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## Effect of Cesium-137 Radiation on the Formation of N-Nitrosopyrrolidine in Bacon

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To date, the majority of information collected on the irradiation of bacon has been based on  $^{60}\text{Co}$  as the radiation source. In this study,  $^{137}\text{Cs}$  was used as the radiation source for the treatment of bacon. Bacon prepared with 550 ppm sodium ascorbate and either 120 or 40 ppm sodium nitrite was irradiated at absorbed doses of 0.75, 1.5, and 3.0 Mrad. Results show that only at 3.0 Mrad was there a significant difference from the nonirradiated control in both residual nitrite and N-nitrosopyrrolidine (NPYR) values and that NPYR levels were significantly less in the 40 ppm nitrite bacon than in the 120 ppm bacon. Radiation of bacon prepared with and without ascorbate also showed an additive effect on nitrite destruction and NPYR reduction. The results suggest  $^{137}\text{Cs}$  may not be as effective as  $^{60}\text{Co}$  in reducing nitrite/NPYR in bacon.

Nitrite, in combination with sodium chloride, is used in the curing of meat to produce its characteristic color and flavor and to control spoilage and growth of pathogenic microorganisms. In particular, the control of *Clostridium botulinum* has been claimed to be very important with respect to bacon to justify the use of 120 ppm ingoing sodium nitrite ( $\text{NaNO}_2$ ). The problem with this is that the higher the ingoing nitrite, the higher the residual nitrite content. For bacon, there is a high correlation between residual nitrite prior to frying and N-nitrosopyrrolidine (NPYR), a known animal carcinogen, after frying. In addition to nitrosamines, the question concerning whether nitrite itself is carcinogenic or not was raised as a result of the Newberne study (Newberne, 1979). While the interpretation of this study's results has now been largely discounted, two recent papers have revised the question of the toxicological significance of nitrite itself. First, the study by Lijinsky et al. (1983), in which nitrite was fed to rats for 2 years, indicated that nitrite increased the incidence of liver neoplasms in female, but not in male rats, and second, the report by Schweinsberg and Bürkle (1985) indicated that nitrite may be a cocarcinogen in the presence of other carcinogens. As a result, different approaches need to be considered to reduce the amount of nitrite used in processing cured meat products.

Radiation sterilization has been proposed as a possible means of reducing levels of nitrite in bacon to the minimum needed for the development of the characteristic cure color and flavor, and also for the requisite protection against *C. botulinum*. Anellis and Werkowski (1968) showed that *C. botulinum* spores are destroyed by irradiation at a dose between 2.0 and 2.9 Mrad. The only known drawback to the use of radiation in bacon is that the process has not yet been approved for use by the Food

and Drug Administration (FDA).  $\gamma$  radiation has recently been approved for use to control insect infestation in certain species (Federal Register, 1985a) and to kill *Trichinella spiralis* in pork (Federal Register, 1985b).

In our earlier work on the effect of radiation sterilization (Fiddler et al., 1981), bacon prepared with 120 ppm  $\text{NaNO}_2$  was irradiated with  $^{60}\text{Co}$  (3.0 Mrad) at  $-40^\circ\text{C}$ . We found that residual  $\text{NaNO}_2$  was reduced to almost nondetectable levels prior to frying and that NPYR after frying was less than the USDA violative level of 10 ppb. We also found that, in bacon prepared with either 20 or 40 ppm  $\text{NaNO}_2$ , radiation yielded NPYR values indistinguishable from nitrite-free bacon. In the present work, we carried out a series of experiments on  $\text{NaNO}_2$ /NPYR in bacon to determine whether  $^{137}\text{Cs}$  would give us the same trends we obtained earlier with  $^{60}\text{Co}$  (Fiddler et al., 1981).

### EXPERIMENTAL SECTION

**Reagents.** A complete list of reagents needed for determining NPYR in fried bacon and its cooked-out fat was reported elsewhere (Pensabene and Fiddler, 1982; White et al., 1974).

**Bacon Processing.** Skinned, matched pork bellies were purchased from a local supplier within 24 h postmortem and stored at  $-18^\circ\text{C}$  until used. Prior to use, the bellies were thawed for 1 week in a cooler at  $1^\circ\text{C}$ . All bellies were pumped to approximately 110% of green weight to achieve ingoing levels of 1.5% sodium chloride, 0.75% sucrose, 0.3% sodium tripolyphosphate, 0 or 550 ppm sodium ascorbate ( $\text{NaAsc}$ ), and 40 or 120 ppm  $\text{NaNO}_2$ . The pumped bellies were stored in polyethylene bags at  $1^\circ\text{C}$  for 18 h and then processed in a smokehouse as described previously (Pensabene et al., 1979). After processing, the bellies were chilled overnight in a  $1^\circ\text{C}$  cooler and then sliced. The randomized slices were vacuum packaged (12 slices/pkg) prior to irradiation at 0.75, 1.5, and 3.0 Mrad with a  $^{137}\text{Cs}$  source and a chamber temperature of  $2-3^\circ\text{C}$ . A complete description of the irradiator, including source strength,

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**Table I. Effect of  $^{137}\text{Cs}$  Radiation on Residual Nitrite and Nitrosopyrrolidine in Bacon Prepared with either 120 or 40 ppm Nitrite<sup>a</sup>**

dose, Mrad	sodium nitrite added <sup>b</sup>			
	120 ppm		40 ppm	
	NaNO <sub>2</sub> , ppm	NPYR, <sup>c</sup> ppb	NaNO <sub>2</sub> , ppm	NPYR, <sup>c</sup> ppb
0	39	14.96	7	5.82
0.75	26	16.42	3	5.43
1.5	19	13.35	5	4.79
3.0	9	7.66	2	2.78

<sup>a</sup>Mean values,  $n = 6$ . <sup>b</sup>Plus 550 ppm sodium ascorbate. <sup>c</sup>Corrected for recovery of the internal nitrosamine standard.

placement of the samples, etc. has been reported elsewhere (Shieh et al., 1985). All experiments were repeated so that processing and irradiation changes could be accounted for in the statistical analysis of the data. Although earlier data (Fiddler et al., 1981) showed that bacon prepared with 20 ppm nitrite and irradiated with  $^{60}\text{Co}$  produced no detectable volatile nitrosamines, in this present study a 40 ppm NaNO<sub>2</sub> level was selected to help ensure a more uniform distribution of the nitrite in pork bellies, especially under commercial processing conditions where the excess cure solution is filtered and reused. Under these conditions, the lower concentration of nitrite (20 ppm) would deplete too rapidly, thereby increasing the likelihood of having uncured spots in the pumped belly. The level of 40 ppm NaNO<sub>2</sub> was also selected so that product safety would not be compromised. This concentration of NaNO<sub>2</sub> delayed swelling and toxicity in bacon inoculated with 2 spores/g of *C. botulinum* when compared to no-nitrite bacon (Rowley et al., 1983). These authors also obtained extensive data on bacon prepared with 40 ppm NaNO<sub>2</sub>, uninoculated and inoculated (2 and 160 spores/g of *C. botulinum*), and irradiated with  $^{60}\text{Co}$  in 0.5-Mrad absorbed dose increments from 0 to 1.5 Mrad.

**Bacon Sampling and Frying.** The entire contents of each package, minus sufficient sample for residual NaNO<sub>2</sub> analysis, were fried in a preheated Presto Teflon-coated electric frying pan for 6 min (3 min/side) at a precalibrated temperature of 177 °C. Both the edible portion and the cooked-out fat were retained for NPYR analysis.

**Bacon Analysis.** (a) *Sodium Nitrite.* Residual NaNO<sub>2</sub> was determined in 10 g of uncooked bacon by the modified Griess-Saltzman procedure (Fiddler, 1977).

(b) *N-Nitrosopyrrolidine Analysis.* NPYR in the fried bacon was determined by the dry-column method of Pensabene and Fiddler (1982) and NPYR in the cooked-out fat by the method of White et al. (1974). All samples were analyzed in duplicate, and all NPYR values have been corrected for the recovery of the internal nitrosamine standards in each individual sample.

**Statistical Analysis.** Statistical analyses were carried out 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 AND DISCUSSION

Two separate studies were conducted on the effect  $^{137}\text{Cs}$  radiation on NPYR formation in fried bacon and its drippings. In the first study, bacon prepared with 550 ppm NaAsc and either 120 or 40 ppm NaNO<sub>2</sub> was irradiated. Three-way ANOVA's (analysis of variance) were performed on the NPYR levels in the edible portion after frying. The mean values and statistical results are shown in Tables I and II, respectively. A highly significant ( $p < 0.01$ ) dif-

**Table II. Analysis of Variance of the Nitrosopyrrolidine Values from the 120-40 ppm Nitrite Experiment<sup>a</sup>**

source	deg of freedom	sum of squares	mean square	F ratio <sup>b</sup>
belly (B)	5	2940.50	588.10	2390.80**
dose (D)	3	477.44	159.25	5.51**
nitrite (N)	1	1690.25	1690.25	16.26*
B × D	15	433.55	28.90	117.50**
B × N	5	519.77	103.95	422.60**
D × N	3	118.01	39.34	3.78*
B × D × N	15	156.02	10.40	42.28**
error	48	11.81	0.25	
total	95	6347.34		

<sup>a</sup>Reproducibility = (mean square error)<sup>1/2</sup> = 0.50 ppb. <sup>b</sup>Key: (\*)  $p < 0.05$ ; (\*\*)  $p < 0.01$ .

ference was found in the NPYR values among bellies, as would be expected due to the inherent variability in pork belly composition. This compositional variability also accounts for the highly significant belly × dose, belly × nitrite, and belly × dose × nitrite interactions. The ANOVA showed a highly significant difference in the NPYR values among doses. Further analysis of the data by least significant difference (LSD) testing revealed no significant difference in NPYR among the 0-, 0.75-, and 1.5-Mrad doses for both nitrite levels. However, the 3.0-Mrad dose was shown to be significantly lower in NPYR content overall, with NPYR decreasing from an average of 14.96 to 7.66 ppb in the 120 ppm nitrite bacon and from 5.82 to 2.78 ppb in the 40 ppm nitrite bacon. Residual NaNO<sub>2</sub> in these same bellies decreased from an average of 39 to 9 ppm and from 7 to 2 ppm in the 120 and 40 ppm NaNO<sub>2</sub> bacon, respectively. *N*-Nitrosopyrrolidine was also found to be significantly lower ( $p < 0.01$ ) in the bacon cured with 40 ppm NaNO<sub>2</sub> than that cured with 120 ppm nitrite. This result was expected since a highly significant correlation between residual nitrite prior to frying and NPYR values in the edible portion after frying was found and is in agreement with previous reports (Pensabene et al., 1979; Sebranek, 1979). A significant dose × nitrite level interaction was observed, which is consistent with the known destruction of nitrite by radiation and its resulting effect on NPYR reduction (Fiddler et al., 1981). Nitrite reduction is probably due to its reaction with the hydroxyl radical produced by the radiolysis of water (Simie, 1983). A highly significant correlation between dose level and NPYR was also found.

Statistical analysis of the NPYR bacon drippings data showed similar results to the edible bacon, but no dose × nitrite level interaction. This observation reflects the limited solubility of nitrite in the adipose tissue, which contains the NPYR precursor(s).

Both residual nitrite in the uncooked bacon and NPYR in the fried, edible bacon were significantly reduced by an adsorbed radiation dose of 3.0 Mrad from  $^{137}\text{Cs}$  compared to the nonirradiated control; the same trend has been observed with  $^{60}\text{Co}$  as the radiation source and an irradiation temperature of -40 °C (Fiddler et al., 1981). When 40 ppm added nitrite bacon was irradiated with  $^{60}\text{Co}$  at 3.0 Mrad, no residual nitrite was detected; this was not the case with  $^{137}\text{Cs}$ . Similarly, in bacon prepared with 120 ppm added nitrite and then irradiated with  $^{60}\text{Co}$  at an absorbed dose of 3.0 Mrad, the average residual nitrite in all experiments was less than 1 ppm, but the residual nitrite values were considerably higher (average 9 ppm) in bacon that had been irradiated with  $^{137}\text{Cs}$ . This was unexpected, since the higher radiation temperature for  $^{137}\text{Cs}$  (+3 vs. -40 °C) would be expected to facilitate nitrite destruction. This apparent difference in results could be due to the difference in  $\gamma$  energies between  $^{60}\text{Co}$  (1.17, 1.33 MeV) and

**Table III. Effect of  $^{137}\text{Cs}$  Radiation on Residual Nitrite and Nitrosopyrrolidine in Bacon Prepared with or without 550 ppm Ascorbate<sup>a</sup>**

dose, Mrad	sodium ascorbate added <sup>b</sup>			
	0 ppm		550 ppm	
	NaNO <sub>2</sub> , ppm	NPYR, <sup>c</sup> ppb	NaNO <sub>2</sub> , ppm	NPYR, <sup>c</sup> ppb
0	67	19.95	18	10.95
0.75	56	20.10	10	10.59
1.5	37	14.14	8	8.95
3.0	20	8.75	3	6.31

<sup>a</sup> Mean values,  $n = 6$ . <sup>b</sup> Plus 120 ppm sodium nitrite. <sup>c</sup> Corrected for recovery of the internal nitrosamine standard.

**Table IV. Analysis of Variance of the Nitrosopyrrolidine Values from the Ascorbate Experiment<sup>a</sup>**

source	deg of freedom	sum of squares	mean square	$F$ ratio <sup>b</sup>
belly (B)	5	2592.12	518.42	1010.82**
dose (D)	3	1018.27	339.42	36.57**
ascorbate (A)	1	1026.19	1026.19	13.58**
B × D	15	139.23	9.28	18.10**
B × A	5	377.84	75.57	147.34**
D × A	3	201.10	67.03	3.95*
B × D × A	15	254.64	16.98	33.10**
error	48	24.62	0.51	
total	95	5634.01		

<sup>a</sup> Reproducibility = (mean square error)<sup>1/2</sup> = 0.72 ppb. <sup>b</sup> Key: (\*)  $p < 0.05$ ; (\*\*)  $p < 0.01$ .

$^{137}\text{Cs}$  (0.66 MeV) or to the difference in dose rate (Jarrett, 1982). Thomas et al. (1981) reported a similar effect in which thiamin degradation decreased as the temperature of the  $^{137}\text{Cs}$  irradiation was reduced. Thiamin was selected for study since it is very irradiation sensitive. They also found that radiation of pork with electrons (high dose rate) leads to greater thiamin retention, i.e., less destruction, than  $\gamma$  radiation (low dose rate), suggesting that the energetics and the dose rate may also be important in other component destruction, including nitrite. The same trend was observed with the NPYR values as for the nitrite.

Bacon was prepared with 120 ppm NaNO<sub>2</sub>, with and without 550 ppm NaAsc and irradiated to 3.0 Mrad by use of the  $^{137}\text{Cs}$  source. Statistical analyses were performed on NPYR data, with mean values and statistical results shown in Tables III and IV, respectively. As expected, highly significant differences in NPYR values were found among the bellies. In addition, significant belly × dose, belly × ascorbate level, and belly × dose × ascorbate level interactions were observed. Highly significant differences in NPYR levels were also found among dose levels. As in the other experiment, LSD testing revealed no significant difference among 0-, 0.75-, and 1.5-Mrad doses, with 3.0 Mrad yielding significantly lower NPYR values than the other doses. NPYR values were significantly lower in the 550 ppm added ascorbate bacon than in the no-ascorbate bacon. Also, a significant dose × ascorbate level interaction was observed. This was similar to the previous  $^{60}\text{Co}$  results, which demonstrated that radiation and ascorbate combined give lower NPYR values than either treatment alone (Fiddler et al., 1981). This suggests that radiation is not

destroying or oxidizing the ascorbate to such an extent that it can no longer compete with the NPYR precursor for the nitrite. In the drippings, no significant difference was found between NPYR values at either ascorbate level, nor was a dose × ascorbate level interaction found. These results, as in the case of the NaNO<sub>2</sub> experiment, were probably due to the limited solubility of NaAsc in the adipose tissue. In addition, highly significant correlations were found between NPYR values and residual NaNO<sub>2</sub> and between NPYR values and dose levels.

In both the nitrite and ascorbate experiments, there was a reduction in NPYR levels at 3.0 Mrad. However, the average decrease was 46% compared to 63% when  $^{60}\text{Co}$  was used previously as the radiation source. Since the experiments were not carried out at the same time on matched pairs of pork bellies, this difference may not be significant. Therefore, a direct comparison between  $^{60}\text{Co}$  and  $^{137}\text{Cs}$  isotope sources is warranted.

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**Registry No.** *N*-Nitrosopyrrolidine, 930-55-2; ascorbate, 50-81-7; nitrite, 14797-65-0; cesium-137, 10045-97-3.

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