N-Nitrosodimethylamine Formation in Cooked Frankfurters Containing Alaska Pollock (Theragra chalcogramma) Mince and Surimi

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ABSTRACT

Studies were conducted to determine the feasibility of using Alaska pollock mince as a partial substitute for red meats in the formulation of frankfurters. Studies included the effects of fish form (unwashed and washed mince and surimi), percentage substitution (15 and 50) and five different methods of cooking the fish-meat franks on their N-nitrosodimethylamine (NDMA) content. The amount of formed NDMA depended on the method of cooking. Broiling and frying generated the highest levels. Microwave and boiling generated the lowest levels of NDMA, which were equivalent to the level in uncooked franks. Broiling was observed to increase the amount of dimethyl- and trimethylamine formed.

Key Words: nitrosamines, surimi, frankfurters, nitrite, fish

INTRODUCTION

THE FIRST REPORTED incident involving a nitrosamine in a foodstuff was associated with a fish-derived product. N-Nitrosodimethylamine (NDMA), a potent liver carcinogen in animals, was identified in nitrite-preserved herring meal as the cause of liver damage in mink and later sheep (Ender et al., 1964; Sakshaug et al., 1965). Since that time, low concentrations of NDMA have been found in fresh, processed (smoked, nitrite and nitrate treated) and salted and dried fish (Hotchkiss, 1989). With fresh fish, cooking increased or caused NDMA to be formed where none was originally present (Crosby et al., 1972; Iyengar et al., 1976; Yamamoto et al., 1984; Sen et al., 1985). This effect was more pronounced with salt dried seafoods, especially when broiled with gas (Maki et al., 1980; Matsui et al., 1980; Huang et at., 1984).

There is increasing interest within the fish and meat industry to develop new food products that take advantage of raw materials, such as surimi. One example of this technology is surimi-based shellfish analogs that have rapidly grown in consumption in the U.S. in a very short time. Surimi is a refined form of mechanically deboned fish that has been washed, with cryoprotetive agents added to protect against protein denaturation during frozen storage. Unlike soy protein, surimi has an elastic and chewy texture because of its unique gel-forming characteristics resulting from the high concentration of myofibriller protein present. This property, plus its water-holding capacity, makes surimi ideal for use in comminuted luncheon meats and sausages such as frankfurters. Just as with the initial use of poultry in frankfurters, surimi could be added to products (in concentrations up to 15%) without changing the product's name, provided that the fish protein is identified as one of the ingredients on the label. The use of surimi in a cured meat product, however, requires regulatory approval. There is concern about the use of surimi for this purpose because amines have historically been associated with fish and fish products with respect to deterioration of quality. These amines, in the presence of nitrite in the meat, increase the likelihood of nitrosamine formation. The concentration of amines present, particularly dimethylamine (DMA), varies with the fish species and other factors. Alaska (Walleye) pollock is the principal source of surimi. This species accumulates relatively high levels of trimethylamine oxide (TMAO) and, as a member of the gadoid (codfish) family, pollock contains an enzyme which can convert TMAO to equimolar amounts of DMA and formaldehyde during frozen storage. Trimethylamine oxide occurs commonly in marine fish and can function as an osmoregulatory compound (Martin et al., 1982). During iced storage, TMAO can be converted by bacteria to trimethylamine (TMA), which is responsible for "stale fish" odor. In the codfish species TMAO breaks down in frozen storage, unless the temperature is quite low, and the formaldehyde formed combines with fish protein causing texture deterioration. The DMA formed is a potential precursor for NDMA (Scanlan et al., 1974). Since Alaska pollock mince undergoes thorough washing in surimi production, most of the amines should be removed, thereby reducing the possibility for NDMA to be formed. However, no data are currently available, particularly with respect to a cured surimi-meat product such as a frankfurter. An additional concern is the possible formation of NDMA during cooking, since both NDMA and N-nitrosopyrrolidine have been shown to form in bacon during frying (Fazio et al., 1971; Crosby et al., 1972; Sen et al., 1973). In 1970, Lijinsky and Epstein postulated that human cancer might be caused by nitrosamines in the body from ingested nitrites and secondary amines and that cooking could be a major source of the secondary amines. While the principal concern was with *in vivo* nitosamine formation, cooking could also generate additional amine precursors to contribute to preformed nitrosamines in foods.

Our objective was to determine the effects of various cooking methods on NDMA formation in frankfurters prepared with 15 and 50% surimi and its precursor, washed and unwashed mince.

MATERIALS & METHODS

Materials

N-Nitrosoazetidine (NAZET) was synthesized from azetidine and sodium nitrite and purified as described previously (Pensabene et al., 1972). NDMA and all other reagents were purchased from local suppliers and used without further purification (Pensabene and Fiddler, 1988).

Frankfurters

Samples of unwashed and washed mince and surimi were collected from each batch of fresh Alaska pollock used. These forms of mince were obtained from commercial sources. Mince obtained during surimi processing prior to addition of the cryoprotecting agents was designated as "washed." In some cases the surimi was prepared under conditins previously, described (Babbitt et al., 1985). Minces and surimi were frozen in 10 kg blocks and shipped by overnight express form Alaska to Charleston, then stored at -40°C until processed into frankfurters.

Table 1—Effect of cooking method on N-nitrosodimethylamine in Alaska pollock fish-meat frankfurters

	N-Nitrosodimethylamine (ppb)					
	None	Boiled	Microwave	Roll-a-Grill	Fried	Broiled
All meat	N.D.[a]	N.D.[a]	0.2[a]	N.D.[a]	0.3[a]	0.2[a]
15%						
Mince						
Unwashed	1.4[a]	1.3[a]	1.7[a]	3.3[b]	5.7[c]	3.9[b]
Washed	0.5[a]	0.4[a]	0.4[a]	0.6[a]	1.1[c]	0.9[b]
Surimi	0.5[a]	0.4[a]	0.3[a]	0.5[a]	0.8[b]	1.5[c]
50%						
Mince						
Unwashed	5.8[a]	6.1[a]	6.8[a]	12.1[b]	18.1[c]	17.9[c]
Washed	0.9[a]	0.9[a]	1.0[a]	1.3[a,b]	2.0[b]	1.8[b]
Surimi	0.8[a]	0.7[a]	1.1[a]	1.2[a,b]	1.8[b]	4.0[c]

n = 16 for each method of cooking.

[a-c] Across the table, means with the same letter are not significantly different from each other (P<0.05).

Unwashed and washed minced fish and surimi-meat frankfurters in which 0%, 15% or 50% of the meat was substituted by Alaska pollock were prepared by the National Marine Fisheries Service, Southeast Fisheries Center, Charleston Laboratory, following a typical industry processing and hot smoking procedure (Brooker, 1985), with use of 156 ppm sodium nitrite ($NaNO_2$) and 550 ppm sodium erythorbate. The finished products were shipped to ERRC as cold packs in insulated containers. Upon receipt, the frankfurters were removed from their casings and refrigerated at 4°C for 18–24 hr. Frankfurters wre analyzed for residual $NaNO_2$ and then cooked by each of five methods. The cooked samples were ground, then frozen (-20°C) until analyzed for volatile nitrosamines.

Cooking procedure

Boiling. Six frankfurters were placed in 3 L of boiling water, the water removed from the heat, and the frankfurters allowed to cook for 5 min.

Microwave. Six frankfurters were heated in a 1300 W microwave oven for 2 min at the high power setting.

Roll-a-Grill. The unit was preheated on the high setting for 15 min, then six frankfurters were placed on the gril, turned on rollers, and cooked for 15 min.

Broiling. The electric oven was preheated for 15 min. Six frankfurters were cut lengthwise, placed on a broiling tray 4.5 inches from the heating element, and cooked fo 3 min. Frankfurters were removed from the oven, turned, then broiled for an additional 2 min.

Frying. The electric pan was preheated for 10 min to a calibrated temperature of 171°C (340°F). Six frankfurters were cut lengthwise and fried for 8 min (4 min/side).

Sodium nitrite analysis

Residual $NaNO_2$ content was determined on 10g of uncooked, ground sample by the modified Griess-Saltzman procedure (Fiddler, 1977).

Amine analysis

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

N-Nitrosodimethylamine analysis

The complete details for the analysis of volatile nitrosamines, particularly NDMA, using a solid phase extraction procedure have been described elsewhere (Pensabene and Fiddler, 1988). All samples were analyzed in duplicate, and all of the NDMA values were corrected for the recovery of the NAZET internal standard in each individual sample. N-Nitrosodimethylamine was the only volatile nitrosamine detected in any of the samples tested. ("N.D." denotes "none detected" or < 0.2 ppb, the minimum level of reliable measurement based on the gas chromatography-Thermal Energy Analyzer (GC-TEA) system response). To confirm the presence of NDMA, especially in the surimi-containing frankfurters, methylene chloride extracts were subjected to the photolytic confirmation procedure described by Doerr and Fiddler (1977). On both Carbowax and Chromosorb 103 gas chromatographic columns, the TEA peak corresponding to the NDMA standard retention time disappeared. The identity of NDMA was further confirmed by capillary Gc-mass spectrometry in a few samples.

Statistical analysis

The General Linear Models (GLM) procedure of the Statistical Analysis System PC software distributed by SAS Institute, Inc. was used to analyze the data and the results were interpreted according to the methods of Snedecor and Cochran (1979).

Safety note

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

RESULTS & DISCUSSION

FRANKFURTERS where the meat was substituted with 0, 15 and 50% Alaska pollock unwashed and washed mince or surimi were analyzed for NDMA before and after cooking by five different methods. These represent typical means of cooking prior to consuming this type of product. The results are shown in Table 1. The all-meat franks contained the least amount of NDMA (0.2 ppb, n = 132) when compared to the fish containing franks, even when subjected to methods of cooking where temperatures were highest. These values were similar to the low levels we have reported previously (Fiddler et al., 1972). For all three pollock forms, substitution at the 50% level resulted in higher NDMA values than substitution at 15%. Surimi and washed mince-containing franks had lower NDMA than their unwashed mince counterparts at both substitution levels either uncooked or cooked by the five different methods. The analysis of variance of overall data indicated significant differences among the cooking methods. The overall repeatability for NDMA was 3.0 ppb. The results from Duncan's Multiple-Range test of the NDMA means for each cooking method, independent of % substitution and fish form indicated that boiled and microwaved franks were significantly different from uncooked franks. This was probably due to the less than optimal temperature of 100°C that would not favor NDMA formation. Those franks subjected to higher cooking temperatures as in frying and broiling were higher in NDMA than those cooked by the other methods, but were not significantly different from each other. Both those methods lead to rapid water loss and are associated with product browning; one through direct transfer of heat from cooking medium to product surface and the other by indirect heat transfer, although at a higher temperature. The mean temperature of the air 11.4 cm from the heat source in the oven was 250°C compared to a surface temperature of 171°C in the frying pan. Cooking by a commercial Roll-a-Grill device where there was direct contact between turning heated (148°C) metal rollers and the frankfurters for longer periods of time than the other cooking methods gave intermediate NDMA values. The least amount of NDMA was formed by microwave cooking and the highest by frying the fish-meat franks, similar to results reported for N-nitrosopyrrolidine formation in bacon (Pensabene et al., 1974; Vecchio et al., 1986; Miller et al., 1989; Osterdahl et al., 1990). The regression analysis also showed that regardless of the means of cooking there was a correlation betwen NDMA and the following individual factors in the uncooked franks: residual $NaNO_2$, DMA, TMA and TMAO. While secondary amines like DMA are typically thought to be the principal precursors of NDMA, TMAO and TMA can also form NDMA (Ohshima and Kawabata, 1978). TMAO can nitrosate directly without going through a dealkylation step as for TMA. These reactions may be facilitated by the elevated temperatures that occur during cooking. There was no apparent difference in NDMA between the surimi and the washed mince containing franks when the overall data were analyzed.

Table 2—Amines in Alaska pollock fish-meat frankfurters

	DMA, ppm Uncooked	DMA, ppm Broiled	TMA, ppm Uncooked	TMA, ppm Broiled	TMAO, ppm Uncooked	TMAO, ppm Broiled
All meat	1.9	2.6	0.5	0.7	(1.5)[b]	(2.1)[b]
15%						
Mince						
Unwashed	41.2[a]	71.5	6.9[a]	17.1	300.6	332.3
Washed	7.0	8.9	2.0[a]	4.8	52.0	64.1
Surimi	4.3[a]	7.3	1.9[a]	6.2	47.3	53.2
50%						
Mince						
Unwashed	223.4[a]	366.9	20.0[a]	46.3	1089.2	1228.5
Washed	24.1	27.7	2.8[a]	6.2	144.2	162.4
Surimi	12.7[a]	32.4	2.8[a]	12.7	131.2	152.1

n = 16.
[a] Denotes uncooked and broiled values are significantly different ($P<0.05$).
[b] TMAO results reflect the TMA formed from reduction of phospholipids.

Since surimi rather than washed mince is employed as an intermediate raw material for shelfish analogs, surimi would most likely be used in preparing formulated cured meat products. Additional investigations on conditions that could affect the amine content of the mince or surimi, such as abusive or prolonged storage are warranted, particularly where the highest concentrations of NDMA were formed as a result of cooking. Broiling was the cooking method selected to study amine formation because of our findings and the previously cited references indicating that broiling caused or increased NDMA formation in fresh fish.

Table 2 shows that higher levels of DMA, TMA and TMAO were present in the fish-containing franks compared to all-meat control. These amines were also higher in the unwashed mince franks than those containing the two other forms of mince. No TMAO was present in the meat franks. TMAO is typically determined by the difference in TMA before and after reduction of TMAO. Therefore, the numbers indicated for the meat franks reflect the TMA formed by reduction of endogenous components, most likely phospholipids. TMAO was considerably higher than DMA, and DMA was higher than TMA as one would expect from the utilization of frozen Alaska pollock, one of the gadoid species (Babbitt et al., 1984). The washed mince and surimi-containing franks had significantly lower levels of DMA, TMA and TMAO than the unwashed-mince counterparts as expected, but not different from each other at either 15 or 50% substitution level in the uncooked or electric broiled samples. This was true whether or not the values were corrected for weight loss during cooking. All three forms of fish-containing franks showed a significant increase in TMA content as a result of broiling. More importantly, DMA also increased in the unwashed mince and surimi franks with broiling; the effect was particularly apparent in the 50% surimi substituted franks (12.7 ppm uncooked vs 32.4 ppm broiled). Because of the variability in TMAO values, a decrease in TMAO with the corresponding increase in DMA and TMA was not clearly demonstrated. The elevation of DMA and TMA levels in the fish-meat franks was consistent with many published reports indicating that thermal treatment and various methods of cooking of seafoods dramatically increased their amine content (Hughes, 1958; Kawamura et al., 1971; Ryu et al., 1974; Kawabata et al., 1982; Lin et al., 1984; Lin and Huang, 1985).

In conclusion, the method of cooking meat frankfurters substituted with Alaska pollock mince and surimi could affect the level of NDMA in the cooked product. High temperature cooking as in broiling and frying yielded the highest levels of NDMA. Thermal treatment not only facilitated nitrosamine formation, but also generated additional amine precursors. Note that the entire study was conducted with fresh mince and surimi, however, other factors such as age, storage, and mince/surimi processing may affect the amount of amine precursors available.

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