NITROSAMINES IN IRRADIATED BACON

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INTRODUCTION

NITRITE, an intentional additive, imparts desirable flavor, color, and texture characteristics to meat products, and provides protection against 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). United States 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, particularly in light of the study that implicated nitrite itself as being carcinogenic (Newberne, 1979).

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 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 Irradiated bacon did receive FDA approval in 1963, but this was rescinded in 1968, despite the lack of adverse health effects in limited animal feeding studies (GAO, 1978).

To date, results of collaborative studies between the U.S. Army Natick Laboratories and the USDA, Eastern Regional Research Center have indicated that irradiation sterilized (Radappertization) low-nitrite-containing ham and corned beef can be prepared without confirmable levels of volatile NAs. This information, plus limited information on pre-fried (partly fried) bacon, together with sensory and other chemical data, have been made available only in publications of limited distribution (Wierbicki et al., 1974, 1976; 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

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 approximately 10% of its green weight to achieve target levels of 1.5% sodium chloride, 0.75% sugar, 0.3% sodium tripolyphosphate, 550 ppm sodium ascorbate, and 0, 20, or 120 ppm sodium nitrite. The pumped bellies were stored in polyethylene bags at 1°C for 18 hr, 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 hr, and sliced, and 10 representative slices from each section were packed in 202 x 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 Co^{60} source, with 3.0 Mrad at -40 ± 5°C, then returned to ERRC in a frozen state. The samples were thawed at 3°C for 48 hr prior to being analyzed for residual nitrite and nitrosamines.

Bacon Sampling and Frying

THE ENTIRE contents of each can, minus a 10 g sample for residual nitrite determination, 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.

Nitrosamine Analysis

A MODIFICATION of the procedure described by Fine et al. (1975) was employed for NA analysis. A 25 g ground fried bacon sample, to which was added 1 ml of a dichloromethane (DCM) solution containing 0.25 μg N-nitrosomethylethylamine (NMEA) and 0.25 μg N-nitrosohexamethyleneimine(NHMI) as internal standards, was placed in a 500 ml distillation flask equipped with a thermometer well. Twenty-five ml of mineral oil and 2 ml of 0.2N NaOH were added, and the sample was distilled under vacuum (0.5 mm) 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 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., in press). The minimum level of reliable measurement was determined to be 0.5 ppb nitrosamine based on TEA response. The mean recovery values for the internal standards obtained from all of the samples analyzed were 96% for NMEA and 93% for NHMI.

Sodium Nitrite

RESIDUAL sodium nitrite content was determined in 10 g of bacon by the Griess-Saltzman procedure modified by Fiddler (1977).

RESULTS AND DISCUSSION

THE EFFECTS of irradiation on residual nitrite and NA formation in fried bacon cured with sodium nitrite at the current legal level of 120 ppm are shown in Table 1. One-tailed paired t-tests were performed on the measured nitrosamine and residual nitrite values in the matched belly pairs (Snedecor and Cochran, 1974). The statistical analysis is shown in Table 2. 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 after being radappertized. 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; 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).

The second series of experiments was designed to determine the effect of ingoing nitrite concentration on residual nitrite levels and formation of NAs in irradiated bacon (Table 3). Three comparisons were made in each of the three experiments: 0 vs 20 ppm, 0 vs 120 ppm, and 20 vs 120 ppm $NaNO_2$. The statistical analysis is presented in Table 4.

0 vs 20 ppm $NaNO_2$

RESIDUAL nitrite was not detected in the nitrite-free irradiated bacon, whereas in bacon cured with 20 ppm $NaNO_2$ only 4 of 18 samples exhibited detectable residual nitrite (mean concentration, 0.22 ppm). This difference was not statistically significant. There was no detectable NDMA in fried bacon cured with either 0 or 20 ppm $NaNO_2$. No detectable NPYR was found in the fried edible portion of nitrite-free bacon. Two of 18 bacon samples prepared with 20 ppm $NaNO_2$ 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 $NaNO_2$

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 $NaNO_2$, and a mean of 0.44 ppb NDMA for the 120 ppm $NaNO_2$ samples was not significant at the $p < 0.01$ level. The mean difference of 2.00 ppb NPYR (0.11 ppb at 20 ppm $NaNO_2$ and 2.11 ppb in bacon with 120 ppm $NaNO_2$) was significant.

0 vs 120 ppm $NaNO_2$

RESULTS from this treatment group followed a pattern similar to that of the previous group with 20 and 120 ppm $NaNO_2$ bacon. Mean residual $NaNO_2$ levels were not significantly different (5.11 ppm), while the NA values were affected by the ingoing nitrite concentration. The difference of <1 ppb NDMA (0 vs 0.89 ppb) in the fried bacon was not significant; however, a difference of 2.33 ppb NPYR (0 vs 2.33) was significant. The NA values for the cooked-out drippings showed greater differences and generally paralleled the fried bacon data insofar as significance was concerned.

From the above results, it is concluded that radappertization with Co^{60} 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. Irradiated bacon prepared with an ingoing level of 20 ppm $NaNO_2$ contains little or no residual nitrite and, after it is fried, concentrations of the nitrosamines are indistinguishable from nitrite-free bacon. The favorable sensory and quality characteristics of irradiated bacon cured with 20 ppm $NaNO_2$ (Wierbicki and Heiligman, 1980) shows promise for future consumer acceptance of this product.

NOTE: Reference to brand or firm name does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

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

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TABLE 1. NITRITE AND NITROSAMINES IN IRRADIATED AND NON-IRRADIATED BACON

				Residual $NaNO_2$ (ppm)		Fried Edible Portion			
						NDMA (ppb)		NPYR	
Belly	Side	Section	Treatment	Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 1	Exp. 2
1	right	brisket	None	7	5	2	2	7	6
	left		Irrad.	n.d.*	1	n.d.	1	2	3
	right	center	None	2	3	2	3	7	10
	left		Irrad.	n.d.	n.d.	n.d.	1	4	4
	right	flank	None	22	13	1	1	8	6
	left		Irrad.	n.d.	n.d.	1	1	2	3
2	right	brisket	None	22	20	2	6	12	15
	left		Irrad.	n.d.	n.d.	1	2	3	5
	right	center	None	14	18	7	4	7	13
	left		Irrad.	n.d.	n.d.	n.d.	1	3	4
	right	flank	None	12	13	2	5	9	13
	left		Irrad.	n.d.	n.d.	n.d.	1	4	2
3	right	brisket	None	18	5	3	2	14	7
	left		Irrad.	n.d.	n.d.	2	n.d.	8	3
	right	center	None	18	6	3	2	10	9
	left		Irrad.	n.d.	1	1	1	4	3
	right	flank	None	14	4	3	2	8	6
	left		Irrad.	n.d.	n.d.	1	1	2	2

* n.d. - none detected.

TABLE 2. EFFECTS OF IRRADIATION ON NITRITE AND NITROSAMINE FORMATION IN FRIED BACON (STATISTICAL EVALUATION)

	Control ($\bar{x}_1$)	Irradiated ($\bar{x}_2$)	Mean difference ($\bar{x}_1 - \bar{x}_2$)	t-statistic (df)
Residual $NaNO_2$ (ppm)	12.0	0.11	11.89	5.97 (17)*
NDMA (ppb)	2.89	0.83	2.06	5.2[illegible] (17)*
NPYR (ppb)	9.28	3.39	5.89	10.31 ([illegible])*

* $p < 0.01$

TABLE 3. EFFECT OF NITRITE LEVEL ON NITROSAMINE FORMATION IN IRRADIATED BACON

			$NaNO_2$ (ppm)				Nitrosamines in Fried Edible Portion (ppb)					
				Residual			NDMA			NPYR		
Belly	Side	Section	Added	Exp. 1	Exp. 2	Exp. 3	Exp. 1	Exp. 2	Exp. 3	Exp. 1	Exp. 2	Exp. 3
1	right	brisket	20	n.d.*	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	left		120	3	n.d.	2	1	n.d.	n.d.	3	2	2
	right	center	0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	left		20	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1
	right	flank	0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	left		120	3	1	n.d.	2	n.d.	n.d.	2	1	1
2	right	brisket	0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	left		20	1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	right	center	0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	left		120	17	2	1	1	n.d.	1	3	2	3
	right	flank	20	3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	left		120	19	n.d.	n.d.	1	1	n.d.	3	1	2
3	right	brisket	0	1	n.d.	1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	left		120	23	1	n.d.	3	1	n.d.	4	2	3
	right	center	20	2	n.d.	n.d.	n.d.	n.d.	n.d.	1	n.d.	n.d.
	left		120	33	1	n.d.	1	n.d.	n.d.	3	1	2
	right	flank	0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	left		20	n.d.	1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

* n.d. - none detected.

TABLE 4. EFFECT OF NITRITE CONCENTRATION ON RESIDUAL NITRITE AND NITROSAMINE FORMATION IN FRIED IRRADIATED BACON (STATISTICAL EVALUATION)

0 vs. 20 ppm $NaNO_2$

	$NaNO_2$ level (ppm) 0 ($\bar{x}_1$)	20 ($\bar{x}_2$)	Difference ($\bar{x}_2 - \bar{x}_1$)	t-Statistic (df)
Residual $NaNO_2$ (ppm)	0	0.22	0.22	1.51 (8)
NDMA (ppb)	0	0	0	0 (8)
NPYR (ppb)	0	0.11	0.11	1.00 (8)

20 vs. 120 ppm $NaNO_2$

	$NaNO_2$ level (ppm) 20 ($\bar{y}_1$)	120 ($\bar{y}_2$)	Difference ($\bar{y}_2 - \bar{y}_1$)	t-Statistic (df)
Residual $NaNO_2$ (ppm)	0.56	6.44	5.88	1.65 (8)
NDMA (ppb)	0	0.44	0.44	2.53 (8)
NPYR (ppb)	0.11	2.11	2.00	8.49 (8)*

0 vs. 120 ppm $NaNO_2$

	$NaNO_2$ level (ppm) 0 ($\bar{z}_1$)	120 ($\bar{z}_2$)	Difference ($\bar{z}_2 - \bar{z}_1$)	t-Statistic (df)
Residual $NaNO_2$ (ppm)	0.22	5.33	5.11	1.84 (8)
NDMA (ppb)	0	0.89	0.89	2.53 (8)
NPYR (ppb)	0	2.33	2.33	7.00 (8)*

* $p < 0.01$