

# Alaska Pollock (*Theragra chalcogramma*) Mince and Surimi as Partial Meat Substitutes in Frankfurters: N-Nitrosodimethylamine Formation

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## ABSTRACT

Studies were conducted to determine the feasibility of using 3 forms of Alaska pollock (AP) as a partial substitute for meat in a nitrite-cured product. The effect of several pre- and post-processing storage conditions on N-nitrosodimethylamine (NDMA) were studied in frankfurters substituted with 15 and 50% unwashed and washed mince and surimi. In franks made with unwashed mince, frozen stored prior to processing, NDMA increased during frozen storage; little change was observed in franks containing washed mince and surimi. Refrigerated storage of franks generally showed a decrease in NDMA with all 3 forms of fish. Overall, the use of washed mince and surimi at the 15% substitution level gave mean values less than 2 ppb NDMA, even after boiling. The results indicate that 50% unwashed AP mince should not be used as a partial substitute for meat in frankfurters.

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

## INTRODUCTION

SURIMI is mechanically deboned, washed and dewatered minced fish to which cryoprotecting agents, sugar and sorbitol, have been added to prevent myofibrillar protein denaturation during freezing. The details of surimi production have been extensively reviewed by Lee (1984, 1986) and Flick et al. (1990). The bland odor, color and excellent gel-forming properties of surimi, have made it ideal for the manufacture of shellfish analogs such as crab, shrimp and lobster. The acceptance and growth of markets for these products in the U.S. have been remarkable. As a result, the use of surimi as a functional ingredient for other foods has been explored. Surimi and minced fish have been proposed as a partial replacement for meat in typical all-meat luncheon meats, including frankfurters (Agnello, 1983, Mitchell, 1984; Lanier 1985a,b; Rasekh, 1987; Morris, 1988). Label approval has been granted for both a 15% surimi-pork and a surimi-beef breaded and fried nugget product (Thompson, 1988). Approval for other similar types of product are expected.

About 90% of the surimi is made from walleye or Alaska pollock (of the gadoid family) because of its abundant supply (Vondruska et al., 1988). Under conditions of frozen storage, gadoid fish produce equimolar amounts of dimethylamine (DMA) and formaldehyde from trimethylamine oxide (TMAO) (Tokunaga, 1965; Castell et al., 1971). Babbitt et al., (1984) found that this reaction was particularly pronounced in Alaska pollock mince compared to the intact fish muscle. Because Alaska pollock can contain TMAO, up to 10 mg/g (Hebard et al., 1982), there is a potential for notable amounts of it and DMA to be present even after the mince undergoes washing in surimi production. These amines, in turn, could cause N-nitrosodimethylamine (NDMA), a well known animal carcin-

ogen (Magee and Barnes, 1956), to form in the presence of nitrite. No data on NDMA were available on surimi or mince employed as a partial substitute for red meats in a product, such as a frankfurter, that is made with nitrite as an essential ingredient. We have recently reported the effect of 5 different cooking methods on NDMA in frankfurters containing 15 and 50% unwashed and washed mince and surimi (Fiddler et al., 1991a). Washed mince is the same form of fish as surimi, except the cryoprotecting agents have not been added.

Our objective was to evaluate the conditions, pre- and post-processing that would have the most effect on amine-NDMA formation. This would help to determine amounts and circumstances under which the minced fish and surimi might safely be used.

## MATERIALS & METHODS

### Alaska pollock

Pollock were caught between December, 1987 and April, 1991. The fish were processed into mince and surimi immediately after harvesting at the National Marine Fisheries Service (NMFS) laboratory, Kodiak, Alaska. The mince and surimi blocks were shipped frozen to NMFS laboratories in Charleston, SC or Pascagoula, MS, where the frankfurters were prepared. The mince and surimi blocks for the pre-processing storage study were stored at  $-20^{\circ}\text{C}$  until needed.

### Fish-meat frankfurters

Frankfurters, in which 0, 15 or 50% of the meat was substituted with unwashed and washed mince and surimi, were prepared by the NMFS laboratories following a typical industry formulation and processing procedure that used natural smoke (Brooker, 1985). In addition, 156 ppm sodium nitrite ( $\text{NaNO}_2$ ) and 550 ppm sodium erythorbate were added to the formulation prior to processing. The finished products were shipped overnight to the Eastern Regional Research Center (ERRC) in insulated containers with cold packs. Upon receipt, the frankfurters were removed from their casings and stored at  $4^{\circ}\text{C}$  for 18–24 hr. Frankfurters were analyzed for residual  $\text{NaNO}_2$  and then broiled as follows: preheat the electric oven ( $280^{\circ}\text{C}$ ) for 15 min then cut 6 frankfurters lengthwise, place them on a broiling tray 11.5 cm from the heating element, and broil for 3 min. Remove the frankfurters from the oven, turn them, then broil for an additional 2 min. The uncooked (no additional cooking after smokehouse processing) and broiled samples were ground through a 1.6 mm plate, then frozen ( $-20^{\circ}\text{C}$ ) until analyzed for volatile nitrosamines. For the post-processing study, the frankfurters were stored in a refrigerator ( $4^{\circ}\text{C}$ ) for 0, 14, 35 and 56 days prior to broiling.

### Sodium nitrite, amine, and total N-nitroso analyses

Residual  $\text{NaNO}_2$  was determined in 10.0g of ground sample prior to and after broiling by the modified Griess-Saltzman procedure (Fiddler, 1977).

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

"Apparent Total N-nitroso Compounds" was determined by the procedure described by Havery (1990) and modified by Fiddler et al., (unpublished). Briefly, a 10.0g sample was homogenized with a Tis-sumizer model SDT-1810 (Tekmar, Co.) at level 7 on the controller in a 35 mL centrifuge tube for 5 min using 15 mL acetonitrile. The

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sample mixture was centrifuged for 45 min at 15,000 rpm (26,200 x g) in a Sorval model RC-5B refrigerated centrifuge set at 2°C. The supernatant was decanted into a 25 mL graduated cylinder to measure the volume. To a 2.0 mL aliquot 0.6 mL of 10% sulfamic acid in 1N sulfuric acid was added and mixed for 5 sec using a shaker. Then a 50 µL aliquot was taken using a 100 µL syringe and injected into a stream of 2% potassium iodide (flow at 1.5 mL/min) via a Rheodyne 7125 injection valve. The stream was mixed with 10% sulfuric acid in glacial acetic acid (flow at 0.5 mL/min) through a reaction coil heated at 70°C in a water bath. The resulting nitric oxide was swept through an ice-water trap and 2 dry ice-acetone cold traps by a stream of helium (flow at 15mL/min) sufficient to maintain a Thermal Energy Analyzer (Thermedics, Inc) pressure of about 10 mm mercury. The response was calculated based on peak heights.

#### N-Nitrosodimethylamine analysis

The complete details for analysis of volatile nitrosamines, particularly NDMA, using a solid phase extraction procedure have been described (Pensabene and Fiddler, 1988). All samples were analyzed in duplicate, and all of the NDMA values were corrected for the recovery of the N-nitrosoazetidine (NAZET) internal standard in each individual sample. N-Nitrosodimethylamine was the only volatile nitrosamine detected in 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 20M-TPA on Gas Chrom P and Chromosorb 103 GC 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, by the method of Kimoto and Fiddler (1982).

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

#### Statistical analysis

All analyses of samples were performed in duplicate. A randomized full block experimental design was used. The data were analyzed for differences within factors using the ANOVA portion of the General Linear Models (GLM) procedure of the Statistical Analysis System (SAS) PC Software distributed by SAS Institute, Inc. (SAS Institute, Inc., 1985). If levels within a factor were significantly different, they were examined further using Duncan's test for differences among individual means at the  $p < 0.05$  level. Correlations between various factors and NDMA or amines in either the uncooked or electric broiled samples were examined using a multivariate linear regression procedure. This, and MEANS procedures for determining the means, minima and maxima were contained in SAS. The results were interpreted according to the methods of Snedecor and Cochran (1979).

## RESULTS & DISCUSSION

OVERALL DATA were compiled related to fish form and percent substitution in franks (Table 1). These are from individual experiments where franks were made with Alaska pollock mince and surimi from freshly caught, refrigerated, and frozen fish and from experiments where fish-meat franks were subjected to refrigerated storage, post processing. The data are given for frankfurters, uncooked and after broiling. This method of cooking has been previously reported to yield the highest levels of NDMA in surimi-containing frankfurters (Fiddler et al., 1991a). Studies were made at the 50% substitution level in addition to the 15% level, in the event of potential future use of minced fish or surimi at higher levels. Bacus (1978) reported that fish could effectively replace 15 to 25% lean beef in a frankfurter-type formulation. A beef flavored, turkey-containing frankfurter made with 50% surimi has been described (Bischoff, 1986).

The NDMA values were significantly ( $p < 0.05$ ) higher in the broiled versus the uncooked frankfurters regardless of form of fish substituted. These data provided additional evidence that dietary exposure to nitrosamines could not be accurately

assessed by only analyzing uncooked cured meat products. Cooking changes the matrix in question and may cause release of additional precursors or promote the nitrosation reaction through the application of heat. Higher NDMA levels were found in the 50% substituted franks, especially those containing unwashed mince. Clearly, the use of Alaska pollock unwashed mince at that level would not be acceptable since ca 20 ppb mean NDMA value was obtained after broiling. The higher values from this form reflect the larger amount of amine precursors present that had not yet been removed during surimi production. The difference in amine values between unwashed and washed mince reflects the efficacy of the washing step; 92% of the DMA was removed at the 50% and 87% removed at the 15% substitution level. For the broiled franks containing 50% washed mince the range of NDMA was from N.D. to 7.1 and for 50% surimi from N.D. to 15.4 ppb. Higher levels of NDMA were found in the broiled franks containing surimi than in those containing washed mince, especially at 50% substitution, where the mean was 4.2 vs 1.8 ppb (Table 1). We hypothesized that the carbohydrate based cryoprotecting agents may have a role in catalysis of the nitrosation of DMA since it was previously observed in a model system containing sucrose at pH 5.0 (Kurechi et al., 1980). The elevation of NDMA was not apparent in the overall data at the 15% surimi substitution level where the NDMA mean value of 1.4 ppb was not significantly different ( $p > 0.05$ ) from the 1.1 ppb obtained from the corresponding washed mince samples. The range of NDMA detected in franks made with these two forms at this level was N.D. to 4.9 ppb. The repeatability for NDMA in uncooked and cooked frankfurters was 0.4 ppb. These results are similar to the mean 0.2 ppb NDMA reported for all-meat frankfurters broiled under the same conditions (Fiddler et al., 1991a). The mean NDMA for franks containing 15% unwashed Alaska pollock was relatively low, 5.6 ppb (range 1.7 to 10.9 ppb). But, several samples had a strong amine odor during cooking indicating the necessity of using washed mince or surimi even at that substitution level from a practical point of view. In addition to the marked increase in NDMA due to broiling, the results also showed an increase in DMA and TMA that was particularly apparent in franks containing unwashed mince, despite the loss due to volatility of these compounds. An increase in amine content as a result of high temperature cooking of seafoods and fish has been noted by others (Hughes, 1958; Ito et al., 1971; Lin and Hurng, 1985). A concomitant decrease in TMAO was not apparent, probably due to the large variation of TMAO content in the samples. Regression analysis of the data showed highly significant ( $p < 0.01$ ) correlations between NDMA in the uncooked franks and DMA, TMA and TMAO, but not with residual sodium nitrite, which varied from n.d. to 110 ppm. Similarly, NDMA values in broiled franks correlated with amine content in both the uncooked and broiled samples. Since no significant ( $p > 0.05$ ) correlation was found between residual sodium nitrite and NDMA level, nitrite was not considered an important factor. Therefore, the key to controlling the amount of NDMA present in the frankfurters, where a fish substrate is used as a partial substitute for meat, is to monitor and control the amine content after establishing guidelines. Further reduction of NDMA is possible by the application of other techniques. Pacheco-Aguilar et al., (1989) found that a single wash at 3:1 water-flesh ratio at pH 5.0-5.3 was effective in producing surimi of good quality from Pacific Whiting (*Merluccius productus*). An initial wash of this type applied to Alaska pollock may be more effective in removing precursor amines than other washing procedures usually employed. Alternatively, the use of more sodium ascorbate than the 550 ppm regulatory limit in formulating frankfurters may be more effective in inhibiting nitrosamine formation during cooking.

Given the amine/NDMA correlations, a predictive equation and its r-square was determined by stepwise selection of independent variables. Only data from fresh samples (zero time)

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Table 1—N-nitrosodimethylamine and methylamines in Alaska pollock mince and surimi-meat frankfurters

Form	Uncooked (ppm)			(ppb) NDMA	Broiled (ppm)			(ppb) NDMA
	DMA	TMA	TMAO		DMA	TMA	TMAO	
50% Mince <sup>a</sup> (n = 54) <sup>a</sup>								
Unwashed	243.1 <sup>A</sup>	16.3 <sup>A</sup>	690.5 <sup>A</sup>	10.5 <sup>A</sup>	301.9 <sup>A</sup>	53.1 <sup>A</sup>	798.9 <sup>A</sup>	19.7 <sup>A</sup>
Washed	19.1 <sup>B</sup>	3.8 <sup>B</sup>	78.2 <sup>B</sup>	1.1 <sup>B</sup>	29.1 <sup>B</sup>	7.8 <sup>B</sup>	88.6 <sup>B</sup>	1.8 <sup>B</sup>
Surimi	13.5 <sup>B</sup>	2.5 <sup>B</sup>	67.2 <sup>B</sup>	1.3 <sup>B</sup>	26.8 <sup>B</sup>	10.3 <sup>B</sup>	84.0 <sup>B</sup>	4.2 <sup>C</sup>
15% Mince <sup>a</sup> (n = 44)								
Unwashed	60.9 <sup>A</sup>	8.2 <sup>A</sup>	232.6 <sup>A</sup>	2.5 <sup>A</sup>	86.1 <sup>A</sup>	16.4 <sup>A</sup>	236.1 <sup>A</sup>	5.6 <sup>A</sup>
Washed	7.7 <sup>B</sup>	2.1 <sup>B</sup>	34.6 <sup>B</sup>	0.6 <sup>B</sup>	9.3 <sup>B</sup>	3.8 <sup>B</sup>	42.0 <sup>B</sup>	1.1 <sup>B</sup>
Surimi	4.1 <sup>B</sup>	1.5 <sup>B</sup>	25.7 <sup>B</sup>	0.5 <sup>B</sup>	6.8 <sup>B</sup>	3.8 <sup>B</sup>	29.2 <sup>B</sup>	1.4 <sup>B</sup>

<sup>a</sup> For each percentage (15 or 50%), and within each column (individual analytes), the means with the same letter are not significantly different ( $p < 0.05$ ) from each other. n = Number of samples at each form and percentage (in duplicate).

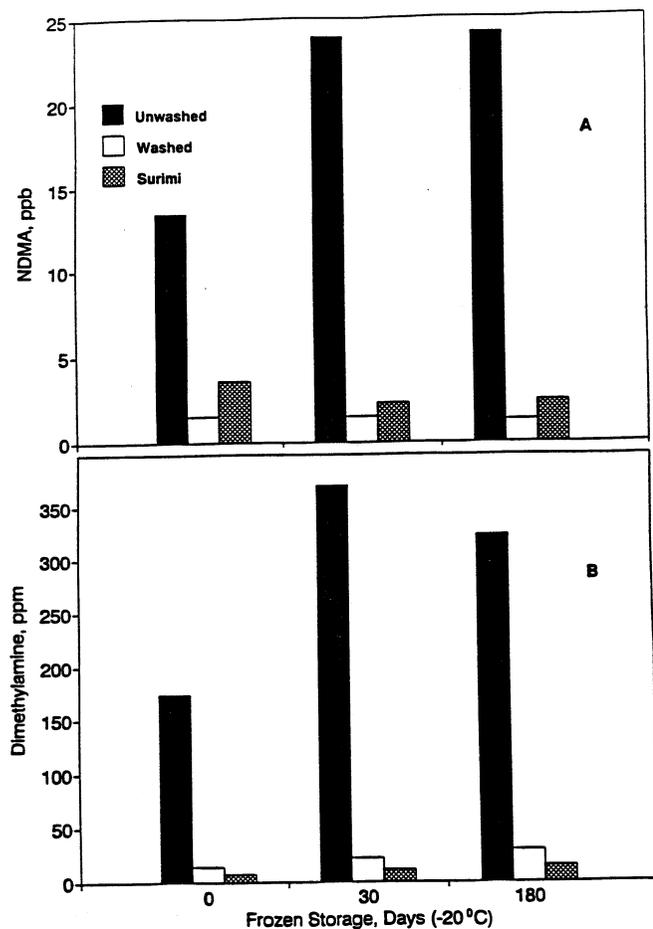


Fig. 1.—Effect of frozen mince and surimi storage ( $-20^{\circ}\text{C}$ ) on NDMA in broiled (A) and DMA in uncooked (B) 50% substituted frankfurters. (n=6, for each form and time)

from each experiment were used to minimize interactions from other factors that could interfere with the correlation determination. Additionally, only the 50% fish substituted frankfurter values were used, since these samples had the most meaningful nitrosamine values i.e. non-zero or not close to zero. Similar correlations were observed with data from the 15% substituted franks. All forms of fish were used since the surimi and washed mince contributed the low values and the unwashed mince contributed the high ones. There was a highly significant ( $p < 0.01$ ) correlation between the DMA and TMA in the uncooked franks and the nitrosamine values in the broiled franks. The equation is represented without an intercept since the intercept is probably more dependent on other starting materials than on the added fish substrate. TMAO was not included since it was nonsignificant ( $p > 0.05$ ) in the multivariate correlation.

The equation from the 50% substituted frankfurters:

$$\text{NDMA, ppb} = 0.0667 \times \text{DMA, ppm} + 0.6273 \times \text{TMA, ppm}$$

$$r\text{-squared} = 0.9383$$

From it a general predictive equation was derived

$$\text{NDMA, ppb} = \frac{\% \text{Subst.}}{100} \times (0.1334 \times \text{DMA, ppm} + 1.2546 \times \text{TMA, ppm})$$

Where ppm, "DMA" and "TMA" are contained in the original fish substrate to be used as the replacement for meat and "%Subst." is the intended % of fish substitution. For example, for a 15% substituted frank to contain less than 3 ppb NDMA, the DMA and TMA content should be less than 75 ppm and 8.0 ppm, respectively. This is one of a several combinations that could be derived from this equation.

Surimi is made commercially from the freshest fish, otherwise there is a significant deterioration of the desired functional properties (Lee, 1984). In preparing shellfish analogs, surimi paste is typically kept frozen, then thawed prior to its use. It is likely that surimi would be used in the formulation of comminuted cured meat products in the same manner. Alaska pollock has been shown to form large amounts of DMA during frozen storage especially when ground (Tokunaga, 1965). Unlike TMA, whose formation parallels microbial growth, DMA forms optimally at temperatures below the freezing point of gadoid fish (Spinelli and Koury, 1981; Babbitt et al., 1984). Because of the potential for increased formation of NDMA, 4 different experiments were conducted in which unwashed and washed mince and surimi were frozen up to 180 days at  $-20^{\circ}\text{C}$  prior to use in making frankfurters. Only the results from the 50% substituted franks are shown (Fig. 1) since they most clearly show observed effects. The data from the 15% franks, especially those substituted with washed mince and surimi, were very low and showed no trend. Only the NDMA mean values for broiled samples were used because they represent the "worse case". Similarly, the mean DMA data for uncooked franks are given because this amine is the most important precursor for NDMA and since this best represents a starting material for the finished product model.

In franks made with the three forms of fish frozen up to 180 days prior to processing into frankfurters, NDMA increased significantly ( $p < 0.05$ ) and leveled off after 30 days in those made with unwashed mince (Fig. 1A). This reflects the presence of significantly ( $p < 0.05$ ) larger concentrations of amines, especially DMA that is also formed during frozen storage (Fig. 1B). The increase in DMA appears to be much less pronounced for washed mince and surimi with little or no effect on NDMA. This experiment most clearly showed the higher NDMA in the surimi versus the washed mince despite the significantly ( $p < 0.05$ ) lower DMA content in the surimi.

Total volatile bases (TVB), of which DMA is a major component, increased markedly in raw surimi and surimi-derived flaked artificial crab when stored at 4, 10 and  $22^{\circ}\text{C}$  (Hollingworth et al., 1990). In the artificial crab, the TVB did not increase until after 30 days storage at  $4^{\circ}\text{C}$ , while the non-heat

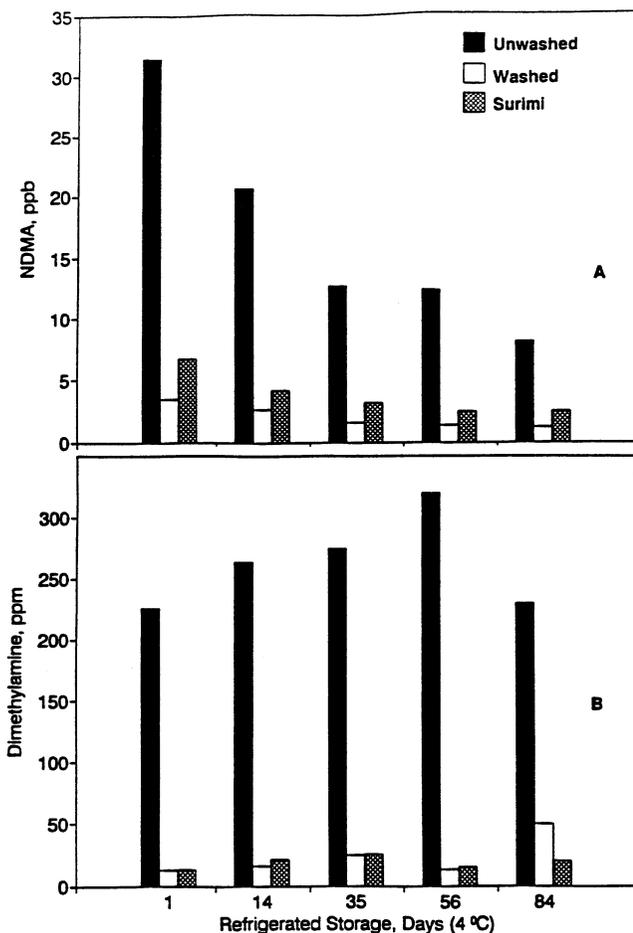


Fig. 2.—Effect of post-processing storage (4°C) on NDMA in broiled (A) and DMA in uncooked (B) 50% substituted frankfurters. (n=6, for each form and time)

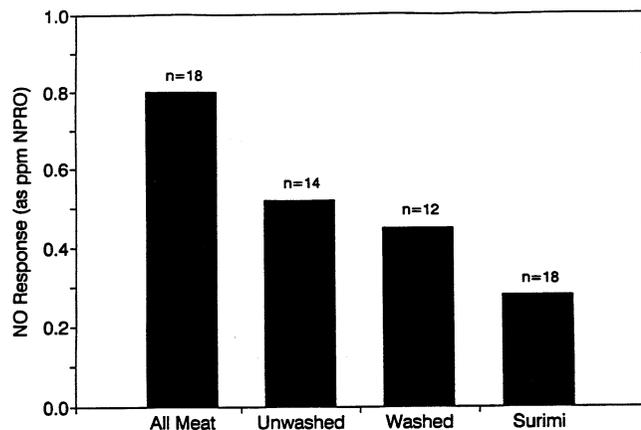


Fig. 3.—Total apparent N-Nitroso compounds in broiled 50% substituted frankfurters.

treated surimi did so after 13 days. Our results on minced fish and surimi-containing frankfurters stored at 4°C post-processing are shown in Fig. 2. N-Nitrosodimethylamine decreased significantly ( $p < 0.05$ ) with time up to 35 days, then stayed relatively constant up to 56 days. This was despite an increase in DMA in the washed mince and surimi-containing franks from ca. 13 to 25 ppm and in the unwashed mince franks from 225 to 325 ppm during this same time. This reduction in NDMA was not apparent when the uncooked franks data were analyzed. The results may reflect the depletion of nitrite in the

product with time with less available for reaction at the time of broiling. As with frozen storage of fish substrate, NDMA values were significantly ( $p < 0.05$ ) higher in surimi franks vs the corresponding washed mince containing franks. This was true at both the 15 and 50% substitution levels.

The fish-containing franks were analyzed for their "apparent total N-nitroso compound" (ATNC) content according to the method of Havery (1990). The residual nitrite was destroyed by treatment with 1% sulfamic acid in 1N sulfuric acid to eliminate nitric oxide response due to nitrite. Independent of form used and whether the samples were broiled or not, 15% substitution (n=34) gave a mean value of 0.59 ppm, based on a response compared to a standard of N-nitrosoproline (NPRO). Both were significantly ( $p < 0.05$ ) lower than the all meat controls (n=20) whose mean was 0.75 ppm. Interestingly, the 50% substitution (n=56) level was significantly less at 0.30 ppm. Analysis of the data from the uncooked franks (n=30) according to form, independent of the % substitution, showed that the washed mince and surimi (0.36 and 0.33 ppm, respectively) gave significantly lower ATNC than the all-meat controls. Results from the 50% substituted franks indicated the same effect in the broiled samples (Fig. 3). This was also true for the 15% broiled samples. There was no indication of an increase in ATNC as a result of cooking. These findings suggested that the potential for additional non-volatile nitrosamine formation in combination fish-meat frankfurters was not increased by the use of Alaska pollock mince and surimi. This was supported by our previous findings on nitrosoamino acids in which the N-nitrosothiazolidine-4-carboxylic acid (NTHZC) content of surimi-meat frankfurters was similar to, or lower, than in the all-meat controls, even at 50% substitution (Pensabene et al., 1991). NTHZC would be expected to be higher because it is formed from cysteine and formaldehyde, another decomposition product of TMAO.

Alaska pollock surimi and washed mince in frankfurters was suitable in terms of volatile nitrosamine content when substituted for 15% or less meat. Although surimi-based products have a long history of safe consumption, the microbial safety of such materials for a new application should be demonstrated before widespread use. The use of minced fish and surimi could upgrade the nutritional quality of comminuted cured meats and make possible the formulation of low calorie, lower fat products because of their superior water-fat binding and textural imparting properties.

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