A Research Note

N-Nitrosothiazolidine and its 4-Carboxylic Acid in Frankfurters Containing Alaska Pollock

JOHN W. PENSABENE, WALTER FIDDLER, ROBERT A. GATES, MALCOLM HALE, MICHAEL JAHNCKE, and JAN GOOCH

ABSTRACT

In marine fish of the gadoid family trimethylamine oxide can decompose to formaldehyde. It can then react with cysteine and cysteamine, that may in turn react with nitrite, if present, to form N-nitrosothiazolidine-4-carboxylic acid (NTHZC) and N-nitrosothiazolidine (NTHZ). The NTHZC and NTHZ content of Alaska pollock surimi-meat frankfurters were similar to or lower than those found in an all-meat control, even at 50% substitution. No correlation was found between age of the fish protein prior to processing into surimi meat frankfurters and any measured variables.

INTRODUCTION

THE CONCERNS about nitrosamine formation in nitrite-cured combination fish-meat products have focused primarily on N-nitrosodimethylamine (NDMA), a known animal carcinogen (Barnes and Magee, 1954). This is because high concentrations of dimethylamine (DMA), the principal precursor of NDMA, can be formed in fish, particularly those in the gadoid family (Castell et al., 1971). This occurs when trimethylamine oxide (TMAO) undergoes decomposition. The other decomposition product, formaldehyde, can react with cysteine and its decarboxylated derivative, cysteamine, to form N-nitrosothiazolidine-4-carboxylic acid (NTHZC) and N-nitrosothiazolidine (NTHZ), respectively, after nitrosation (Pensabene and Fiddler, 1985). Presence of these nitrosamines in significant quantities may indicate the presence of other potentially carcinogenic nitrosamines in foods, even though NTHZ has been found noncarcinogenic (Lijinsky et al., 1988). Wood smoke and liquid smoke condensate, commonly used to impart flavor to processed meats, can also be a source of formaldehyde (Pensabene and Fiddler, 1983a).

The occurrence of NTHZC and NTHZ in smoked, cured, all-meat products has been studied extensively (Pensabene and Fiddler, 1983b; Pensabene and Fiddler, 1985; Sen et al., 1985, 1986; Fiddler et al., 1989). Sen et al. (1986) found up to 1600 ppb NTHZC in 1 of 8 positive samples in 20 smoked fish samples. Siddiqi et al. (1988a) found 23 and 82 ppb NTHZC in dried and smoked fish, respectively. They (Siddiqi et al., 1988b) also found 3294 ppb NTHZC in another smoked fish sample vs. 225 ppb in dried fish. Tozawa and Kawabata (1986) reported between 1.2 and 13.5 ppb NTHZ in 12 of 32 direct dried, commercial fish meal samples. However, there are no reports of NTHZC and NTHZ in combination fish-meat products.

The USDA Food Safety and Inspection Service (FSIS) has been petitioned to amend the standard of identity for cooked sausage to permit inclusion of fish protein into frankfurters as an optional ingredient. This would not only make use of underutilized fish protein, but also has the potential to increase the nutritional and sensory quality. However, for regulatory approval, ascertaining the potential for nitrosamine formation in nitrite cured products containing added fish protein is important.

The objective of our study was to investigate the effect of the form of fish protein, percent meat substitution, and frozen storage conditions on the formation of NTHZC and NTHZ in combination fish-meat frankfurters.

Authors Pensabene, Fiddler, and Gates are with the USDA/ARS, Eastern Regional Research Center, 600 E. Mermaid Lane, Philadelphia, PA 19118. Authors Hale, Jahncke and Gooch are with National Marine Fisheries Service, 217 Ft. Johnson Road, Charleston, SC 29412.

MATERIALS & METHODS

Fish-meat frankfurters

Frankfurters in which 0, 15, or 50% of the meat was substituted with Alaska pollock unwashed or washed mince or surimi, were prepared with 156 ppm sodium nitrite and 550 ppm sodium erythorbate using standard industry guidelines, including a smoking cycle, by the Charleston Laboratory of the National Marine Fisheries Service and then shipped to ERRC as described previously (Brooker, 1985; Pensabene and Fiddler, 1988). Prior to processing into frankfurters, some of the fresh mince and surimi was stored an additional 30 or 180 days at −20°C.

Sodium nitrite analysis

Residual sodium nitrite ($NaNO_2$) was determined in 10.0 g of uncooked, ground sample by the modified Griess-Saltzman procedure (Fiddler, 1977).

TMAO analysis

TMAO was determined in the combination fish-meat frankfurters using a procedure recently developed for volatile amines in such products (Fiddler et al., 1991).

NTHZC and NTHZ analysis

NTHZC was determined in the combination fish-meat frankfurters using a recently developed method to isolate and quantitate 10 different N-nitrosoamino acids (Pensabene and Fiddler, 1990). NTHZ was determined in the combination fish-meat frankfurters using a modification of the method developed for all-meat products (Pensabene and Fiddler, 1982). During the initial column extraction procedure, two separate columns were used instead of one, as described for analysis of NDMA in such products (Pensabene and Fiddler, 1988).

Statistical analysis

The General Linear Models (GLM) procedure (Analysis of Variance and Linear Regression Analysis at $p < 0.05$) of the Statistical Analysis System PC software distributed by SAS Institute, Inc. (1985) was used to analyze results. These results were then interpreted according to methods of Snedecor and Cochran (1979).

Safety note

Precaution should be taken in handling nitrosamines, since they are potential carcinogens.

RESULTS & DISCUSSION

AS PART of a larger comprehensive study on combination fish-meat frankfurters for the presence of N-nitrosamines, frankfurters prepared with Alaska pollock mince and surimi

Table 1—N-Nitrosothiazolidine and N-nitrosothiazolidine-4-carboxylic acid in Alaska pollock-meat frankfurters

Form	% Fish	$NaNO_2$, ppm		TMAO, ppm		NTHZ, ppb		NTHZC, ppb	
		Range	Avg	Range	Avg	Range	Avg	Range	Avg
All Meat[a]	0	15-68	41	0.8-4.3	1.5	2.8-6.5	4.4	113-270	188
Unwashed[b]	15	30-85	59	160-390	301	7.8-25.7	13.3	359-957	531
	50	36-110	80	546-1454	1089	8.8-22.2	15.8	210-877	412
Washed[b]	15	27-75	50	11-97	52	3.1-8.9	5.5	176-363	263
	50	19-94	57	34-293	144	3.3-10.8	5.8	65-250	144
Surimi[b]	15	14-80	42	8-88	47	2.6-7.0	4.5	97-239	152
	50	21-101	47	26-279	131	2.3-9.9	4.9	50-171	102

[a] n = 26
[b] n = 46

were analyzed for NTHZ and NTHZC after smokehouse processing. Results are shown in Table 1. Unwashed fish mince is mechanically separated (deboned) fish. Washed mince undergoes several washing steps, then has the water removed. Surimi is the term for a product obtained from washed mince to which cyroprotecting agents have been added to prevent protein denaturation during freezing. To date, the major N-nitrosoamino acid detected in all samples of this product type was NTHZC. Although N-nitrosoproline (NPRO) was commonly found in all-meat products (Pensabene et al., 1979; Tricker et al., 1984; Sen and Kubacki, 1987), this nitrosoamino acid was detected only in trace amounts in a few samples. This was expected since the reaction rate for nitrosation of thiazolidine-4-carboxylic acid (THZC) is much faster than that for proline under similar conditions (Siddiqi et al., 1988a). Statistical analysis of the data showed that NTHZ and NTHZC levels were significantly lower ($p < 0.05$) in the frankfurters made with washed mince or surimi than those containing unwashed pollock. This demonstrated the effectiveness of the washing procedure in removing both formaldehyde and cysteamine and cysteine the precursors for these nitrosamines. For NTHZ, there was no significant difference between the all-meat control and the surimi-meat containing frankfurters (4.4 vs 4.9 ppb), with a maximum of 10 ppb found in frankfurters at the 50% substitution level. With the exception of heavily smoked cured meat products, NTHZ is usually present at less than 10 ppb (Pensabene and Fiddler, 1983b). The washed mince containing frankfurters were slightly higher than either the surimi or all-meat control frankfurters. There was no significant difference in the NTHZC content in the frankfurters prepared from washed mince and the all-meat control, with the surimi containing frankfurters significantly lower than both. The highest average values for NTHZ and NTHZC in the surimi-meat frankfurters, 4.9 and 152 ppb, respectively, were comparable to those found in commercial all-meat frankfurters, 8.5 ppb NTHZ and 58 ppb NTHZC, and cure-pumped bacon, 5.4 ppb NTHZ and 370 ppb NTHZC (Pensabene and Fiddler, 1985).

For NTHZC, a significant difference ($p < 0.05$) was observed between 15 and 50% substitution levels, but no difference was shown in NTHZ. However, the NTHZC values in 50% unwashed and washed mince and surimi-meat frankfurters were lower than those at 15% substitution. The reason for this unexpected difference is unknown. Neither cysteine, present in relatively high concentrations in both meat and fish (Dennis et al., 1979; Opstoedt et al., 1984; Pensabene et al., 1987), nor its formaldehyde reaction product, THZC, appear to be the limiting factors in the amount of nitrosamine formed. Another possibility is the amount of nitrite available to generate nitrosating compounds. A significant correlation ($p < 0.05$) existed between nitrosamine levels and residual sodium nitrite and there was sufficient nitrite present that it would not be the limiting reactant. However, the amount of formaldehyde present may be a limiting factor due to its highly reactive nature. For example, since fish protein is particularly high in lysine and formaldehyde is strongly attracted to the epsilon amino group of this amino acid (Anson and Edsall, 1945), this may explain why less NTHZC was formed in samples containing 50% pollock than in those with 15%. In addition, Gill et al. (1979) have shown that formaldehyde cross-links proteins in muscle tissue of gadoids, forming a three-dimensional network. This in turn causes textural toughness in the fish. These types of reactions suggest that formaldehyde may be reacting to a greater degree with the fish components than with the meat to make less available for reaction with cysteine.

Other studies have shown that NTHZ and NTHZC were formed in cured, all-meat products as a result of smokehouse processing (Pensabene and Fiddler, 1983b; Sen et al., 1986; Ikins et al., 1986). In addition to the formaldehyde generated from wood smoke, there may have been an additional contribution of formaldehyde in combination fish-meat products due to the breakdown of TMAO in the fish (Castell et al., 1971). This might cause additional formation of NTHZ and NTHZC. For this reason, the TMAO content was determined at the same time as the thiazolidine-containing nitrosamines. Similar to the nitrosamine results, there was no difference in levels of TMAO in washed mince and surimi (52 vs 47 ppm at the 15% substitution level and 144 vs 131 ppm at the 50% level), with both forms significantly lower than their unwashed mince counterparts. The apparent low values for TMAO in the all-meat frankfurters (1.5 ppm) were not from TMAO, since no TMAO was present in meat. Typically, TMAO is measured indirectly in fish as trimethylamine (TMA) after reduction (Fiddler et al., 1991). For all-meat frankfurters, TMAO results reflect the TMA formed from reduction of phospholipids. Our results again clearly showed that washing Alaska pollock mince used to form surimi was effective in removing TMAO and formaldehyde. We also found, as expected, a significant difference ($p < 0.05$) in TMAO values between 15 and 50% substitution levels.

Formaldehyde increases with the age of the fish held either "on ice" or frozen due to increased decomposition of TMAO (Castell et al., 1971; Babbitt et al., 1972). We found the age of pollock (0, 30, 180 days) correlated with NTHZC only in frankfurters made from unwashed mince; there was no correlation with age in those containing washed mince or surimi. There was also no correlation between age and NTHZ or TMAO. Because of this, surimi frozen up to 180 days at −20°C, then used to make frankfurters gave NTHZ and NTHZC values not significantly different from the all-meat control (mean values: 4.6 ppb NTHZ, 138 ppb NTHZC in 180 day surimi vs 3.8 ppm NTHZ and 169 ppb NTHZC in the 180 day all-meat controls).

CONCLUSION

THE NTHZ AND NTHZC CONTENT of surimi-meat frankfurters were similar to or lower than those found in all-meat controls, even at 50% substitution. The surimi containing frankfurters had the lowest levels of TMAO, NTHZ and NTHZC. Based on these findings, use of unwashed Alaska pollack mince would not be recommended in combination fish-meat frankfurters.

REFERENCES

Anson, M.L. and Edsall, J.T. 1945. *Advances in Protein Chemistry*, Volume II, p. 316. Academic Press, Inc., New York.

Babbitt, J.K., Crawford, D.L., and Law, D.K. 1972. Decomposition of trimethylamine oxide and changes in protein extractability during frozen storage of minced and intact hake (*Merluccius productus*) muscle. J. Agric. Food Chem. 20: 1052.
Barnes, J.M. and Magee, P.N. 1954. Some toxic properties of dimethylnitrosamine. Brit. J. Industr. Med. 11: 167.
Brooker, J.R. 1985. An evaluation of fish meat as an ingredient in hot dogs-summary report. NMFS, Washington, DC.
Castell, C.H., Smith, B., and Neal, W. 1971. Production of dimethylamine in muscle of several species of gadoid fish during frozen storage, especially in relation to presence of dark muscle. J. Fisheries Res. Board of Canada. 28: 1.
Dennis, M.J., Davies, R., and McWeeny, D.J. 1979. The transnitrosation of secondary amines S-nitrosocysteine in relation to N-nitrosamine formation in cured meats. J. Sci. Food Agric. 30: 639.
Fiddler, R.N. 1977. Collaboration study of modified AOAC method for analysis for nitrite in meat and meat products. J. Assoc. Off. Anal. Chem. 60: 594.
Fiddler, W., Doerr, R.C., and Gates, R.A. 1991. Gas chromatographic method for determinations of dimethylamine, trimethylamine, and trimethylamine oxide in fish-meat frankfurters. J. Assoc. Off. Anal. Chem. 74: 400.
Fiddler, W., Pensabene, J.W., Foster, J.M., and Gates, R.A. 1989. N-Nitrosothiazolidine and N-nitrosothiazolidine-4-carboxylic acid in dry-cured bacon. J. Food Safety. 9: 225.
Gill, T.A., Keith, R.A., and Smith-Lall, B.J. 1979. Textural deterioration of red hake and haddock muscle in frozen storage as related to chemical properties and changes in the myofibrillar proteins. J. Food Sci. 44: 661.
Ikins, W.G., Gray, J.I., Mandagere, A.K., Booren, A.M., Pearson, A.M., and Stachiw, M.A. 1986. N-Nitrosamine formation in fried bacon processed with liquid smoke preparations. J. Agric. Food Chem. 34: 980.
Lijinsky, W., Kovatch, R.M., Keefer, L.K., Saavedra, J.W., Hansen, T.J., Miller, A.J., and Fiddler, W. 1988. Carcinogenesis in rats by cyclic nitrosamines containing sulfur. Food Cosmet. Toxicol. 26: 3.
Opstvedt, J., Miller, R., Hardy, R.W., and Spinelli, J. 1984. Heat-induced changes in sulfhydryl groups and disulfide bonds in fish protein and their effect on protein and amino acid digestibility in rainbow trout. J. Agric. Food Chem. 32: 929.
Pensabene, J.W., Doerr, R.C. and Fiddler, W. 1987. Determination by liquid chromatography with electrochemical detection of cysteamine and cysteine, possible precursors of N-nitrosothiazolidine. J. Assoc. Off. Anal. Chem. 70: 1033.
Pensabene, J.W., Feinberg, J.I., Piotrowski, E.G., and Fiddler, W. 1979. Occurrence and determination of N-nitrosoproline and N-nitrosopyrrolidine in cured meat products. J. Food Sci. 44: 1700.
Pensabene, J.W. and Fiddler, W. 1982. Dual column chromatographic method for determination of N-nitrosothiazolidine in fried bacon. J. Assoc. Off. Anal. Chem. 65: 1346.
Pensabene, J.W. and Fiddler, W. 1983a. Factors affecting the N-nitrosothiazolidine content of bacon. J. Food Sci. 48: 1452.
Pensabene, J.W. and Fiddler, W. 1983b. N-Nitrosothiazolidine in cured meat products. J. Food Sci. 48: 1870.
Pensabene, J.W. and Fiddler, W. 1985. Effect of N-nitrosothiazolidine-4-carboxylic acid on formation of N-nitrosothiazolidine in uncooked bacon. J. Assoc. Off. Anal. Chem. 68: 1077.
Pensabene, J.W. and Fiddler, W. 1988. Determination of volatile N-nitrosamines in frankfurters containing minced fish and surimi. J. Assoc. Off. Anal. Chem. 71: 839.
Pensabene, J.W. and Fiddler, W. 1990. Determination of ten N-nitrosoamino acids in cured meat products. J. Assoc. Off. Anal. Chem. 73: 226.
SAS. 1985. *SAS User's Guide: Statistics*. SAS Institute, Inc., Cary, NC.
Sen, N.P., Seaman, S., and Baddoo, P. 1985. N-Nitrosothiazolidine and nonvolatile N-nitroso compounds in foods. Food Technol. 39: 84.
Sen, N.P., Baddoo, P.A., and Seaman, S.W. 1986. N-Nitrosothiazolidine and N-Nitrosothiazolidine-4-carboxylic acid in smoked meats and fish. J. Food Sci. 51: 821.
Sen, N.P. and Kubacki, S.J. 1987. Review of methodologies for the determination of nonvolatile N-nitroso compounds in foods. Food Add. Contam. 4: 357.
Siddiqi, M., Tricker, A.R., and Preussmann, R. 1988a. The occurrence of preformed N-nitroso compounds in food samples from a high risk area of esophageal cancer in Kashmir, India. Cancer Letters. 39: 37.
Siddiqi, M., Tricker, A.R., and Preussmann, R. 1988b. Formation of N-nitroso compounds under simulated gastric conditions from Kashmir foodstuffs. Cancer Letters. 39: 259.
Snedecor, G.W. and Cochran, W.G. 1979. Statistical Analysis, 6th edition, Iowa State University Press, Ames, IA.
Tozawa, H. and Kawabata, T. 1986. Volatile N-nitrosamines detected in commercial fish meals. Bull. Japanese Soc. Sci. Fisheries. 52: 1969.
Tricker, A.R., Perkins, M.J., Massey, R.C., Bishop, C., Key, P.E., and McWeeny, D.J. 1984. Incidence of some non-volatile N-nitroso compounds in cured meats. Food Add. Contam. 1: 245.
Ms received 9/27/90; revised 1/3/91; accepted 1/29/91.

We thank Judith M. Foster and Janet Crowell for technical assistance.
Presented in part at the 50th annual meeting of the Institute of Food Technologists, June 16–20, 1990, Anaheim, CA.
Reference to brand name or firm does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature.