SOME CURRENT OBSERVATIONS ON THE OCCURRENCE AND FORMATION OF N-NITROSAMINES

W. Fiddler, E. G. Piotrowski, J. W. Pensabene and A. E. Wasserman

Eastern Regional Research Laboratory*
Philadelphia, Pennsylvania 19118

INTRODUCTION

Nitrosamines are of world wide interest from the standpoint of being a potential public health hazard. As a class of chemical compounds nitrosamines have been shown to have tumorigenic, mutagenic and carcinogenic properties in test animals. While the hazard to man has not been completely demonstrated, there is the possibility that humans would also be susceptible to the action of these compounds. Therefore, it is important that the food supply should not contain nitrosamines. Nitrosamines are formed under acidic conditions by the reaction of secondary amines with nitrite. Cured meat products have come under suspicion, since nitrite is added directly in the cure, or is indirectly formed by bacterial reduction of nitrate during processing. While the occurrence of free secondary amines is not common in meat, there are precursor compounds available which can decompose to form them.

There have been reports in the earlier literature of nitrosamines in fresh meat (1) and cured meat samples (2-4) in which gas-liquid chromatographic (GLC) retention times, colorimetric or thin-layer chromatographic procedures were used to identify the nitrosamines. Analysis by such methods alone, however, cannot be accepted as unambiguous confirmation of nitrosamines since they are not specific, and naturally occurring compounds are known to give erroneous results (5). Therefore, many of the earlier claims of finding nitrosamines in cured meats by nonspecific procedures should be considered as questionable. Because of the known carcinogenicity of nitrosamines and their possible presence in the food supply it is of utmost importance that they be determined accurately and their identity be confirmed unambiguously. At present GLC in combination with mass spectrometry (MS) is the best method for the identification of nitrosamines when they are present in very low concentrations, particularly in the presence of interfering naturally occurring components from complex food products. Several workers (5-7) have recently reported finding and confirming DMNA in cured meat samples using GLC-MS methods. Analyses carried out by our Laboratory resulted in confirming the presence of 11-84 μg/kg DMNA in 3 of 40 commercial frankfurter samples. Two of the positive samples were from one manufacturer where high residual $NaNO_2$ values were found ($>$200 mg/kg). However, there were 15 negative samples from the same processor that also contained high residual $NaNO_2$. There appeared to be no correlation between $NaNO_2$ concentration and DMNA occurrence, at least in this case.

---

*Agricultural Research Service, U. S. Department of Agriculture

The occurrence of all confirmed samples containing nitrosamines has been random, appearing only in a single sample of a product type or manufacturer. The question is, therefore, why do nitrosamines form in some cured meat samples and not in others? There are many processing and storage conditions which could contribute to nitrosamine formation. The effect of each of the variables is unknown.

Since 16 billion frankfurters were consumed in the U. S. last year, the presence of even a few samples containing nitrosamines requires an investigation into the cause of their formation. For this reason we undertook a study of the effect of some cure ingredients on nitrosamine formation in frankfurters.

EXPERIMENTAL

The frankfurters were prepared in a conventional manner from lean beef and pork, pork fat, ice, sugar and salt. The desired amounts of $NaNO_2$, $NaNO_2$ plus $NaNO_3$ or $NaNO_2$ plus sodium ascorbate (NaAsc) were thoroughly mixed with the emulsion which was then stuffed into frankfurter casings, linked, cooked and lightly smoked in an air conditioned smokehouse using a commercial program of increasing heat and controlled humidity to a final internal temperature of 160°F. This required approximately two hours. One set of frankfurters containing different levels of cure components were removed, the remainder were smoked and maintained at an internal temperature ca. 160°F for an additional two hours. The additional processing time is not normally used commercially in the U. S. but was utilized in our studies to give results that might be more definitive than the two hour processing time. After processing, the frankfurters were held overnight at 38°F then vacuum packaged, frozen and kept in a freezer at 5°F until analyses were carried out.

The processing studies using $NaNO_2$ alone or in combination with $NaNO_3$ and NaAsc were performed at least three times. The frankfurters were analyzed for residual $NaNO_2$ and $NaNO_3$ by the official AOAC procedure (Griess reagent) and the nitrate specific ion electrode respectively. Analysis for DMNA was done by a modification of the procedures developed by the Food and Drug Administration (8) where DMNA is determined with a GLC alkali flame ionization detector and confirmed by GLC-MS when possible. Because of the difficulty encountered in obtaining sufficient material for confirmation by mass spectrometry only concentrations of DMNA of 10 μg/kg or greater as determined by the GLC procedure were considered to be significant.

RESULTS AND DISCUSSION

In the study of the effect of varying the concentration of $NaNO_2$ alone on DMNA formation, a wide range of nitrite was used. The results have recently been reported (9). Some representative data are shown in Table 1. At levels of $NaNO_2$ up to 750 mg/kg, or 5 times the permissible level that can be added to comminuted meat products, no significant DMNA was observed when a normal 2 hour processing time was used. Concentrations

of DMNA of 10 μg/kg or greater were found in franks made with $NaNO_2$ levels of 1500 mg/kg or higher, with either the 2 or 4 hour processing time. For most of the levels of added nitrite at which apparent or confirmed DMNA could be demonstrated, there was a slight increase in DMNA concentration when the frankfurters were cooked and smoked an additional 2 hours. From this study with $NaNO_2$ alone it appears that 156 mg/kg $NaNO_2$ added (or 1/4 oz per 100 lb meat), which is the legal limit in the United States, is insufficient to produce significant amounts of DMNA in frankfurters, under our processing conditions.

Sodium nitrate is usually included in cure salts, often serving as the precursor for nitrite where bacterial action may reduce the nitrate. It is permissible in the U. S. to add up to 1720 mg/kg meat (2-3/4 oz. per 100 lb.) of nitrate salts in the manufacture of frankfurters. The role of nitrate, in combination with nitrite, on the formation of DMNA was investigated, using 1700 mg/kg, approximately the legal quantity, and 10 times that amount, or 17,000 mg/kg. Some representative results are shown on Table 2. Seventeen thousand mg/kg $NaNO_3$ alone produced no observable DMNA. When the normal amount of $NaNO_2$ (150 mg/kg) was added to the emulsion, with either 1700 or 17,000 mg/kg $NaNO_3$ no DMNA was formed even after 4 hours of processing. Frankfurters prepared with 1500 mg/kg $NaNO_2$ contained 10-11 μg/kg DMNA after 2 hours of processing in the absence of $NaNO_3$ as well as in the presence of 1700 or 17,000 mg/kg $NaNO_3$. After exposure to 4 hours of heating and smoking however, the concentration of DMNA produced with 17,000 mg/kg $NaNO_3$ is somewhat higher than the controls. It is possible, therefore, that at very high levels of nitrate there may be a slight enhancing effect on DMNA production.

Sodium ascorbate or erythorbate is usually used as a cure component to speed up cure color formation. The amount allowable in the U. S. is 7/8 oz. to 100 lb. or 547 mg/kg with respect to the meat. Frankfurters were prepared with $NaNO_2$ and NaAsc, and the representative results of analyses are shown in Table 3. With frankfurters prepared with 150 and 1500 mg/kg $NaNO_2$ in combination 550 mg/kg NaAsc or 10 times this amount and processed for 2 hours, no DMNA was found. When the frankfurters were cooked and smoked an additional two hours no DMNA was detected in the product containing 150 mg/kg $NaNO_2$ and either 550 or 5500 mg/kg NaAsc. However the level of DMNA, produced as expected in frankfurters prepared with 1500 mg/kg $NaNO_2$ alone, was reduced when 550 mg/kg NaAsc was added, and even further lowered in the presence of 5500 mg/kg NaAsc. This finding is not surprising since it is well known that NaAsc reduces nitrite to nitric oxide during curing (10). Nitric oxide forms nitric oxide myoglobin which then forms the cure color pigment, nitric oxide hemochrome upon heating (11), thus reducing the concentration of nitrite available for nitrosamine formation. For each concentration of $NaNO_2$ used, 150 or 1500 mg/kg, addition of increasing amounts of NaAsc yields products having a lower residual $NaNO_2$ concentration. In fact, the per cent loss of $NaNO_2$ is greater for samples containing $NaNO_2$ in combination with NaAsc than with $NaNO_2$ alone or with $NaNO_3$.

Model system experiments simulating the conditions used for the processing of the frankfurters were carried out in a pH 6.0 buffer solution containing dimethylamine HCl and heated for 2 hours at 160°F (71°C). The concentrations of cure salts used were based on the amount that would be added to meat if the frankfurters were prepared according to permissible levels. The results of the model experiments tend to confirm the results of the processing study. It appears that high levels of $NaNO_3$ increases the rate of nitrosation of dimethylamine, while NaAsc inhibits DMNA formation.

The results to date of our study of frankfurter cure ingredients suggests that one possible variable which can contribute to DMNA formation is localized high concentrations of nitrite in emulsions due to inadequate mixing during processing. In addition, if cured meat products having the necessary properties of shelf-life stability, flavor, color and texture can be prepared without $NaNO_3$, it would be desirable to eliminate or limit the amount of this compound used. Also the use of NaAsc would be desirable since it reduces the residual $NaNO_2$ concentration and inhibits nitrosamine formation thereby reducing a potential health hazard.

REFERENCES

1. Mohler, K. and O. L. Mayrhofer. 1968. Z. Lebensm.-Unters.-Forsch. 135: 313-318.

2. Ender, F. and L. Ceh. 1968. Fd. Cosmet. Toxicol. 6: 569-571.

3. Mohler, K. and O. L. Mayrhofer. 1969. Proceedings 15th European Meeting of Meat Research Workers, Helsinki, Finland, p. 302-304.

4. Freimuth, U. and E. Glaser. 1970. Nahrung. 14: 357-361.

5. Sen, N. P. 1972. Fd. Cosmet. Toxicol. 10: 219-223.

6. Fazio, T., R. H. White and J. W. Howard. 1971. J. Ass. Offic. Anal. Chem. 54: 1157-1159.

7. USDA Press Release, 411-72.

8. Howard, J. W., T. Fazio and J. O. Watts. 1970. J. Ass. Offic. Anal. Chem. 53: 269-274.

9. Fiddler, W., E. G. Piotrowski, J. W. Pensabene and A. E. Wasserman. 1972. 32nd Annual Meeting of Institute Food Technologists, Minneapolis, Minn.

10. Fox, J. B. & J. S. Thomson. 1963. Biochem. 2: 465-470.

11. Fox, J. B. 1966. J. Agr. Fd. Chem. 14: 207-210.

Table 1. Effect of $NaNO_2$ on DMNA Formation in Frankfurters

$NaNO_2$ Added, mg/kg	Processing Time: 2 hrs $NaNO_2$ Loss, %	2 hrs DMNA[a] µg/kg	4 hrs $NaNO_2$ Loss, %	4 hrs DMNA[a] µg/kg
150	55	tr	65	tr
750	52	3	59	8
1050	45	8	55	12
1500	46	10	52	14
2500	45	19 MS	46	19 MS

Table 2. Effect of $NaNO_2$ and $NaNO_3$ on DMNA Formation in Frankfurters

$NaNO_2$ added, mg/kg	$NaNO_3$ added, mg/kg	Processing Time: 2 hrs $NaNO_2$ loss, %	2 hrs DMNA[a] µg/kg	4 hrs $NaNO_2$ loss, %	4 hrs DMNA[a] µg/kg
0	17,000	--	tr	--	tr
150	1700	54	tr	70	tr
150	17,000	52	--	67	tr
1500	0	53	11	56	22
1500	1700	54	10	59	15
1500	17,000	54	10	62	32

[a] Corrected for recovery from a sample with 20 µg/kg DMNA added.

Table 3. Effect of $NaNO_2$ and NaAsc on DMNA Formation

$NaNO_2$ added, mg/kg	NaAsc added, mg/kg	Processing Time: 2 hrs $NaNO_2$ loss, %	2 hrs DMNA[a] µg/kg	4 hrs $NaNO_2$ loss, %	4 hrs DMNA[a] µg/kg
0	550	--	--	--	--
150	550	79	--	85	--
150	5500	90	--	92	tr
1500	0	55	11	56	22
1500	550	64	--	69	7
1500	5500	78	--	86	4

[a] Corrected for recovery from a sample with 20 µg/kg DMNA added.