Table V. AFB_1 Uptake by Developing Seeds of Maize[a]

	ng/g fresh weight
pericarp (aleurone)	18.3
embryo	18.2
endosperm	0.7
total	37.2

[a] Ten microliters (0.5 μCi) of [^{14}C]AFB_1 (sp act., 180 mCi/mmol) was injected through a small incision in the stem subtending the developing ear 14 days following pollination. Thirty-three days later the seeds were dissected into pericarp, embryo, and endosperm. Data represent the total [^{14}C]AFB_1 recovered by chloroform extraction.

Thirty-three days later an equivalent of 18 ng of [^{14}C]-AFB_1/g fresh weight was recovered from the pericarp and embryo with only traces (0.7 ng) in the endosperm (Table V). A radiochromatographic scan with a Packard Model 385 scanner of the chloroform extract revealed a radioactive zone that cochromatographed with authentic AFB_1; however, positive identification by chemical derivatization was not made. The isolation of AFB_1 from the seed illustrates that aflatoxin can be recovered from intact grain in which there was no evidence of fungal contamination. Furthermore, the accumulation of [^{14}C]AFB_1 by the pericarp and embryo may explain why aflatoxin is frequently recovered in the gultin fraction following the wet milling process of grain.

The isolation of toxin from seedlings grown in Hoagland's solution adulterated with AFB_1 and the recovery of toxin from grain following the injection of AFB_1 into the internode subtending the developing ear raise the question whether these processes occur under field conditions. If they do, precautions should be exercised in the disposal of aflatoxin contaminated commodities back into the soil where subsequent crops are to be grown.

The results of this study indicate additional research is urgently needed to establish whether aflatoxin exists in field soils where it can be absorbed by roots. Research is also needed on the effect of different soil types and environment on the degradation of aflatoxin by microflora.

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Effect of Preprocessing Procedures for Green Bellies on *N*-Nitrosopyrrolidine Formation in Bacon

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The effect of pork belly storage on nitrosopyrrolidine (NPYR) formation in fried bacon was investigated. Bacon made from fresh bellies produced significantly less ($p < 0.05$) NPYR than that made from bellies that had been either stored for 1 week in a refrigerator or frozen for 3 months and then thawed prior to use. Bellies thawed in water produced less NPYR than bellies thawed in a refrigerator or at room temperature. A high correlation ($p < 0.01$) between residual nitrite and NPYR was also observed.

Recent research has focused on devising methods for the inhibition of nitrosamine formation in fried bacon, especially *N*-nitrosopyrrolidine (NPYR). Different approaches to solving the problem have been followed, including the determination of the precursor species (Gray, 1976; Nakamura et al., 1976; Gray and Collins, 1978; Gray et al., 1978; Cassens et al. 1979; Bharucha et al., 1979); determination of the variables affecting production of these compounds (Herring, 1973); determination of the cooking methods and the temperatures that reduce nitrosamine formation (Pensabene et al., 1974; Sen et al., 1976a; Mattson, 1978; Wasserman et al., 1978). Attention has also been given to reduction of levels of nitrite in the cure mixture (Sen et al., 1974; Havery et al., 1978) and the addition of water- and lipid-soluble reductants to the cure (Herring, 1973; Mottram et al., 1975; Hwang and Rosen, 1976; Sen et al., 1976b; Walters et al., 1976; Coleman, 1978; Fiddler et al., 1978). In the latter case, inhibition of ni-

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trosamine formation occurs because the reducing agents compete with amines for the nitrosating species, leaving less nitrite available for N-nitrosation. Of these factors, the processing and curing components of bacon manufacturing have received the most emphasis. The report to the Secretary of Agriculture by the Expert Panel on Nitrites, Nitrates, and Nitrosamines (*Fed. Regist.*, 1975) recommended omission of sodium nitrate from most cured meat products and, in the case of bacon, reduction of ingoing sodium nitrite levels from 156 to 120 ppm and the inclusion of 550 ppm of sodium ascorbate or erythorbate. The Food Safety and Quality Service, U.S. Department of Agriculture, recently permitted the use of acid-producing microorganisms, such as lactobacilli, in the processing of bacon (*Fed. Regist.*, 1979). This reduces the residual nitrite in bacon prior to frying, which should help prevent nitrosamine formation while controlling *Clostridium botulinum* outgrowth. A heretofore unexplored approach for reducing nitrosamines in fried bacon is the control of preprocessing conditions that occur between slaughter and the time of curing. Refrigeration and freezing represent the major preprocessing procedures to which green pork bellies are subjected. Information concerning the relationship between preprocessing cold storage and nitrosamine formation is meager and conflicting (Herring, 1973); yet a significant portion of the 1.5 billion pounds of bacon marketed in the United States annually is from bellies that undergo extended cold storage. In 1978, for example, up to 82 million pounds of pork bellies were held in public freezer warehouses every month (USDA, 1978). This report therefore describes the investigation of two methods of storage and three methods of thawing green pork bellies prior to being processed into bacon and their relation to nitrosamine formation.

EXPERIMENTAL SECTION

Skinned, matched pork bellies were purchased from a local supplier within 1 day of slaughter and cut into three sections: brisket, center, and flank. In the storage experiment, one section of the belly was stored in a freezer at −18 °C for 3 months and then thawed in a refrigerator at 1 °C for 1 week, another section was stored in a refrigerator at 1 °C for 1 week, and the third section was processed fresh, without further storage. In the thawing experiment, all the sections of the belly were stored in a freezer at −18 °C for 2 weeks and then one section was thawed in a refrigerator at 1 °C for 1 week, one section was thawed in the meat processing room at 13 °C for 24 h, and the third section was thawed in water at 13 °C for 6 h. Treatments were carried out in triplicate because of the variability in pork belly composition, and direct comparison of the effects of a treatment could be achieved only by subjecting the same section of paired bellies to the treatments under consideration.

All sections were pumped to approximately 10% of green weight to achieve ingoing target levels of 1.5% sodium chloride, 0.5% sugar, 0.3% sodium tripolyphosphate, 550 ppm of sodium ascorbate, and 120 ppm of sodium nitrite. The processing and frying conditions have been reported previously (Pensabene et al., 1979). The edible portions and drippings were analyzed separately.

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

Nitrosamine Analysis. (*a*) *Fried Bacon.* A modification of the procedure described by Fine et al. (1975a) was employed. A 25-g ground fried bacon sample, to which was added 1 mL of a CH_2Cl_2 solution containing 0.25 μg of *N*-nitrosomethylethylamine (NMEA) and 0.25 μg of *N*-nitrosohexamethylenimine (NHMI) as internal standards, was placed in a 500-mL, two-neck distillation flask equipped with a thermometer and a stopcock connecting tube. Fifty milliliters of mineral oil and 4 mL of 0.1 N NaOH were added, and the sample was distilled under vacuum (0.5 mm) until a temperature of 140 °C was achieved. The distillate was collected in a glass trap immersed in liquid nitrogen. The distillate was quantitatively transferred to a 250-mL separatory funnel with 5 mL of H_2O then 5 mL of CH_2Cl_2. After the addition of 4 mL of 0.1 N HCl, the distillate was extracted 3× with an equal volume of CH_2Cl_2. The combined extracts were dried by passage through anhydrous sodium sulfate and concentrated to 1.0 mL in a Kuderna-Danish apparatus. This procedure was checked for artifactual nitrosamine formation with sample blanks, and no nitrosamine was detected. The standards used to correct for *N*-nitrosodimethylamine (NDMA) and NPYR recovery were NMEA and NHMI, respectively, since these internal standards were found to be recovered at the same rate and had GLC retention times close to the nitrosamines of interest. The average recovery of the internal nitrosamine standards was 94% for NMEA and 89% for NHMI.

(*b*) *Drippings.* *N*-Nitrosamines in the fat drippings were isolated and separated by the method of White et al. (1974). The average recovery of the internal nitrosamine standards was 88% for NMEA and 82% for NHMI.

Determination. The concentrations of volatile nitrosamines were determined quantitatively by GLC–Thermal Energy Analyzer (TEA) under conditions similar to those described by Fine et al. (1975b) except that the cold trap for the TEA was immersed in a liquid nitrogen–ethanol slurry at −117 °C. The minimum detectability level was 0.5 ppb.

Confirmation. The nitrosamines in the drippings were confirmed by use of a Varian Aerograph Model 2700 gas chromatograph, equipped with a 6 ft × $^1/_4$ in. (o.d.) glass column packed with 15% Carbowax 20M-TPA, connected to a Varian MAT 311A mass spectrometer. The helium flow rate was 15 mL/min. The temperatures used were the following: detector, 200 °C; injector port, 200 °C; GLC–MS interface, 180 °C. The column was programmed from 90 to 130 °C at 4 °C/min for NDMA and from 165 to 200 °C at 4 °C/min for NPYR. The mass spectrometer was operated in the peak-matching mode adjusted to a resolution of 1 in 10000 or 12000. The mass spectra were obtained at an ionizing voltage of 70 eV and an ion source temperature of 150 °C. The mass/charge ratios for NDMA (74.04799) and for NPYR (100.06366) were determined by use of m/e 69.99857 and 99.99361 perfluorokerosene reference peaks, respectively, and by measuring the difference in m/e. The signal was recorded on both an oscilloscope and a recording oscillograph.

Nitrite. Residual sodium nitrite values were determined before frying by the Fiddler (1977) modification of the Griess-Saltzman procedure.

Statistical Analysis. Pairwise t tests of the matched samples (Snedecor and Cochran, 1974) were carried out to determine the significance of the treatment effects on NPYR formation. This method of analysis is appropriate for the matched-pair design of this experiment, since it eliminates the large variability in composition that occurs among the various sections within a pork belly (Pensabene et al., 1979).

RESULTS AND DISCUSSION

The concentrations of NPYR measured in the fried bacon samples obtained from pork bellies subjected to three different storage conditions are shown in Tables I–III. Only the data for NPYR are presented since this

Table I. Effect of Belly Storage (Fresh vs. Refrigerated) on NPYR Formation in Fried Bacon

matched belly treatment[a]	brisket			center			flank		
		NPYR, ppb[b]			NPYR, ppb[b]			NPYR, ppb[b]	
	RNIT[c]	edible	drippings	RNIT[c]	edible	drippings	RNIT[c]	edible	drippings
fresh	45	3	20	81	3	21	38	1	18
refrigerated	53	10	13	80	7	33	45	3	21
fresh	54	2	23	32	3	16	42	1	26
refrigerated	65	8	27	61	1	27	56	2	21
fresh	62	6	24	91	4	22	92	4	25
refrigerated	66	7	36	82	9	25	82	14	41
fresh	77	4	11	67	4	22	47	4	12
refrigerated	30	3	12	52	12	33	13	3	14
fresh	11	4	25	38	5	21	13	3	22
refrigerated	27	4	18	67	9	35	89	2	21
fresh	33	6	30	15	3	14	24	2	34
refrigerated	43	4	37	84	7	37	67	5	33

[a] 18 replications in 3 experiments (6/section). [b] Corrected for recovery of internal nitrosamine standard. [c] RNIT = residual nitrite, ppm.

Table II. Effect of Belly Storage (Fresh vs. Frozen-Thawed) on NPYR Formation in Fried Bacon

matched belly treatment[a]	brisket			center			flank		
		NPYR, ppb[b]			NPYR, ppb[b]			NPYR, ppb[b]	
	RNIT[c]	edible	drippings	RNIT[c]	edible	drippings	RNIT[c]	edible	drippings
fresh	44	1	24	43	5	26	47	2	25
frozen-thawed	33	5	27	45	19	32	50	13	28
fresh	31	ND[d]	23	53	8	35	60	2	23
frozen-thawed	46	2	22	43	19	33	45	8	23
fresh	114	7	28	81	5	22	66	4	20
frozen-thawed	39	10	45	38	5	30	72	5	34
fresh	44	3	8	76	3	16	73	13	28
frozen-thawed	29	3	25	67	6	34	22	8	32
fresh	11	1	26	30	5	31	33	3	25
frozen-thawed	11	1	36	43	5	33	44	7	33
fresh	27	3	22	55	10	34	14	3	21
frozen-thawed	28	1	43	70	8	50	28	1	27

[a] 18 replications in 3 experiments (6/section). [b] Corrected for recovery of internal nitrosamine standard. [c] RNIT = residual nitrite, ppm. [d] ND = none detected.

Table III. Effect of Belly Storage (Refrigerated vs. Frozen-Thawed) on NPYR Formation in Fried Bacon

matched belly treatment[a]	brisket			center			flank		
		NPYR, ppb[b]			NPYR, ppb[b]			NPYR, ppb[b]	
	RNIT[c]	edible	drippings	RNIT[c]	edible	drippings	RNIT[c]	edible	drippings
refrigerated	42	8	39	59	4	34	53	5	25
frozen-thawed	42	11	26	26	6	30	27	9	27
refrigerated	20	4	27	46	2	23	57	8	36
frozen-thawed	17	9	23	29	3	30	19	10	37
refrigerated	47	3	11	68	13	31	66	9	24
frozen-thawed	60	6	26	85	10	44	68	5	31
refrigerated	24	5	19	16	5	17	51	5	15
frozen-thawed	34	4	34	16	3	27	70	3	8
refrigerated	56	4	28	66	3	21	53	2	35
frozen-thawed	19	3	31	37	1	30	34	4	37
refrigerated	36	2	23	93	4	18	59	6	27
frozen-thawed	13	1	12	43	2	34	48	2	29

[a] 18 replications in 3 experiments (6/section). [b] Corrected for recovery of internal nitrosamine standard. [c] RNIT = residual nitrite, ppm.

is the nitrosamine of major interest in bacon. The Food Safety and Quality Service, U.S. Department of Agriculture, has set a 10-ppb violative level for this nitrosamine. Table IV contains the statistically treated summary of the information in Tables I–III. When data from fried bacon derived from refrigerated bellies were compared to data from fresh bellies, significant increases were observed in the NPYR concentrations of the fried edible portion (3.7 ppb) and drippings (5.4 ppb). Freezing and then thawing the bellies also induced significantly more NPYR formation in the fried edible portion (2.7 ppb) and drippings (8.3 ppb) than did processing with fresh bellies. When the

Table IV. Average Differences[a] in NPYR for Treatment Comparisons in the Storage Study

treatment comparison	ppb edible	ppb drippings
refrigerated minus fresh	3.7 ± 1.0	5.4 ± 2.0
frozen-thawed minus fresh	2.7 ± 1.2	8.3 ± 1.7
frozen-thawed minus refrigerated	0.0 ± 0.7[b]	4.1 ± 2.0

[a] Average values from 18 matched pairs plus or minus standard error. [b] All treatment comparisons without the superscript are significantly ($p < 0.05$) nonzero by a paired t test where t = (average)/(standard error).

freeze–thaw and the refrigerated methods were compared directly, there was no difference in the NPYR concentration of the edible fraction, but a statistically significant increase occurred in the concentration of NPYR in the drippings (4.1 ppb) from the freeze–thaw method.

In the same storage experiments, the only significant increase in NDMA was found in the fried edible portion obtained from bellies that had been frozen and then thawed. No other statistically significant observation was made for the other measures of NDMA.

Storage of pork bellies, therefore, has a definite effect on NPYR formation in bacon. This may result from the increase in both amines and amino acids that occurs during extended storage (Bowers, 1969; Patterson and Mottram, 1974; Patterson and Edwards, 1975). Lakritz et al. (1976) have shown that free proline, a possible precursor of NPYR, increased in the intact and lean tissue of green pork bellies by approximately 50% after refrigerated storage for 1 week. In the adipose tissue, free proline increased 96% compared to that in the fresh control sample. Freezing has an even greater effect on the green bellies, since there is more tissue destruction due to ice crystal formation and salt solubilization of proteins (Luyet, 1959; Callow, 1955). This could lead to even higher levels of the nitrosamine precursors.

Since a significant increase in NPYR was observed when frozen sections of bellies were used, additional studies on the effect of various thawing methods were carried out. The results of these experiments are shown in Table V. These data are statistically treated in Table VI. There was no effect on NPYR concentration in the fried edible portion of the bacon with any of the three thawing treatments. A statistically significant increase in the drippings was observed due to either thawing 24 h at a room temperature of 12 °C (drippings, 5.2 ppb) or thawing in a refrigerator at 1 °C for 1 week (drippings, 9.5 ppb) compared to thawing in water at 13 °C for 6 h. There was no statistical difference in any NPYR measurement when the refrigerated thawing method was compared to the room temperature thawing method.

As in the storage study, NDMA was observed in the samples obtained from the different treatments. However, the only significant increase was observed in the drippings in the refrigeration thawed samples compared to those in the water thawed samples.

In a recent report (Pensabene et al., 1979) we found a significant correlation between residual nitrite (RNIT) and NDMA and NPYR content in the fried edible portion of the bacon. Therefore, the data from all the experiments in this study were analyzed to determine whether the same correlation existed. In the storage experiments, RNIT was significantly correlated ($p < 0.01$, $r = 0.24$, $N = 108$; where r is the correlation value and N is the number of data points) with NPYR in the fried edible bacon, but no significant correlation at the $p < 0.05$ level was found with NDMA in the fried edible bacon. In the thawing study RNIT was significantly correlated with both NPYR ($p < 0.01$, $r = 0.40$, $N = 54$) and NDMA ($p < 0.01$, $r = 0.47$, $N = 54$) in the fried edible bacon.

In conclusion, our data show that bacon made from fresh bellies produces less NPYR than bacon made from refrigerated or frozen and then thawed bellies. If frozen bellies have to be employed, thawing in water at 13 °C is recommended. Clearly, control over residual nitrite is a prime factor in controlling the nitrosamine content in the fried bacon, since a statistically significant correlation has been observed in a number of different experiments.

Table V. Effect of Thawing on NPYR Formation in Fried Bacon

matched belly treatment[a]	brisket RNIT[c]	brisket NPYR, ppb[b] edible	brisket NPYR, ppb[b] drippings	center RNIT[c]	center NPYR, ppb[b] edible	center NPYR, ppb[b] drippings	flank RNIT[c]	flank NPYR, ppb[b] edible	flank NPYR, ppb[b] drippings
water	4	1	21	40	5	30	38	2	35
room temperature	2	2	27	40	4	31	39	2	37
water	18	1	17	94	3	33	58	2	20
room temperature	45	2	24	64	13	37	34	2	33
water	12	2	8	62	8	18	54	5	22
room temperature	22	3	16	38	5	22	46	5	24
water	30	2		34	1	26	64	2	25
refrigerated	20	4		26	3	39	38	5	34
water	22	3	19	39	2	18	68	3	23
refrigerated	31	2	30	33	1	23	35	3	27
water	37	5	23	42	3	12	46	4	11
refrigerated	52	5	33	50	4	21	38	4	26
room temperature	24	5	32	26	3	34	27	1	25
refrigerated	58	4	31	30	2	39	22	3	32
room temperature	42	6	33	58	3	20	33	1	17
refrigerated	35	7	29	41	4	23	26	2	17
room temperature	52	4	24	58	4	27	22	4	22
refrigerated	34	6	20	66	8	36	20	4	18

[a] 9 replications in 3 experiments (3/section). [b] Corrected for recovery of internal nitrosamine standard. [c] RNIT = residual nitrite, ppm.

Table VI. Average Differences[a] in NPYR for Treatment Comparisons in the Thawing Study

treatment comparison	ppb edible	ppb drippings
room temperature minus water thawed	1.0 ± 1.2	5.2 ± 1.3[c]
refrigerated minus water thawed[b]	0.7 ± 0.5	9.5 ± 1.3[c]
refrigerated minus room temperature	1.0 ± 0.5	1.2 ± 1.7

[a] Average values from nine matched pairs plus or minus standard error. [b] Eight matched pairs. [c] t = (average)/(standard error) gives a value which is significantly ($p < 0.05$) nonzero.

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