S7P30.WP

VOLATILE N-NITROSAMINES IN NITRITE-CURED AND NITRITE-FREE TREATED MUSCLE FOODS

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INTRODUCTION

Preservation of meat by salting dates back to antiquity. However, it was not until the late nineteenth century that studies by Polenske (1891), Kisskalt (1899) and Lehman (1899) demonstrated the importance of the nitrite rather than the nitrate anion in the curing process. Since that time, the technology that has developed relative to the use of nitrite has changed meat curing from an inexact art to a precise science (Sebranek, 1979). However, mechanisms for nitrite's multifunctional role in meat preservation have only been partially elucidated.

Nitrite is responsible for a reddening effect (Fox, 1966) and development of the characteristic and well-loved flavour of cured meats, as well as prevention of its deterioration (Igene *et al.*, 1985; Morrissey and Tichivangana, 1985; Freybler *et al.*, 1993). Most importantly, nitrite acts as an antimicrobial agent retarding the germination of spores and toxin formation by anaerobic bacteria such as *Clostridium botulinum* (Sofos *et al.*, 1979; Benedict, 1980). With the advent of modern refrigeration, the importance of nitrite-curing of meat for its preservation has declined. However, cured meats are distinctly attractive in their colour, flavour, as well as texture and are popular because they combine these features with the convenience of storage stability.

Despite all of its desirable effects, nitrite was revealed to be the culprit in the formation of N-nitrosamines in certain cooked cured products. N-nitrosodimethylamine (NDMA) and N-nitrosopyrrolidine (NPYR), examples of such reaction products, were found to be carcinogenic, mutagenic and teratogenic in experimental animals (Magee and Barnes, 1967; Preussmann and Stewart, 1984). Early work by Mirvish (1970) revealed that the rate of N-nitrosamine formation was directly proportional to the content of amines in meat and to the square of the residual nitrite concentration. Consequently, the N-nitrosamine concern has led to technological changes in the meat processing industry. These include elimination of nitrate from curing applications to allow more complete control of nitrosating reactions, reduction of levels of nitrite added, particulary for bacon, and incorporation of higher concentrations of N-nitrosamine-blocking agents such as sodium ascorbate or its isomer, erythorbate, in cures. Although various studies have confirmed the presence of volatile N-nitrosamines in cured meats, there appear to be discrepancies in both the qualitative and quantitative nature of findings reported in the literature. Many factors such as mode of cooking, temperature and duration of heat processing, nitrite concentration, salt concentration, pH and presence and concentration of ascorbate affect the potential for N-nitrosamine formation (Sen *et al.*, 1979).

Surimi and washed minced fish have been proposed as a partial replacement for meat in typical cured luncheon products, as well as frankfurters (Fiddler *et al.*, 1993), however, nitrite is not permitted as an additive in the curing of fish in Canada. Such a substitution would not only make use of under-utilized fish protein, but it also has the potential to increase the nutritional and sensory quality of formulated products (Pensabene *et al.*, 1991). However, enzymatic breakdown of trimethylamine N-oxide in commercially important fish of the gadoid family, such as cod, to produce dimethylamine (DMA) is a concern (Pensabene and Fiddler, 1988; Fiddler *et al.*, 1993). The DMA may in turn participate in the formation of NDMA in the presence of nitrite, nitrous acid or any of their derivatives. Brooker (1985)

reported that higher levels of NDMA were found in hybrid fish-meat as compared with all-meat (control) frankfurters; however, Pensabene and Fiddler (1988) questioned the possibility of artifactual NDMA formation in this study due to the method of analysis employed.

Without nitrite a large class of well-loved muscle foods would be eliminated from the Western world's diet. Therefore, it is prudent to develop alternatives to nitrite in the curing of meat and fish products. In 1975, Sweet proposed the use of composite non-nitrite curing mixtures for duplicating the cumulative action of nitrite. His system consisted of a colorant (erythrosine), an antioxidant/ chelator, an antimicrobial agent and all other curing adjuncts with the exception of nitrite. We also prepared nitrite-free products, however, our colorant of choice was the actual cooked cured-meat pigment (CCMP), which was preformed outside of the meat matrix and then applied to it (Shahidi *et al.*, 1984; 1985; Shahidi and Pegg, 1991a,b). Meat systems examined included pork, cod, cod surimi and hybrid pork/fish products. The present study investigated the effects of nitrite-curing and CCMP-treating of cod and cod-containing meat systems since fish of the gadoid family are known to contain a much higher concentration of DMA and its precursor than red meats (Pensabene and Fiddler, 1988). The present paper reports a summary of the efficiency of nitrite-free curing systems in preventing N-nitrosamine formation in such treated products.

MATERIALS AND METHODS

Meat and Fish Samples

Boneless pork loins were obtained from Newfoundland Farm Products Corp. (St. John's, NF) and their subcutaneous fat was trimmed. Loins were comminuted twice using a Hobart 4146 meat grinder (Hobart MFG Co. Ltd., Don Mills, ON) with a 7.9mm and then with a 4.8mm plate. Comminuted meat samples were transferred to polyethylene pouches (Eastern Paper Co., St. John's, NF), packaged using a Multivac vacuum packager (Model A300/32, Wolfertschwenden, Germany) and then stored at -15°C until used.

Cod (*Gadus morhua*) frozen at -15°C was obtained from Fishery Products International (St. John's, NF). Before use, fillets were thawed and ground in a manner similar to that for meat (see above). Cod surimi, which is the washed flesh of minced cod to which sorbitol at 4% (w/w) and sodium tripolyphosphate at 0.3% (w/w) has been added, was a product of Terra Nova Fisheries (Clarenville, NF). The surimi was obtained frozen in block form and was stored at -60°C until used.

Preparation of Cooked Cured-Meat Pigment (CCMP)

The cooked cured-meat pigment (CCMP) was prepared from haemin, isolated from bovine red blood cells or as a by-product of seal meat processing (Shahidi *et al.*, 1992). Nitric oxide was used as a nitrosylating agent as described by Shahidi *et al.* (1985).

Nitrite- and CCMP-Curing of Meat Systems

Comminuted pork, cod, cod surimi or hybrid formulations was/were mixed with 20% (w/w) of distilled water, 3000mg/kg sodium tripolyphosphate, 2500mg/kg sodium hypophosphite, 550mg/kg sodium ascorbate and 30mg/kg butylated hydroxyanisole. Ground pork was substituted with cod or cod surimi in hybrid formulations at 0, 15 or 50% levels. Sodium nitrite and CCMP were added directly to meat samples at concentrations of 156 and 12mg/kg respectively. Systems were thoroughly homogenized and then cooked at 85±2°C in a thermostated water bath for ca. 40 minutes to reach an internal temperature of 75±2°C. Meats were stirred occasionally with a glass rod during the cooking process. After cooling to room temperature, samples were homogenized in a Waring blender for 30 seconds, transferred to polyethylene pouches (Eastern Paper Co., St. John's, NF) and vacuum packaged. Contents of each sample were then analyzed for presence of volatile N-nitrosamines.

Analysis for Volatile N-Nitrosamines

The complete details for analysis of volatile N-nitrosamines, particularly NDMA, using a vacuum-distillation method have been described by Sen *et al.* (1985). All samples were analyzed in duplicate and the content of NDMA detected by the thermal-energy analyzer (TEA) was corrected based on the recovery of N-nitroso-di-*n*-propylamine (NDPA), an internal standard used in each sample. To confirm the presence of NDMA in some of the prepared systems, sample extracts were subjected to GLC-MS analysis as described by Sen *et al.* (1985).

RESULTS AND DISCUSSION

Composite nitrite-free meat curing systems containing the preformed CCMP have been successful in duplicating the colour, flavour and bacteriostasis of their nitrite-cured counterparts as reported by Shahidi and associates (Wood *et al.*, 1986; Shahidi *et al.*, 1987; 1988; Shahidi and Pegg, 1990; 1991c; 1992). To justify the use of such composite systems, absence of carcinogenic N-nitrosamines in finished products should be verified. Since nitrite or any of its derivatives are not added and since the meats are not smoked, the occurrence of N-nitrosamines is not anticipated in cooked products. However, the preformed CCMP, used as a colorant in nitrite-free curing of meats, is a nitrosylated haem derivative of myoglobin/haemoglobin with the potential to act itself as a nitrosating agent. As illustrated in Figure 1, CCMP or mononitrosyl ferrohaemochrome contains a nitric oxide moiety in one of the dz_2 orbitals of the ferrous iron atom of haem. Since the cured-meat pigment is known to decompose in the presence of light and oxygen, transnitrosation reactions involving other meat constituents are possible. Although the likelihood of occurrence of such reactions is remote, nonetheless, the presence of volatile N-nitrosamines in nitrite-free cured meats containing CCMP was investigated. Uncured and nitrite-cured samples were used as controls.

In this study, only NDMA was detected in some of the nitrite-cured systems although presence of all volatile N-nitrosamines was tested. Table 1 summarizes the content of volatile NDMA in cooked, uncured, nitrite-cured (156mg/kg) and CCMP-treated (12mg/kg) pork, cod, cod surimi and pork/fish hybrid systems. No measurable amount of NDMA was detected in the uncured, nitrite-cured or CCMP-treated pork systems. A concentration of <0.2μg/kg, which is the detection limit of the TEA, is reported. This value is obtained based on the recovery of the internal standard, NDPA.

Only 0.9, 0.3 and 1μg/kg of NDMA, as confirmed by MS, was detected in the nitrite-cured cod, pork/cod (15%) and pork/cod (50%) hybrid formulations, respectively. These results may reflect the very fresh nature and careful processing of the fish used in this study. The precursor of NDMA, dimethylamine (DMA), is formed in the muscles of fish due to the action of endogenous enzymes on trimethylamine N-oxide. Perhaps only partial degradation of trimethylamine N-oxide to DMA had occurred in the fish muscle tissue by the time of its use. Although NDMA was present in nitrite-cured fish-containing products, this and other volatile N-nitrosamines were absent in uncured and nitrite-free, CCMP-treated samples. This implies that either no disproportionation of CCMP had occurred or that insufficient amounts of nitric oxide were produced to participate in transnitrosation reactions. Absence of N-nitrosamines in nitrite-cured cod surimi suggests that washing of cod muscles may be an effective means of removing or reducing the concentration of DMA or its precursors from fish muscles in order to prevent their nitrosation. No volatile N-nitrosamines were detected in uncured and CCMP-treated cod surimi samples. However, NDMA was detected at a concentration of 0.2μg/kg in nitrite-cured pork/cod hybrid formulations at both 15 and 50% substitution of cod. Furthermore, CCMP-treated hybrid analogs were free of any detectable NDMA as observed for cod-containing samples.

Consequently, this study supports the view that composite nitrite-free curing systems containing a colorant (CCMP), an antioxidant/ chelator system (butylated hydroxyanisole/sodium tripolyphosphate/ sodium ascorbate) and possibly an antimicrobial agent (sodium hypophosphite) can be employed to successfully prepare processed meat products with identical characteristics of their nitrite-cured analogs without the fear of N-nitrosamine formation. Additionally, we have demonstrated that nitrite-free curing of fish and fishery by-products in combination with red meats in the production of novel products is now at hand.

ACKNOWLEDGEMENTS

We thank the Natural Sciences and Engineering Research Council (NSERC) of Canada for financial support.

REFERENCES

BENEDICT, R.C. 1980. Biochemical basis for nitrite-inhibition of *Clostridium botulinum* in cured meat. *J. Food Prot.* 43:877-891.

BROOKER, J.R. 1985. An evaluation of fish meat as an ingredient in hot dogs - Summary report. National Marine Fisheries Service, Washington, DC.

FIDDLER, W., PENSABENE, J.W., GATES, R.A., HALE, M., JAHNCKE, M., and BABBITT, J.K. 1993. Alaska pollock (*Theragra chalcogramma*) mince and surimi as partial meat substitutes in frankfurters: N-Nitrosodimethylamine formation. *J. Food Sci.* 58:62-65, 70.

FOX, J.B.,Jr. 1966. The chemistry of meat pigments. *J. Agric. Food Chem.* 14:207-210.

FREYBLER, L.A., GRAY, J.I., ASGHAR, A., BOOREN, A.M., PEARSON, A.M., and BUCKLEY, D.J. 1993. Nitrite stabilization of lipids in cured pork. *Meat Sci.* 33:85-96.

IGENE, J.O., YAMAUCHI, K.Y., PEARSON, A.M., GRAY, J.I., and AUST, S.D. 1985. Mechanisms by which nitrite inhibits the development of warmed-over flavour (WOF) in cured meat. *Food Chem.* 18:1-18.

KISSKALT, K. 1899. Beiträge zur Kenntnis der Ursachen des Rothwerdens des Fleisches beim Kochen, nebst einigen Versuchen über die Wirkung der schwefligen Säure auf die Fleischfarbe. *Arch. Hyg.* 35:11-18.

LEHMAN, K.B. 1899. Über das Haemoglobin, ein neuer weitverbreitetes Blutfarbstoff-Derivat. *Sitzt. Physikal. Med. Ges. Würzburg.* 4:57-61.

MAGEE, P.N., and BARNES, J.M. 1967. Carcinogenic nitroso compounds. *Adv. Cancer Res.* 10:163-246.

MIRVISH, S.S. 1970. Kinetics of dimethylamine nitrosation in relation to nitrosamine carcinogenesis. *J. Natl. Cancer Inst.* 44:633-639.

MORRISSEY, P.A., and TICHIVANGANA, J.Z. 1985. The antioxidant activities of nitrite and nitrosylmyoglobin in cooked meats. *Meat Sci.* 14:175-190.

PENSABENE, J.W., and FIDDLER, W. 1988. Determination of volatile N-nitrosamines in frankfurters containing minced fish and surimi. *J. Assoc. Off. Anal. Chem.* 71:839-843.

PENSABENE, J.W., FIDDLER, W., GATES, R.A., HALE, M., JAHNCKE, M., and GOOCH, J. 1991. N-Nitrosothiazolidine and its 4-carboxylic acid in frankfurters containing Alaska pollock. *J. Food Sci.* 56:1108-1110.

POLENSKE, E. 1891. Über den Verlust, welchen das Rindfleisch an Nährwerth durch das Pökeln erleidet, sowie über die Veränderungen Salpeter-haltiger Pökellaken. Mittheilungen aus dem chemischen Laboratorium des Kaiserlichen Gesundheitsamtes, 13. *Arb. K. Gesundhamt.* 7:471-474.

PREUSSMANN, R., and STEWART, B.W. 1984. N-Nitroso carcinogens. In: SEARLE, C.E. (ed). *Chemical Carcinogens, Second edition.* ACS Monograph 182. American Chemical Society, Washington, DC. pp.643-828.

SEBRANEK, J.G. 1979. Advances in the technology of nitrite use and consideration of alternatives. *Food Technol.* 33(7):58-62, 93.

SEN, N.P., SEAMAN, S., and MILES, W.F. 1979. Volatile nitrosamines in various cured meat products: Effect of cooking and recent trends. *J. Agric. Food Chem.* 27:1354-1357.

SEN, N.P., TESSIER, L., SEAMAN, S.W., and BADDOO, P.A. 1985. Volatile and nonvolatile nitrosamines in fish and the effect of deliberate nitrosation under simulated gastric conditions. *J. Agric. Food Chem.* 33:264-268.

SHAHIDI, F., and PEGG, R.B. 1990. Colour characteristics of cooked cured-meat pigment and its application to meat. *Food Chem.* 38:61-68.

SHAHIDI, F., and PEGG, R.B. 1991a. Novel synthesis of cooked cured-meat pigment. *J. Food Sci.* 56:1205-1208.

SHAHIDI, F., and PEGG, R.B. 1991b. Encapsulation of the pre-formed cooked cured-meat pigment. *J. Food Sci.* 56:1500-1504, 1518.

SHAHIDI, F., and PEGG, R.B. 1991c. Effect of the preformed cooked cured-meat pigment (CCMP) on colour parameters of muscle foods. *J. Muscle Foods.* 2:297-304.

SHAHIDI, F., and PEGG, R.B. 1992. Nitrite-free meat curing systems: Update and review. *Food Chem.* 43:185-191.

SHAHIDI, F., RUBIN, L.J., DIOSADY, L.L., CHEW, V., and WOOD, D.F. 1984. Preparation of dinitrosyl ferrohemochrome from hemin and sodium nitrite. *Can. Inst. Food Sci. Technol. J.* 17:33-37.

SHAHIDI, F., RUBIN, L.J., DIOSADY, L.L., and WOOD, D.F. 1985. Preparation of the cooked cured-meat pigment, dinitrosyl ferrohemochrome, from hemin and nitric oxide. *J. Food Sci.* 50:272-273.

SHAHIDI, F., RUBIN, L.J., and WOOD, D.F. 1987. Control of lipid oxidation in cooked ground pork with antioxidants and dinitrosyl ferrohemochrome. *J. Food Sci.* 52:564-567.

SHAHIDI, F., RUBIN, L.J., and WOOD, D.F. 1988. Stabilization of meat lipids with nitrite-free curing mixtures. *Meat Sci.* 22:73-80.

SHAHIDI, F., SYNOWIECKI, J., and BALEJKO, J. 1992. Utilization of seal meat by-products. *Proc. 38th ICMST.* Clermont-Ferrand, France. pp.1121-1124.

SOFOS, J.N., BUSTA, F.F., and ALLEN, C.E. 1979. Botulism control by nitrite and sorbate in cured meats: A review. *J. Food Prot.* 42:739-770.

SWEET, C.W. 1975. *Additive composition for reduced particle size meats in the curing thereof.* US Patent 3, 899, 600.

WOOD, D.S., COLLINS-THOMPSON, D.L., USBORNE, W.R., and PICARD, B. 1986. An evaluation of antibotulinal activity in nitrite-free curing systems containing dinitrosyl ferrohemochrome. *J. Food Prot.* 49:691-695.

Table 1. Presence of N-Nitrosodimethylamine (NDMA) in Uncured, Nitrite-Cured and CCMP-Treated Cooked Meat Systems[1].

Processing Treatment	NDMA (µg/kg) IN MEAT SYSTEMS[2]						
	Pork	Cod	Cod Surimi	Pork + Cod[3]		Pork + Cod	
				15%	50%	15%	50%
Uncured	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2
Nitrite-Cured	<0.2	0.9	<0.2	0.3	1.0	0.2	0.2
CCMP-Treated	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2

[1] All systems in addition to the meat and/or fish listed above contain 20% (w/w) distilled water, 3000mg/kg sodium tripolyphosphate, 2500mg/kg sodium hypophosphite, 550mg/kg sodium ascorbate and 30mg/kg butylated hydroxyanisole.

[2] The 15 and 50% represents the percentage of cod or cod surimi substituted for pork in hybrid formulations.

[3] Detection limit of the thermal-energy analyzer for N-nitrosamines is 0.2µg/kg.