

## Investigations on Nitrosamines in Irradiation-Sterilized Bacon

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The effect of irradiation sterilization with <sup>60</sup>Co (3.0 Mrad at -40 °C) on nitrosamine formation and preformed nitrosamines in bacon was determined, and the development of a low nitrite, nitrosamine-free irradiated bacon that provides protection against pathogenic microorganisms such as *Clostridium botulinum* was investigated. The data from these experiments suggest that irradiation sterilization with <sup>60</sup>Co reduces residual nitrite in bacon prior to frying, thereby reducing volatile nitrosamines after frying, and destroys preformed volatile nitrosamines, if present, in the bacon prior to irradiation. In bacon prepared with 20 ppm of NaNO<sub>2</sub> and 550 ppm of sodium ascorbate, irradiation produces concentrations of nitrosamines that are indistinguishable from those in nitrite-free bacon (<1 ppb of NPYR).

Nitrite, an intentional additive, imparts desirable flavor, color, and texture characteristics to meat products and provides protection against oxidative rancidity and pathogenic microorganisms, especially *Clostridium botulinum*. In bacon, residual nitrite appears to react with natural meat components during frying to form *N*-nitrosopyrrolidine (NPYR) and, to a lesser extent, *N*-nitrosodimethylamine (NDMA). U.S. bacon manufacturers have tried a variety of corrective procedures in an effort to comply with Federal regulations that have set a 10-ppb violative and a 17-ppb action level for nitrosamines (NAs) in fried bacon. The reduction or elimination of nitrite has become a major issue.

Studies have shown that *C. botulinum* spores are destroyed by irradiation at sterilization doses (Anellis and Werkowski, 1968); thus, the need for nitrite to control growth and toxin formation is eliminated or greatly reduced. For bacon, the irradiation sterilizing dose is between 2.0 and 2.87 Mrad (Anellis et al., 1965). The major drawback to the use of irradiation is that the process has not been approved for use by the FDA. Irradiated bacon did receive FDA approval in 1963, but this was rescinded in 1968, despite the lack of adverse health effects in limited animal feeding studies (General Accounting Office, 1978).

To date, results of collaborative studies between the U.S. Army Natick Laboratories and the USDA's Eastern Regional Research Center have indicated that irradiation sterilized (radappertized) low nitrite containing ham and corned beef can be prepared without the formation of confirmable levels of volatile NAs. These results, plus some information on prefried (partly fried) bacon, together with sensory and other chemical data, have been made available only in publications of limited distribution (Wierbicki and Heiligman, 1974; Wierbicki et al., 1974, 1977; Wierbicki, 1979).

The present study was conducted to determine the effect of irradiation sterilization on NA formation in bacon and to determine whether a low-nitrite NA-free bacon can be developed. The results are reported herein.

### EXPERIMENTAL SECTION

**Reagents.** NPYR, *N*-nitrosomethylethylamine (NMEA), and *N*-nitrosohexamethylenimine (NHMI) were

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synthesized and purified as reported previously (Pensabene et al., 1972). NDMA, dichloromethane (DCM; "Distilled in Glass" solvent from Burdick and Jackson Laboratories), and all other chemicals were purchased from commercial suppliers and used without further purification.

**Bacon Processing.** Skinned matched pairs of pork bellies were purchased from a local supplier within 1 day of slaughter and stored in a freezer at -18 °C until needed. Prior to use, the bellies were thawed for 1 week in a cooler at 1 °C. The bellies were cut into thirds (brisket, center, and flank), and each section was pumped to ~10% of its green weight to achieve target levels of 1.5% sodium chloride, 0.75% sugar, 0.3% sodium tripolyphosphate, 0 or 550 ppm of sodium ascorbate, and 0, 20, or 120 ppm of sodium nitrite. The pumped bellies were stored in polyethylene bags at 1 °C for 18 h and then processed in a smokehouse (Pensabene et al., 1979). The bacon sections were removed from the smokehouse, placed in polyethylene bags, stored at 1 °C for 18 h, and sliced. Ten representative slices from each section were packed in 202 × 404 mm metal cans. These samples were shipped in a container refrigerated with cold packs to the U.S. Army Natick Laboratory. The samples were irradiated with a <sup>60</sup>Co source, with 3.0 Mrad at -40 ± 5 °C, and then returned to ERRC in a frozen state. The samples were thawed at 3 °C for 48 h prior to being analyzed for residual nitrite, ascorbate, and nitrosamines. In the preformed nitrosamine experiment, the bacon was prepared as described above with the same concentrations of cure ingredients. NaNO<sub>2</sub> (40 ppm) was added alone or in combination with 20 ppb each of NDMA and NPYR to ground raw bacon, made with no NaNO<sub>2</sub> and 550 ppm of NaAsc, or ground fried bacon, obtained from 120 ppm of NaNO<sub>2</sub> and 550 ppm of NaAsc, prior to canning and irradiation.

**Bacon Sampling and Frying.** The entire contents of each can, minus sufficient sample for residual nitrite and sodium ascorbate determinations, were fried in a preheated Presto Teflon-coated electric frying pan for 6 min at a calibrated temperature of 177 °C (350 °F). The fried edible portion was retained for nitrosamine analysis.

**Note:** Caution should be exercised in handling nitrosamines since they are potential carcinogens.

**Bacon Analysis.** (a) *Sodium Nitrite.* Residual sodium nitrite content was determined in 10 g of bacon by the Griess-Saltzman procedure as modified by Fiddler (1977). The minimum detectable level was <1 ppm.

(b) *Sodium Ascorbate.* This analysis was carried out by the microfluorometric method described by Deutsch and Weeks (1965) and Newmark et al. (1974). The minimum detectable level was <20 ppm.

(c) *Nitrosamine Analysis.* A modification of the procedure described by Fine et al. (1975) was employed to analyze NAs in the fried edible portion. A 25-g ground

fried bacon sample, to which was added 1 mL of a DCM solution containing 0.25  $\mu\text{g}$  of NMEA and 0.25  $\mu\text{g}$  of NHMI as internal standards, was placed in a 500-mL distillation flask equipped with a thermometer well. A total of 25 mL of mineral oil and 2 mL of 0.2 N NaOH were added, and the sample was distilled under vacuum (0.5 mmHg) until the temperature reached 140 °C. The distillate, collected in a glass trap immersed in liquid nitrogen, was quantitatively transferred to a 125-mL separatory funnel and extracted with 15 mL of DCM. The trap washing and extraction steps were repeated twice, and the combined DCM extracts were dried by passage through anhydrous sodium sulfate and concentrated to 1.0 mL in a Kuderna-Danish apparatus. The concentrations of volatile nitrosamines were determined quantitatively by GLC-Thermal Energy Analyzer (TEA) under conditions similar to those reported elsewhere (Pensabene et al., 1980). The minimum level of reliable measurement was determined to be 0.5 ppb of nitrosamine based on TEA response. The mean recovery values for the internal standards obtained from all of the edible samples analyzed were  $95.1 \pm 9.3\%$  for NMEA and  $92.0 \pm 10.6\%$  for NHMI.

Nitrosamines in the raw bacon were isolated and separated by the method of Fazio et al. (1973). The mean recovery values of the internal standards obtained from all of the samples analyzed were  $81.3 \pm 7.0\%$  for NMEA and  $88.2 \pm 5.9\%$  for NHMI.

Nitrosamines in the fat drippings were isolated and separated by the method of White et al. (1974). The mean recovery values of the internal standards obtained from all of the drippings samples analyzed were  $94.9 \pm 10.2\%$  for NMEA and  $89.5 \pm 12.8\%$  for NHMI.

(d) *Mass Spectral Analysis.* The fried edible portion of the bacon was analyzed by the official mineral oil distillation procedure as employed by the USDA Food Safety and Quality Service in their bacon monitoring program (*Fed. Regist.*, 1980). Utilization of this method produced sample extracts with large concentrations of components that prevented mass spectral confirmation, even with extensive cleanup procedures. NDMA and NPYR were confirmed by GLC-high-resolution mass spectrometry in selected samples of the drippings, particularly in the non-irradiated controls, where the level of nitrosamines was greater than 5 ppb. The details of the procedure and conditions have been described elsewhere (Pensabene et al., 1980).

(e) *Statistical Analysis.* One-tailed paired *t* tests or analysis of variance was performed on the measured nitrosamine and residual nitrite values according to methods described by Snedecor and Cochran (1974). Only the mean and the *t* or *F* statistical values are presented because of the large amount of raw data obtained in these investigations. All the data are available upon request from the principal author.

## RESULTS AND DISCUSSION

A study was conducted on the effect of irradiation on residual nitrite in raw bacon and NA formation in fried bacon cured with sodium nitrite at the current legal level of 120 and 550 ppm of NaAsc. The statistical analysis is shown in Table I. Because of the combination of low concentrations of NAs measured and small variance,  $p < 0.01$  was designated as the level of significance. The 18 belly section pairs had a mean residual nitrite concentration of 12 ppm before being irradiated but a mean of only 0.11 ppm afterward. This difference was statistically significant at the  $p < 0.01$  confidence level. Levels of NDMA in the fried edible portion of bacon were diminished significantly from 2.89 to 0.83 ppb after irradiation;

Table I. Effects of Irradiation on Nitrite and Nitrosamine Formation in Fried Bacon<sup>a</sup>

	control ( $\bar{x}_1$ )	irradiated ( $\bar{x}_2$ )	difference ( $\bar{x}_1 - \bar{x}_2$ )	<i>t</i> statistic (df)
residual NaNO <sub>2</sub> , ppm	12.0	0.11	11.89	6.97 (17) <sup>b</sup>
fried portion				
NDMA, ppb	2.89	0.83	2.06	5.25 (17) <sup>b</sup>
NPYR, ppb	9.28	3.39	5.89	10.31 (17) <sup>b</sup>
drippings				
NDMA, ppb	5.78	3.33	2.45	3.77 (8) <sup>b</sup>
NPYR, ppb	10.67	6.56	4.11	2.94 (8) <sup>b</sup>

<sup>a</sup> Bacon prepared with 120 ppm of NaNO<sub>2</sub> and 550 ppm of NaAsc. <sup>b</sup>  $p < 0.01$ .

likewise, the concentration of NPYR, the NA of primary concern, was reduced from 9.28 to 3.39 ppb ( $p < 0.01$ ). Similar significant reductions of NDMA and NPYR were noted in the cooked-out drippings. These data show that irradiation destroys residual nitrite and, as a direct result, less nitrosation of precursor amine occurs. A high correlation between residual nitrite and nitrosamine formation in fried bacon has been noted previously (Gough and Walters, 1976; Pensabene et al., 1979).

Since no or very low levels of residual nitrite were detected in the irradiated bacon prior to frying in the previous experiment, bacon was processed with and without 550 ppm of NaAsc. A mean value of 14.2 ppm of NaNO<sub>2</sub> was obtained in the ascorbate-free bacon. When NaAsc was employed, a mean value of 51.5 ppm of residual NaAsc was detected with no residual nitrite evident. The NaNO<sub>2</sub> results were significant at the  $p < 0.01$  level ( $t = 5.06$ ,  $df = 10$ ,  $n = 6$ ). Ascorbate (or erythorbate) is known to react with nitrite under acidic conditions to form nitric oxide (Dahn et al., 1960), which is lost by volatilization directly or undergoes oxidation to nitrogen dioxide, which dismutates in the presence of water. The net result is a loss of measured nitrite. This reduces the formation of the nitrosating species, NO<sup>+</sup>, thereby reducing nitrosamine formation. Clearly, the addition of NaAsc is essential in reducing residual nitrite and nitrosamines. The effect of this compound appears to be enhanced by the irradiation process.

A larger study was conducted with bacon prepared with 120 ppm of NaNO<sub>2</sub> to assess the effect of both added NaAsc and irradiation. The results are shown in Table II. Irradiation significantly ( $p < 0.01$ ) reduced residual NaNO<sub>2</sub> measured prior to frying and the NA values in the fried portion and cooked-out drippings after frying as evidenced by the largest *F* values obtained with this treatment. Also, 550 ppm of NaAsc significantly reduced the residual NaNO<sub>2</sub> and NPYR but not NDMA. The combination of irradiation and NaAsc significantly reduced only the residual NaNO<sub>2</sub>, as evidenced by the mean value of <1 ppm. This is consistent with the 99% reduction of NaNO<sub>2</sub> observed in the results shown in Table I. For the NAs, the values obtained by irradiation or NaAsc were so low that no significant interaction could be observed.

For determination of the effect of irradiation sterilization on preformed nitrosamines, bacon produced with 120 ppm of NaNO<sub>2</sub> and 550 ppm of NaAsc was fried, and aliquots were subjected to irradiation. The normally incurred mean levels of NDMA and NPYR in the nonirradiated controls were 2.6 and 2.9 ppb, respectively. Upon irradiation, 0.7 ppb of NDMA and no detectable NPYR were noted. Addition of 20 ppb each of NDMA and NPYR to nonirradiated fried bacon yielded 16.5 and 15.6 ppb, respectively, vs. 2.3 ppb of NDMA and 3.1 ppb of NPYR after irradiation.

Table II. Effect of Added Sodium Ascorbate and Irradiation on Residual Nitrite and Nitrosamine Formation in Fried Bacon<sup>a</sup>

treatment	residual NaNO <sub>2</sub> , ppm <sup>b</sup>		fried portion <sup>b</sup>				drippings <sup>b</sup>			
	control	irradn	NDMA, ppb		NPYR, ppb		NDMA, ppb		NPYR, ppb	
			control	irradn	control	irradn	control	irradn	control	irradn
none	76.7	14.8	4.2	1.0	11.3	4.2	6.4	3.3	13.0	6.3
NaAsc (550 ppm)	26.0	0.8	3.0	0.9	7.7	2.9	5.0	3.1	11.2	4.8

  

source	F Values and Significance				
	residual NaNO <sub>2</sub> , ppm <sup>b</sup>	fried portion <sup>b</sup>		drippings <sup>b</sup>	
		NDMA, ppb	NPYR, ppb	NDMA, ppb	NPYR, ppb
irradiation	90.0 <sup>c</sup>	34.7 <sup>c</sup>	63.8 <sup>c</sup>	19.3 <sup>c</sup>	15.7 <sup>c</sup>
NaAsc	49.7 <sup>c</sup>	2.5	11.2 <sup>c</sup>	2.1	4.9
irradn × NaAsc	16.0 <sup>c</sup>	1.3	2.2	1.2	<1

<sup>a</sup> Bacon prepared with 120 ppm of NaNO<sub>2</sub>. <sup>b</sup> Mean values (n = 3). <sup>c</sup> p < 0.01.

Table III. Effect of Irradiation on Nitrite and Nitrosamines in Raw Bacon

treatment	residual NaNO <sub>2</sub> , ppm <sup>a</sup>		NDMA, ppb <sup>a</sup>		NPYR, ppb <sup>a</sup>	
	control	irradn	control	irradn	control	irradn
none	3.3	0	0.8	0.4	0	0
NaNO <sub>2</sub> (40 ppm)	8.3	6.0	0.7	0.4	0	0
NDMA + NPYR (20 ppb)	2.0	2.0	16.1	1.3	13.4	1.6
NaNO <sub>2</sub> (40 ppm) + NDMA + NPYR (20 ppb)	9.3	2.0	16.2	3.4	14.6	4.5

  

source	F Values and Significance		
	residual NaNO <sub>2</sub> , ppm <sup>a</sup>	NDMA, ppb <sup>a</sup>	NPYR, ppb <sup>a</sup>
irradiation	17.6 <sup>b</sup>	524.1 <sup>b</sup>	128.0 <sup>b</sup>
treatment	13.4 <sup>b</sup>	265.2 <sup>b</sup>	155.0 <sup>b</sup>
irradn × treatment	4.4	162.0 <sup>b</sup>	52.4 <sup>b</sup>

<sup>a</sup> Mean values (n = 3). <sup>b</sup> p < 0.01.

A larger study was undertaken, therefore, with raw bacon to which NaNO<sub>2</sub> and/or NAs were added. The results are shown in Table III. Irradiation caused a significant (p < 0.01) reduction in residual NaNO<sub>2</sub>, which has been noted in other experiments, except where 40 ppm of NaNO<sub>2</sub> was added without NAs. In this case the variation in nitrite values was too high to obtain a meaningful difference. Since NDMA and NPYR have not been previously found in raw bacon (Fazio et al., 1973), and our values were <1 ppb, no irradiation effect was apparent. Interestingly, addition of 40 ppm of NaNO<sub>2</sub> to raw bacon prior to irradiation also did not produce NAs. Where 20 ppb each of NDMA and NPYR was added with and without 40 ppm of NaNO<sub>2</sub>, a significant (p < 0.01) reduction in NAs was observed after irradiation. No NAs were found in the other two treatments. The magnitude of the reduction in residual nitrite or NAs was dependent on the treatment, as indicated by the significant interactions. All the data suggested that irradiation, in sterilization doses, destroyed NAs. This could be important. For example, if N-nitrosoproline, a nonvolatile NA found in uncooked bacon (Kushnir et al., 1975; Janzowski et al., 1978), is destroyed prior to frying, then it can no longer serve as the precursor for NPYR as suggested by several authors (Kushnir et al., 1975; Gray et al., 1977; Baker and Ma, 1978). This may also be true if the NA precursors were other NAs. From our experiments with bacon, there is no evidence of the formation of NAs or inhibition of NA decomposition in the presence of NaNO<sub>2</sub> by  $\gamma$  irradiation as demonstrated by Challis et al. (1981) in aqueous model systems in which relatively large concentrations of reactants were employed.

Another series of experiments was conducted in order to determine the effect of ingoing nitrite concentration on

Table IV. Effect of Nitrite Concentration on Residual Nitrite and Nitrosamine Formation in Fried Irradiated Bacon

	0 vs. 20 ppm of NaNO <sub>2</sub> at NaNO <sub>2</sub> level, ppm			
	0 ( $\bar{x}_1$ )	20 ( $\bar{x}_2$ )	difference ( $\bar{x}_2 - \bar{x}_1$ )	t statistic (df)
	residual NaNO <sub>2</sub> , ppm	0	0.22	0.22
NDMA, ppb	0	0	0	0 (8)
NPYR, ppb	0	0.11	0.11	1.00 (8)

  

	20 vs. 120 ppm of NaNO <sub>2</sub> at NaNO <sub>2</sub> level, ppm			
	20 ( $\bar{y}_1$ )	120 ( $\bar{y}_2$ )	difference ( $\bar{y}_2 - \bar{y}_1$ )	t statistic (df)
	residual NaNO <sub>2</sub> , ppm	0.56	6.44	5.88
NDMA, ppb	0	0.44	0.44	2.53 (8)
NPYR, ppb	0.11	2.11	2.00	8.49 (8) <sup>a</sup>

  

	0 vs. 120 ppm of NaNO <sub>2</sub> at NaNO <sub>2</sub> level, ppm			
	0 ( $\bar{z}_1$ )	120 ( $\bar{z}_2$ )	difference ( $\bar{z}_2 - \bar{z}_1$ )	t statistic (df)
	residual NaNO <sub>2</sub> , ppm	0.22	5.33	5.11
NDMA, ppb	0	0.89	0.89	2.53 (8)
NPYR, ppb	0	2.33	2.33	7.00 (8) <sup>a</sup>

<sup>a</sup> p < 0.01.

residual nitrite levels and formation of NAs in irradiated bacon. Three comparisons were made in each of three experiments: 0 vs. 20, 0 vs. 120, and 20 vs. 120 ppm of NaNO<sub>2</sub>. The statistical analysis for the data obtained from

the fried edible portion is presented in Table IV.

**0 vs. 20 ppm of NaNO<sub>2</sub>.** Residual nitrite was not detected in the nitrite-free irradiated bacon, whereas in bacon cured with 20 ppm of NaNO<sub>2</sub> only 4 or 18 samples exhibited detectable residual nitrite (mean concentration, 0.22 ppm). This difference was not statistically significant. No detectable NDMA was found in fried bacon cured with either 0 or 20 ppm of NaNO<sub>2</sub>. No detectable NDMA was found in fried bacon cured with either 0 or 20 ppm of NaNO<sub>2</sub>. No detectable NPYR was found in the fried edible portion of nitrite-free bacon. Two of eighteen bacon samples prepared with 20 ppm of NaNO<sub>2</sub> exhibited detectable levels of NPYR. The mean value of the 18 samples, 0.11 ppb, was not significantly different from zero.

**20 vs. 120 ppm of NaNO<sub>2</sub>.** Mean residual nitrite concentrations of 0.56 and 6.44 ppm, respectively, were not significantly different. No NDMA was detected in the edible fried bacon prepared with 20 ppm of NaNO<sub>2</sub>, and a mean of 0.44 ppb of NDMA for the 120 ppm of NaNO<sub>2</sub> samples was not significant at the  $p < 0.01$  level. The mean difference of 2.00 ppb of NPYR (0.11 ppb at 20 ppm of NaNO<sub>2</sub> and 2.11 ppb in bacon with 120 ppm of NaNO<sub>2</sub>) was significant.

**0 vs. 120 ppm of NaNO<sub>2</sub>.** Results from this treatment group followed a pattern similar to that of the previous group with 20 and 120 ppm of NaNO<sub>2</sub> bacon. Mean residual NaNO<sub>2</sub> levels were not significantly different (5.11 ppm), while the NA values were affected by the ingoing nitrite concentration. The difference of <1 ppb of NDMA (0 vs. 0.89 ppb) in the fried bacon was not significant; however, a difference of 2.33 ppb of NPYR (0 vs. 2.33) was significant.

The nitrosamine values for the cooked-out drippings from bacon receiving the three treatments showed greater differences, since the content is generally higher in the drippings than in the fried product. The NPYR results were significantly different in the 20 vs. 120 ppm of NaNO<sub>2</sub> and 0 vs. 120 ppm of NaNO<sub>2</sub> treatments, supporting the fried bacon findings.

From the above results, it is concluded that radappertization with <sup>60</sup>Co utilizing 3.0 Mrad at -40 °C reduces residual nitrite in bacon prior to being fried. The lower nitrosamine concentration after frying correlates with this reduction in nitrite. The reduction of nitrite by sodium ascorbate appears to be enhanced by the irradiation process. Irradiation sterilization doses appear to destroy volatile nitrosamines when present prior to irradiation. Irradiated bacon prepared with an ingoing level of 20 ppm of NaNO<sub>2</sub> contains little or no residual nitrite, and, after it is fried, concentrations of the nitrosamines are indistinguishable from that of nitrite-free bacon. Irradiated bacon cured with 20 ppm of NaNO<sub>2</sub> has also been shown to have favorable sensory and quality characteristics (Wierbicki and Heiligman, 1980).

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