Nitrosamine Formation and Penetration in Hams Processed in Elastic Rubber Nettings: *N*-Nitrosodibutylamine and *N*-Nitrosodibenzylamine

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N-Nitrosodibutylamine (NDBA) was previously detected on the outer surface of commercial boneless hams processed in elastic rubber nettings. More recently, *N*-nitrosodibenzylamine (NDBzA) has been reported in boneless ham products, reflecting a change in the formulation of the rubber used in the manufacture of the nettings. As a result, a study was carried out on the penetration of these two nitrosamines and their secondary amine precursors into hams. Most of the NDBA and NDBzA was detected in the first 0.5 in. from the surface, with a marked drop off (67–75%) after the first 0.25 in., indicating that the nitrosamines were formed at the product surface in contact with the elastic rubber netting. Detectable levels of both nitrosamines were found at a depth of 1.25 in. A gradual rather than a sharp decline was noted for both amines, DBA and DBzA, after the first 0.25 in. A comparison of NDBzA values on the outer surface of the ham versus a center slice was also made.

Keywords: *Nitrosamines; cured meats; rubber; amines*

INTRODUCTION

Packaging materials have become an essential part of food processing, storage, and preservation and, more recently, in the cooking of foods. Studies on the migration of plastic-derived contaminants into foods have been carried out primarily on unreacted monomers, stabilizers, and plasticizers (Carter, 1977). However, a new element was added to concerns about the safety of packaging materials with the finding of nitrosamines in foods as a result of surface contact. For example, Hoffmann et al. (1982) detected up to 17 200 ppb of morpholine (MOR) and up to 5.4 ppb of *N*-nitrosomorpholine (NMOR) in waxed food containers used for butter, yogurt, and cheese products, with levels of up to 3.3 ppb of NMOR found in the food. An average of 12 ppb of NMOR was also found in 9 of 34 samples of paper packaging materials typically used for dry foods (Hotchkiss and Vecchio, 1983). Sen and Baddoo (1986) found this same nitrosamine in coated and waxed margarine wrapping paper and presented evidence for the penetration of NMOR into the outer layer of the product. Wax, used for coating fruits and vegetables, can also be considered a packaging material, since it is used to protect and extend the shelf life of the produce. Both MOR and NMOR were detected in six brands of liquid wax used for apples; only MOR was detected in the treated apples (Sen and Baddoo, 1989). Sen et al. (1987) reported finding *N*-nitrosodibutylamine (NDBA) and, to a lesser extent, *N*-nitrosodiethylamine (NDEA) in hams processed in elastic rubber nettings; up to 504 ppb of NDBA was detected in the used nettings. These two nitrosamines are not normally found in this type of cured meat product, and the netting was identified as the source of the nitrosamines (Sen et al., 1987).

Elastic rubber nettings are widely used in the manufacture of both cured and noncured boneless meat products, primarily to provide pressure to help bind the pieces of meat together during thermal processing. The amine and other nitrosamine precursors in the rubber of the netting could react with the nitrite in the meat at the product surface. In addition, some nitrosamine is already present in the rubber of the elastic netting and could also penetrate into the hams during thermal processing or storage. A combination of the two factors is likely. Sen et al. (1987) reported on the NDBA content of the outermost 5 mm layer, the next 5 mm layer and the center from five samples of netted cured and smoked pork products that included two cottage rolls, a pork shoulder, a picnic ham, and a conventional ham. The outermost portions contained up to 60 ppb of NDBA, the next outermost up to 45 ppb, and the center up to 2.2 ppb. Given the variety of these product types and sizes, and the differences in processing conditions employed, there are no data on the full extent of penetration of the nitrosamine into the product.

While finding nitrosamines in rubber products is not new (Spiegelhalder and Preussmann, 1982), detection of significant quantities of such in a food product, which resulted from contact with a rubber-containing material, is new. Other investigations reported *N*-nitrosodibenzylamine (NDBzA) in boneless ham products (Sen et al., 1988, 1993; Pensabene and Fiddler, 1994), in addition to NDBA (Sen et al., 1987; Peterson, 1993). This finding reflects a change in the formulation of the rubber used in the manufacture of the nettings.

Most of the published information has been obtained on NDBA in the outermost 0.5 cm of the cured meat product. There is little specific information on the extent of nitrosamine penetration into hams, especially for NDBzA, and none on the precursor amines, dibutylamine (DBA) and dibenzylamine (DBzA). We therefore carried out these investigations on commercial boneless hams; the results are reported in this paper.

MATERIALS AND METHODS

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

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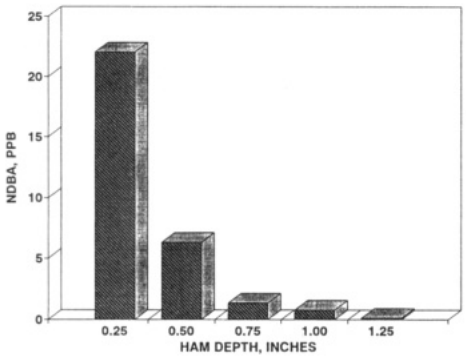

Figure 1. Formation/migration of NDBA into hams.

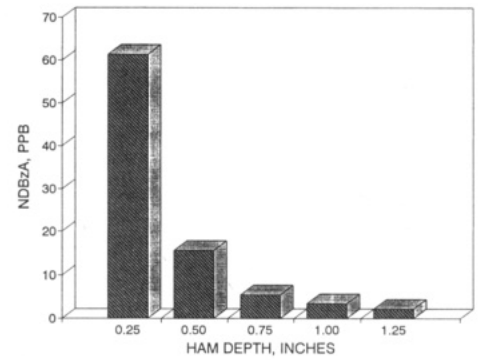

Figure 2. Formation/migration of NDBzA into hams.

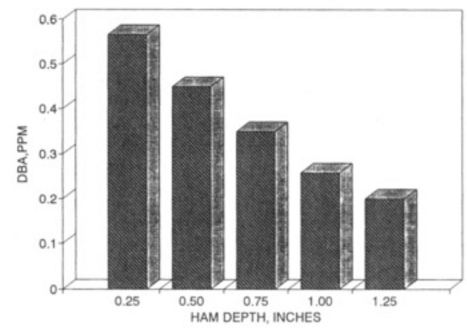

Figure 3. Migration of DBA into hams.

Materials. Ham samples were obtained directly from commercial processing plants. For the penetration study, the outer surface of the ham was removed in 0.25 in. sections, using a Globe Model 110 slicing machine, to a depth of 1.50–2.00 in. To obtain uniform slices, the ham was rotated after each cut so that eight slices from each layer were removed. Each layer was ground through a $^{1}/_{16}$ in. plate using a Hobart Model 4612 meat grinder, thoroughly mixed, vacuum packaged, and then stored in a −20 °C freezer until analyzed. For the outer vs cross-sectional slice study, a single 0.25 in. center cross-sectional slice was removed from the intact ham prior to removal of the outer 0.25 in. surface of the remaining ham. The samples were ground and stored as noted above.

Analysis. Duplicate 10.0 g samples were analyzed for nitrosamines using a solid phase extraction procedure and GC thermal energy analyzer detection system described in detail elsewhere (Pensabene et al., 1992; Pensabene and Fiddler, 1994). The nitrosamine values have been corrected for the recovery of the 10 ppb of nitrosamine internal standard (*N*-nitrosodipropylamine) in each individual sample. The minimum level of reliable measurement (signal/noise > 2) for NDBA and NDBzA was 1.0 ppb.

Nitrosamines in the netting (5–10 g) were determined by cutting the netting into 5–10 mm pieces and soaking them for 18 h in 100 mL of dichloromethane (DCM), drying the DCM by passing it through 35 g of anhydrous sodium sulfate in a coarse sintered glass funnel, and concentrating the DCM to 1.0 mL. The nitrosamines were quantitated as described above. Artifact formation during netting extraction was checked by adding 1.0 ppm of morpholine to the sample prior to extraction; no NMOR was detected.

Dibutylamine (DBA) was analyzed, in duplicate, in 10.0 g of comminuted ham samples according to a procedure described previously (Fiddler and Doerr, 1993). To determine dibenzylamine (DBzA), the same procedure was employed with the following modifications: the acid used for sample extraction was 0.5 N acetic acid and the gas chromatographic column was changed to a 1.8 m × 2.6 mm glass column packed with 5% SP-2401 DB on 100–120 mesh Supelcoport operating at 160 °C isothermal. The minimum detectable level for DBA was 0.1 ppm and for DBzA, 0.05 ppm. Conditions used for mass spectrometric confirmation have been published elsewhere (Pensabene and Fiddler, 1994).

Data Analysis. Data were analyzed by the General Linear Model and Means procedures of the Statistical Analysis System PC software (SAS Institute, 1985). These results were then interpreted according to methods of Snedecor and Cochran (1979) and Youden and Steiner (1975).

RESULTS AND DISCUSSION

In our investigations, two consecutive 0.25 in. layers were trimmed from the outside of 24 commercially produced hams. Each layer was then analyzed for NDBA and DBA. The NDBA data are shown in Figure 1. Most of the NDBA was found in the outermost 0.25 in. A marked 67% decrease was found in the next 0.25 in. below the product surface, indicating that most of the nitrosamine was formed on the surface of the product or transferred from the netting to the product. A highly significant ($p < 0.01$) correlation was found between the level of nitrosamine found in the nettings and the amount found on the outer surface of the ham. To further examine the penetration of the nitrosamine into the ham, 0.25 in. slices were removed, down to 1.25 in., from 15 of the hams which had sufficient cross-sectional width to allow this. After the first 0.50 in. of ham, a fairly rapid decrease to 0.3 ppb at a depth of 1.25 in. was noted. At a depth of 0.75 in. only 7 of the 15 hams contained detectable amounts of nitrosamines. This dropped to 5 at 1.00 in. and only 1 at a depth of 1.25 in. This was from an initial incidence of 14 of 15 hams positive for NDBA on the outer surface. The mean values at each individual depth were found to be significantly ($p < 0.05$) different from each other. These findings indicate that penetration of the nitrosamine from the surface into the inner parts of the ham is slow but significant.

As mentioned previously, the use of reformulated rubber required a similar assessment of the formation/migration of NDBzA to determine whether it had characteristics different from NDBA. For comparative purposes, the results are shown in Figure 2. While the overall pattern was similar, i.e., a marked decrease in NDBzA at a depth of 0.50 in., the exterior surface levels were higher than that found for NDBA in the ham samples tested. Here the decrease was 75%, from 61.4 to 15.6 ppb of NDBzA after the first 0.25 in. And, here again, a gradual decrease to small but detectable levels of NDBzA was noted even to a depth of 1.25 in.

While the principal concern is similar to the migration of nitrosamines from rubber baby bottle nipples into milk and infant formula (Havery and Fazio, 1982), there is also a question of the migration of their precursor amines into a product containing nitrite. To examine this aspect, eight samples of ham, whose surface values ranged from 9.0 to 82.1 ppb of NDBA, were selected for further study of the dibutylamine (DBA). Figure 3 shows a gradual decrease from the exterior surface of

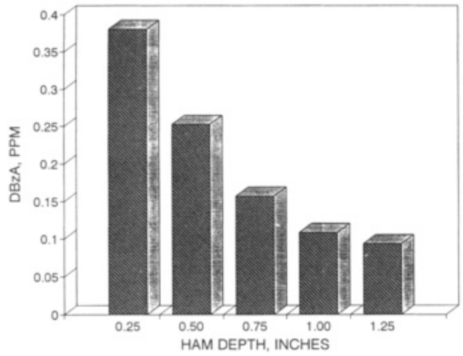

Figure 4. Migration of DBzA into hams.

the ham to the interior (means 0.57−0.23 ppm). The lack of a marked drop off after the first 0.25 in. was consistent with what one would expect for simple migration of the amine into the product. This provided additional evidence for nitrosamine formation primarily on the ham surface. Only one sample had no detectable amine at a depth of 1.25 in. DBA was confirmed by GC/MS at this depth in several of the other samples. The data also suggest DBA penetrates into the product to a greater extent than the nitrosamine migrates or forms. Because of the limited number of samples, no significant correlation was found between the DBA and NDBA surface values. This is also an indication that other factors, such as nitrite concentration, smoke components, water activity, and surface pH, in addition to the concentration of the DBA may influence NDBA formation on the surface of the ham. A similar study on DBzA was carried out with nine hams containing NDBzA. The results are shown in Figure 4. DBzA exhibited a similar pattern of decline to that observed for DBA. A mean value of 0.38 ppm of DBzA in the outermost portion dropped to 0.11 ppm at 1.00 in. and to 0.09 ppm at 1.25 in. At a depth of 1.50 in., DBzA could be detected in only two of the samples. The exterior levels were significantly ($p < 0.05$) higher for DBA than for DBzA. The rates of penetration are similar for NDBA and NDBzA. However, this rate is significantly ($p < 0.05$) lower for DBzA than for DBA. This is probably due to a difference in solubility of the two amines in the product, with the DBA being significantly more soluble than the DBzA. As expected, highly significant ($p < 0.01$) correlations were found between NDBA, NDBzA, DBA, and DBzA and the depth of slice. A highly significant ($p < 0.01$) correlation was also found between the level of NDBA found in the used netting and the quantity found on the outer surface.

Given the depth of penetration of the nitrosamines, the question arose whether a cross-sectional slice could be used for sampling rather than a surface portion. There is an economic justification for determining the nitrosamine content of a cross-sectional slice. In the case of surface sampling, most of what remains from the ham after sampling is not salable. With a cross-sectional slice sample, most of the ham would still be available for sale, which removes the economic burden from the ham processor. With the exception of end pieces, it is recognized that an apparent dilution of the nitrosamine content would be caused by the increased volume of the ham taken from a cross-sectional slice, since the nitrosamine is primarily a surface contaminant (FSIS, 1991). Unlike carcinogenic NDBA, where a risk assessment has been made for this nitrosamine in hams (Custer, 1991), none has been made on the noncarcinogenic (Druckrey et al., 1967), but genotoxic

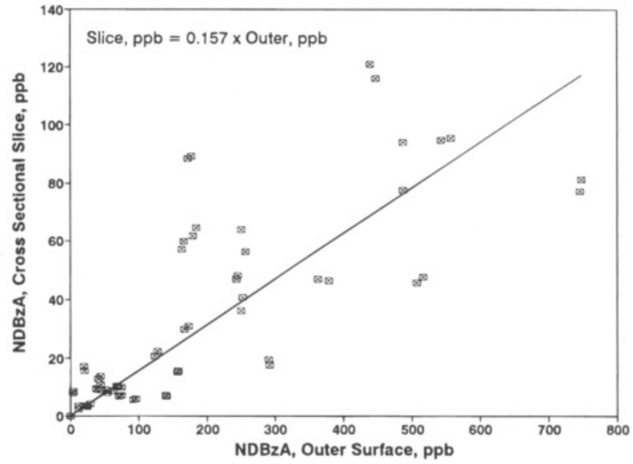

Figure 5. *N*-Nitrosodibenzylamine: outer vs cross-sectional slice.

(Boyes et al., 1990; Schmerzer et al., 1990), NDBzA. This assessment of NDBA addressed the industry claim that people only consumed a small portion of the outer surface when eating a slice of ham. Analysis of the NDBA data reported by FSIS for 10 hams showed no significant correlation ($p < 0.05$) between the outer surface and the inner slice values (FSIS, 1991). However, a high correlation was found between the ham inner slice/outer surface NDBA concentration ratio and the cross-sectional area of the slice. This suggested that there might be a direct correlation between the inner slice and the outer surface NDBA levels once the cross-sectional area factor is removed from the statistical analysis. In our study of NDBzA, we attempted to reduce or eliminate the influence of this factor by taking a 0.25 in. thick center slice from hams having approximately the same cross-sectional area. Thirty-three hams with diameters within the range of the 5.4 in. mean, plus or minus one standard deviation (0.8 in.; range 4.6−6.2 in.), were chosen for the correlation analysis. Of all the sample NDBzA results, only one was determined to be an outlier and removed. Figure 5 is a plot of the data. The outer surface ham NDBzA values ranged from none detected to 748 ppb, with a mean of 177 ppb. The corresponding slice content ranged from none detected to 121 ppb of NDBzA, with a mean of 31.3 ppb. The repeatability and CV were 2.4 ppb and 5.2%, respectively. Regression analysis of the data showed a highly significant correlation ($p < 0.01$) between the outer surface and the slice values, resulting in the equation shown in Figure 5. This equation indicates that approximately 16% of the outer surface NDBzA value will be found in the center cross-sectional slice; in other words, the surface to slice ratio for NDBzA is about 6:1. This is without taking into account the differences in volume and weight between the slice and outer surface portion.

In conclusion, we have demonstrated that there is significant penetration of nitrosamines and their precursor amines into boneless hams as a result of the use of elastic nettings containing rubber. Therefore, means should be developed to reduce or eliminate nitrosamines in cured products employing this type of netting in their production. This might be accomplished by reformulation of the rubber, the use of other types of approved nettings, or identifying and changing the pre- or post-processing conditions that have an effect on nitrosamine formation.

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LITERATURE CITED

Boyes, B. G.; Rogers, C. G.; Matula, T. I.; Stapley, R.; Sen, N. P. Evaluation of genotoxicity of N-nitrosodibenzylamine. *Mutat. Res.* **1990**, *241*, 379–385.

Carter, S. A. The potential health hazard of substances leached from plastic packaging. *J. Environ. Health* **1977**, *40*, 73–76.

Custer, C. Study shows nitrosamines in elastic netted hams. *FSIS Food Saf. Rev.* **1991**, *1*, 16–18.

Druckrey, H.; Preussmann, R.; Ivankovic, S.; Schmähl, D. Organotropic carcinogenic effects of 65 different N-nitroso compounds on BD Rats. *Z. Krebsforsch.* **1967**, *69*, 103–201.

Fiddler, W.; Doerr, R. C. Gas chromatographic-chemiluminescence detection (thermal energy analyzer/nitrogen mode) method for the determination of dibutylamine in hams. *J. AOAC Int.* **1993**, *76*, 578–581.

FSIS. NDBA levels detected in hams. *FSIS Food Saf. Rev.* **1991**, *1*, 19–20.

Havery, D. C.; Fazio, T. Estimation of volatile N-nitrosamines in rubber nipples for baby bottles. *Food Cosmet. Toxicol.* **1982**, *20*, 939–944.

Hoffmann, D.; Brunnemann, K. D.; Adams, J. D.; Rivenson, A.; Hecht, S. S. N-Nitrosamines in tobacco carcinogenesis. In *Nitrosamines in Human Cancer*; Magee, P. N., Ed.; Banbury Report 12; Cold Spring Harbor Laboratory: Cold Spring Harbor, NY, 1982; pp 211–225.

Hotchkiss, J. H.; Vecchio, A. J. Analysis of direct contact paper and paperboard food packaging for N-nitrosomorpholine morpholine. *J. Food Sci.* **1983**, *48*, 240–242.

Pensabene, J. W.; Fiddler, W. Gas chromatographic/thermal energy analyzer method for N-nitrosodibenzylamine in hams processed in elastic rubber netting. *J. AOAC Int.* **1994**, *77*, 981–984.

Pensabene, J. W.; Fiddler, W.; Gates, R. A. Solid-phase extraction method for volatile N-nitrosamines in hams processed with elastic rubber netting. *J. AOAC Int.* **1992**, *75*, 438–442.

Peterson, A. N-Nitrosodibutylamine and other volatile nitrosamines in cured meat packaged in rubber nettings. *J. Food Sci.* **1993**, *58*, 47–48.

Schmerzer, P.; Pool, B. L.; Lefevre, P. A.; Callender, R. D.; Ratpan, F.; Tinwell, H.; Ashby, J. Assay-specific genotoxicity of N-nitrosodibenzylamine to rat liver in vivo. *Environ. Mol. Mutat.* **1990**, *15*, 190–197.

Sen, N. P.; Baddoo, P. A. Origin of N-nitrosomorpholine contamination in margarine. *J. Food Sci.* **1986**, *51*, 216–217.

Sen, N. P.; Baddoo, P. A. An investigation on the possible presence of morpholine and N-nitrosomorpholine in wax-coated apples. *J. Food Saf.* **1989**, *9*, 183–191.

Sen, N. P.; Baddoo, P. A.; Seaman, S. W. Volatile nitrosamines in cured meat products packaged in elastic rubber nettings. *J. Agric. Food Chem.* **1987**, *35*, 346–350.

Sen, N. P.; Seaman, S. W.; Baddoo, P. A.; Weber, D. Further studies on the formation of nitrosamines in cured pork products packaged in elastic rubber nettings. *J. Food Sci.* **1988**, *53*, 731–734.

Sen, N. P.; Baddoo, P. A.; Seaman, S. W. Nitrosamines in cured pork products packaged in elastic rubber nettings: an update. *Food Chem.* **1993**, *47*, 387–390.

Snedecor, G. W.; Cochran, W. G. *Statistical Analysis*, 6th ed.; Iowa State University Press: Ames, IA, 1979.

Spiegelhalder, B.; Preussmann, R. Nitrosamines and Rubber. In *N-Nitroso Compounds: Occurrence and Biological Effects*; IARC Scientific Publication 41; IARC: Lyon, France, 1982; pp 231–243.

Statistical Analysis System Institute. *SAS User's Guide: Statistics*; Cary, NC, 1985.

Youden, W. J.; Steiner, E. H. *Statistical Manual of the Association of Official Analytical Chemists*; AOAC: Arlington, VA, 1975.

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