A., OBIEDZIWSKI, M. and OLKIEWICZ, M. (1976): Die Identifikation von phenolischen Verbindungen in einem polnischen Räucherrauchextrakt. 22. Europ. Fleischforscherkongreß, Malmö, Vol. II, F. 8, 1-7.

FIDDLER, W., DOERR, R.C., WASSERMAN, A.E. and SALAY, J.M., (1966): Composition of Hickory Saw dust Smoke. Furans and Phenols. J. Agric. Food Chem. 14, 659.

FUJIMAKI, M., KIM, K. and KURATA, T. (1974): Analysis and Comparison of Flavour Constituents in Aqueous Smoke Condensates from Various Woods. Agr. Biol. Chem. 38, 45.

GILMAN, H. and McCRACKEN, R. (1927): The Reaction between Nitrosobenzene and Phenylmagnesiumbromide. JACS 49, 1052.

MÖHLER, K. (1978): Das Räuchern. Verlag der Rheinhessischen Druckwerkstätten, Alzey.

POTTHAST, K. (1976): Einfluß verschiedener Techniken des Räucherns und der Anwendung von Räuchermitteln auf den Gehalt von Fleischwaren an cancerogenen Kohlenwasserstoffen, Phenolen und an deren Rauchbestandteilen. Abschlußbericht DFG-Forschungsvorhaben Ha 517/6, 11 u. 14.

TOTH, L. (1981): Präparative Aufarbeitung und Analyse von Phenolfraktionen aus Räucherrauch, geräucherten Fleischerzeugnissen und Räucherpräparaten sowie Untersuchungen zum Einfluß von Rauchbestandteilen auf essentielle Nährstoffe (Vitamine und Aminosäuren) in Fleischerzeugnissen. Abschlußbericht DFG-Forschungsvorhaben Ha 517/22.

WIELAND, W. and ROSEEN, A. (1915): Die Grignardsche Reaktion bei Nitrosoverbindungen. Chem. Ber. 48, 1117.