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Atlantic Menhaden (*Brevoortia tyrannus*) Mince and Surimi as Partial Meat Substitutes in Frankfurters: Effect on *N*-Nitrosamine Formation

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Studies were conducted to determine the feasibility of using Atlantic menhaden mince and surimi as partial meat substitutes for red meats in the formulation of frankfurters. The effects of the following factors on methylamine and volatile nitrosamine content in broiled frankfurters substituted with the menhaden were evaluated: fish form (washed and unwashed mince and surimi), percent substitution (15 and 50%), storage of fresh and frozen fish preprocessing (0–6 months), and refrigerated storage of frankfurters postprocessing (0–56 days). The overall results of these studies indicate that only trace levels of *N*-nitrosodimethylamine, the only volatile nitrosamine detected, were found in these frankfurters.

INTRODUCTION

The remarkable growth in the consumption of shellfish analogs made from Alaska pollock surimi has initiated a search for alternate fish species that may be a good source of surimi. Atlantic and Gulf menhaden (*Brevoortia* sp.) comprise approximately 25% of the total U.S. commercial finfish landings (National Marine Fisheries Service, 1992). Menhaden are small, oily, herring-like fish that resemble alewife and shad. They are harvested in the Atlantic Ocean and Gulf of Mexico and used almost exclusively for animal feed and fish oil with its many applications. In 1986, Zapata Haynie Corp. initiated a National Marine Fisheries Service (NMFS) contract to construct and operate a processing plant. The research and development contract was to help determine the economic and technical feasibility of producing surimi from Atlantic menhaden (*B. tyrannus*), to evaluate the products using this substrate, and to determine the procedures necessary to maintain its quality for human use (Hale and Bimbo, 1987; Bimbo, 1988). Menhaden surimi was found to exhibit excellent gelling properties at temperatures commonly used for restructured seafood (Lanier et al., 1983). The primary quality problem appeared to be the darker color of this surimi compared with that obtained from Alaska pollock, making it unsuitable for the fabrication of shellfish analog products (Hale and Bimbo, 1987). This would not be a problem if menhaden surimi were used as partial substitute for meat in processed products, as proposed by several researchers. In an earlier study, Steinberg et al. (1976) found that washed menhaden was acceptable for this purpose in sausage products.

One of the concerns related to the use of menhaden mince or surimi is that this fish is a member of the herring family. While fresh herring have not generally been associated with the formation of *N*-nitrosodimethylamine (NDMA), a concern is based on previous disclosures of this animal carcinogen in salted herring (Matsui et al., 1980; Sen et al., 1985) and in herring pickled with nitrate (Peterson and Meyland, 1981). In addition, NDMA has been found in herring meal treated with nitrite (Ender et al., 1964) and in meal subjected to nitrogen oxides generated from the combustion of the fuel used for drying (Sen et al., 1972; Skaare and Dahle, 1975; Hurst, 1976).

In this study, *N*-nitrosodimethylamine, dimethylamine (DMA), trimethylamine (TMA), and trimethylamine oxide (TMAO) were measured in frankfurters in which the meat was substituted with 15 and 50% menhaden surimi and its corresponding washed and unwashed mince. The object of this study was to determine the effect of these materials on NDMA formation in a product cured with nitrite.

MATERIALS AND METHODS

SAFETY NOTE: Precaution should be exercised in the handling of nitrosamines, since they are potential carcinogens.

Materials. Atlantic menhaden mince and surimi were prepared at the Zapata Haynie plant in Reedville, VA, and then shipped by overnight express, in frozen 10-kg blocks, to the NMFS laboratory in Charleston, SC. The blocks were then stored at −30 °C until processed into frankfurters. One additional batch of fresh menhaden was obtained from NMFS personnel in North Carolina and then shipped by overnight express to the NMFS–Mississippi State University Seafood Processing Laboratory in Pascagoula, MS, where mince and surimi were prepared the next day using a continuous in-line washing procedure described by Lee (1984). All batches of unwashed or washed mince and surimi-meat frankfurters in which 0, 15, or 50% of the meat was substituted with menhaden were prepared using typical industry hot smoking procedures (Brooker, 1985). The frankfurters contained 156 ppm of sodium nitrite and 550 ppm of sodium erythorbate. The finished products were shipped by overnight express from either Charleston or Pascagoula to the Eastern Regional Research Center in insulated containers containing cold packs. Upon receipt, the frankfurters were removed from their casings and refrigerated at 4 °C for 18–24 h. The frankfurters

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Table I. *N*-Nitrosodimethylamine (NDMA), Dimethylamine (DMA), Trimethylamine (TMA), and Trimethylamine Oxide (TMAO) in Atlantic Menhaden Mince and Surimi-Meat Frankfurters, Combined Data

	uncooked				broiled			
	amines (ppm)				amines (ppm)			
form	DMA	TMA	TMAO	NDMA (ppb)	DMA	TMA	TMAO	NDMA (ppb)
50% (n = 20)[a]								
mince								
unwashed	4.92[A]	9.56[A]	75.96[A]	0.28[A]	8.15[A]	19.62[A]	83.90[A]	3.07[A]
washed	1.11[B]	1.40[B]	9.50[B]	0.06[B]	1.93[B]	2.43[B]	10.75[B]	0.78[B]
surimi	1.33[B]	2.11[B]	2.11[B]	0.03[B]	1.67[B]	3.30[B]	11.34[B]	0.95[B]
15% (n = 20)[a]								
mince								
unwashed	2.36[A]	3.48[A]	29.81[A]	0.13[A]	2.85[A]	5.94[A]	32.25[A]	0.76[A]
washed	1.85[A]	1.18[B]	5.07[B]	0.05[B]	1.34[B]	1.16[B]	4.78[B]	0.25[B]
surimi	0.77[B]	0.65[B]	2.81[B]	0.03[B]	1.12[B]	2.02[B]	4.44[B]	0.43[C]

[a] Number of samples at each form and percentage analyzed in duplicate. The mean values are from combined data from fresh, frozen, and refrigerated experiments. For each compound at each percentage and cooking treatment, the means with the same letter are not significantly ($p < 0.05$) different from each other.

were analyzed for residual sodium nitrite and then broiled and stored as described previously (Fiddler et al., 1992). For the postprocessing study, the frankfurters were vacuum packaged and then stored in a 4 °C refrigerator for 0, 14, 35, and 56 days prior to broiling.

Analysis. Residual sodium nitrite was determined by the modified Griess–Saltzman procedure (Fiddler, 1977) in duplicate 10-g comminuted samples prior to and after broiling.

Dimethylamine, TMA, and TMAO were analyzed in duplicate according to the headspace GC-FID method previously described (Fiddler et al., 1991a).

Duplicate 10.0-g samples of comminuted fish-meat frankfurter were analyzed for volatile nitrosamines using a solid-phase extraction procedure described elsewhere (Pensabene and Fiddler, 1988). The NDMA values have been corrected for the recovery of the internal nitrosamine standard in each individual sample. The minimum level of reliable measurement based on gas chromatographic thermal energy analyzer response was 0.2 ppb.

Data Analysis. Results were analyzed by one- and two-way analysis of variance using SAS package programs. The Duncan multiple-range test was performed for comparisons among the means. Regression analyses (linear model) were performed to reveal possible effects of the levels of DMA, TMA, TMAO, and residual nitrite on NDMA formation. They were interpreted according to the protocol of Snedecor and Cochran (1979).

RESULTS AND DISCUSSION

Of the 10 volatile nitrosamines for which we tested, only NDMA was detected in the menhaden-meat frankfurters. Fiddler et al. (1991b) have shown that of six common methods of cooking, broiling produced the highest amount of NDMA in Alaska pollock surimi-containing frankfurters. Therefore, broiling was selected for assessment of NDMA formation in this study since it represented the "worse case" scenario. Fish broiled by this method, particularly by natural gas, resulted in a marked increase in NDMA when compared to uncooked fish (Matsui et al., 1980).

Table I shows the data combined from experiments in which the frankfurters were made from either freshly prepared or frozen mince and surimi and from an experiment in which the fish-meat frankfurters were subjected to refrigerated storage, postprocessing. Broiled franks substituted with 50% unwashed mince contained a mean of 3.1 ppb of NDMA (range 1.3–8.9 ppb). At this same percentage, substitution with washed mince and surimi resulted in broiled franks containing mean concentrations <1 ppb of NDMA, with a maximum of 2.4 and 2.1 ppb, respectively. The 15% substituted franks had NDMA mean values of 0.8 ppb in those made with unwashed mince, 0.3 ppb for washed mince, and 0.4 ppb for surimi, after broiling. With a repeatability of 0.5 ppb of NDMA for the analytical method, use of any of these three forms of Atlantic menhaden would yield little or no detectable NDMA at the 15% substitution level. The increase in NDMA due to broiling the frankfurters containing all three forms of Atlantic menhaden was most apparent at the 50% substitution level, especially in those made with unwashed mince (0.3 ppb raw to 3.1 ppb broiled).

Regression analysis of the data showed highly significant ($p < 0.01$) correlations between NDMA and TMAO in the uncooked frankfurters and NDMA with DMA, TMA, and TMAO in broiled frankfurters. This is not unexpected since there have been a number of reports of NDMA formation from TMA and TMAO. Oshima and Kawabata (1978) described the complex mechanisms involved. For TMA, there is oxidative cleavage of one *N*-alkyl bond to form DMA before nitrosation and a pathway not involving DMA. The mechanism for the reaction of TMAO involves its reduction to TMA, which then proceeds as above, or the reaction of an imminium ion with nitrite to form an adduct that directly forms NDMA and formaldehyde. There was no correlation between NDMA and residual nitrite in either the uncooked or cooked frankfurters.

While the difference is not statistically significant, NDMA tended to be higher in the broiled surimi-containing frankfurters than in their washed mince counterparts. Earlier studies with Alaska pollock showed significantly larger differences between the washed mince and surimi (Fiddler et al., 1992). Overall, NDMA in Atlantic menhaden mince and surimi-containing frankfurters was considerably lower than the corresponding Alaska pollock franks reported previously. This reflects the lower amine in the fresh unwashed menhaden mince (n = 6) that contained mean values of 12.4 ppm of DMA, 22.8 ppm of TMA, and 217.5 ppm of TMAO. Atlantic menhaden are clupeoids, whose TMAO content is much lower than that of fish of the gadoid species, which includes members of the cod family such as Alaska pollock (Shewan, 1951).

Frankfurters made from frozen (−20 °C) mince and surimi were analyzed after 2, 30, and 180 days. A significant increase in NDMA was observed only in broiled 50% unwashed mince-containing frankfurters after 30 days (2.1 to 5.2 ppb) as shown in Figure 1. Following 180 days the same samples contained 5.0 ppb of NDMA. No significant NDMA difference among times of frozen storage was noted with the washed mince and surimi. Generally, at the 15% substitution level there was no

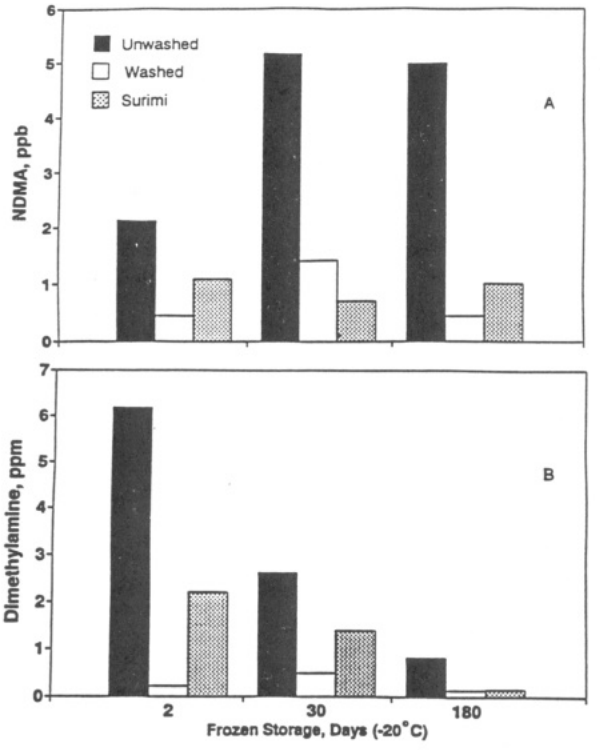

Figure 1. Broiled frankfurters substituted with 50% frozen (−20 °C) Atlantic menhaden mince and surimi: effect of frozen storage on (A) *N*-nitrosodimethylamine (NDMA), and (B) dimethylamine (DMA).

significant increase in NDMA for all three forms of menhaden whose mean values varied from 0.2 to 0.8 ppb. At both the 15 and 50% levels there was no increase in DMA or TMA in the uncooked and broiled frankfurters over the 180 days despite a decrease in TMAO. These data support the view that the conversion of TMAO to DMA during frozen storage is species dependent, forming primarily in the gadoid species (Castell et al., 1971), and that the enzyme and its cofactors responsible for such are absent in menhaden (Lundstrom et al., 1982). This is with the recognition that TMAO was measured as TMA before and after chemical reduction of the sample extract that may contain some phospholipids and other components that would be indicated as TMAO. In this study, part of the problem with assessing the role of the amines on NDMA formation was the low levels found in the uncooked menhaden-containing frankfurters. For example, DMA was 3.7 ppm compared to 178.0 ppm found in previous studies on Alaska pollock (Fiddler et al., 1992). For the all-meat controls, the mean values for DMA were 1.1 ppm in the uncooked and 3.0 ppm in the cooked frankfurters and 0.1 ppb of NDMA, the limit of detection, in the broiled ones.

Independent of percent of substitution and form of fish, frankfurters stored postprocessing under refrigerated conditions and subsequently broiled (n = 12) showed a significant decrease in NDMA from 1.0 ppb on day 1 to 0.7 on day 14; no difference was seeen after days 35 and 56. In this series of experiments, highly significant ($p < 0.01$) correlations were found between the NDMA in the uncooked and broiled frankfurters and the DMA, TMA, and TMAO content, both uncooked and broiled. This occurred despite the relatively low amounts of all three amines present.

In conclusion, the results of this study show that only low levels of NDMA were formed from menhaden mince and surimi. We occasionally noted an off-odor when broiling frankfurters containing 50% unwashed mince; no odor was obtained with the 15%. Therefore, the use of washed mince or surimi is recommended. An additional reason for the use of washed mince or surimi is that analysis of washed mince showed that over 85% of the DMA, TMA, and TMAO was removed during the washing step, thus lowering the potential for NDMA formation. For example, the mean DMA was reduced from 12.4 ppm in the fresh unwashed mince to 1.4 ppm in the washed mince, a level below that found in most meat products (Pfundstein et al., 1991). With the current interest in reducing the fat and caloric content of meat products, menhaden-derived fish protein in these forms offers an excellent opportunity for the use of this abundant yet underutilized species. Its use as water-binding agents and extenders would be a logical extension of current regulations permitting soy protein isolates, whey, and casein for these purposes (Food Safety Inspection Service, USDA, 1991).

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Registry No. Supplied by the Author: NDMA, 62-75-9; DMA, 124-40-3; TMA, 75-50-3; TMAO, 184-78-7; $NaNO_2$, 7632-00-0.

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