

Comparison of Three Methods for Determination of *N*-Nitrosopyrrolidine in Fried Dry-Cured Bacon

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The recently developed Eastern Regional Research Center (ERRC) dry column chromatographic procedure for determining *N*-nitrosopyrrolidine (NPYR) in fried cure-pumped bacon was evaluated for its applicability to fried dry-cured bacon. The method was then compared with 2 established procedures for volatile nitrosamine analysis in cured meat products: the multidetection thermal energy analyzer (MD) method and the mineral oil distillation (MOD) screening procedure. No significant difference ($P < 0.05$) in NPYR values was found between the ERRC and MD procedures, but significant differences were found between the ERRC and MOD procedures and between the MOD and MD procedures. No artifactual nitrosamine formation was found in the ERRC procedure, but significant amounts were found in samples analyzed by the MOD procedure. The ERRC method was demonstrated to be rugged and very rapid. It is proposed that the ERRC method replace the MOD method as the official screening procedure for NPYR in fried bacon.

In 1981, approximately 20 million pounds of dry-cured bacon was manufactured in the United States, mostly in the South (C. S. Custer, USDA, Food Safety and Inspection Service, Processed Products Inspection Division, personal communication). Dry-cured bacon is produced by the direct application of the curing ingredients to the pork belly, which results in slow diffusion of the curing salts into the meat. This produces bacon that is lower in water activity, more variable in in-going nitrite levels, higher in salt concentration, and with greater shelf-life stability than cure-pumped bacon. In addition, the increased curing time yields greater chemical degradation of the product, particularly the lipids, which imparts a distinctive flavor to dry-cured bacon. Many people consider this flavor highly desirable. The difference in curing may influence nitrosamine formation and may also interfere with current nitrosamine analytical techniques.

Recently, attention has been focused on nitrosamine formation in fried dry-cured ham and bacon as a result of surveys of these products for *N*-nitrosopyrrolidine (NPYR) (1, 2), the nitrosamine found consistently in fried cure-pumped bacon. Thirty-eight of 55 bacon samples and 21 of 124 ham and shoulder samples surveyed had NPYR values over the 10 ppb nitrosamine violation level established for cure-pumped bacon in 1978 by the Food Safety and Inspection Service (FSIS)

(3); several samples had exceptionally high levels, up to 320 ppb (2). These surveys have prompted FSIS to establish a monitoring system designed to collect a data base for dry-cured bacon. This will enable FSIS to determine the extent of nitrosamine occurrence in this product and evaluate the possibility that current regulations on cure-pumped bacon may be extended to dry-cured bacon. The FSIS survey results (1) also prompted research to develop methods to inhibit nitrosamine formation in fried dry-cured bacon. One such method was recently reported by Reddy et al. (4).

The analytical methods most commonly used for monitoring volatile nitrosamines in fried bacon are the multidetection (5) and the mineral oil distillation (6, 7) procedures. However, only products with apparent nitrosamines above the violation level are subjected to the lengthy multidetection method for subsequent mass spectral confirmation. The mineral oil procedure developed by Fine et al. (6) is used to screen all samples for possible violative levels, but this procedure has 2 disadvantages: possible artifactual formation and a lengthy analysis time for a screening procedure. A new rapid method for screening cure-pumped bacon, which reduces the possibility of artifactual nitrosamine formation, was developed by Pensabene et al. (8, 9) at the USDA, Eastern Regional Research Center (ERRC). The applicability of the ERRC dry-column method for screening dry-cured bacon was evaluated by comparative analysis with the 2 currently used procedures, the mineral oil screening and the more elaborate multidetection-thermal energy analyzer (TEA) procedure. Results of the 3-way comparison and their treatment are reported here.

METHODS

Note: Nitrosamines are potential carcinogens, therefore, care should be exercised in handling these materials.

Reagents and Apparatus

Reagents and apparatus used were previously described (5, 7, 8), except for the following:

- (a) *N*-Nitrosoazetidine (NAZET) internal standard.—0.10 μg NAZET/mL dichloromethane (DCM).
- (b) *N*-Nitrosodipropylamine (NDPA) internal standard.—0.25 μg NDPA/mL DCM.
- (c) *N*-Nitrosopyrrolidine (NPYR) standard.—0.25 μg NPYR/mL DCM.

(d) *Dry-cured bacon*.—Random samples obtained from local producers or FSIS, and fried according to protocol set forth in FSIS survey (1).

(e) *Other reagents*.—From local suppliers. Used without further purification.

Procedures

Sodium nitrite was determined as described in 24.041–24.042 (10).

In all procedures, nitrosamines were quantitated by using a gas chromatograph interfaced to a thermal energy analyzer set to parameters specified in the ERRC procedure (8), except the GC temperature was programmed from 140 to 220°C at 4°/min.

The ERRC procedure was described in detail by Pensabene et al. (8). A flow diagram of this method is shown in Figure 1.

The multidetection method described by Fazio et al. (5) was used, with modification. NPYR was quantitated by GC-TEA prior to the column chromatography step, which was omitted.

The mineral oil distillation procedure for *N*-nitrosamines in fried bacon was used as described in 24.CO1–24.CO7 (7).

To check for artifactual nitrosamine formation, a reagent blank was performed for each method; no nitrosamines were detected.

NAZET was used as an internal standard in the ERRC procedure because NDPA is not recovered with this method. It was found to be highly correlated to NPYR (8). NAZET is decomposed by distillation techniques, so NDPA was used in the other 2 procedures. NDPA was selected because it is listed as the internal standard in the AOAC official method (7).

Statistical Analysis

Nitrosopyrrolidine results were corrected (normalized) for recovery of the specific internal standard. Analysis of variance (ANOVA) and least significant difference testing were performed on the measured nitrosamine according to the

methods of Snedecor and Cochran (11). The ruggedness test was patterned after the AOAC procedure for ruggedness evaluation (12). Where only a statistical summary is presented, the more extensive raw data are available on request.

Results and Discussion

A ruggedness test of the ERRC procedure was performed on dry-cured bacon containing 8.4 ppb NPYR. Extreme deviations in the normal grinding, packing, and solvent elution steps were employed and most variations did not result in significant differences in NPYR determinations. However, when columns were allowed to run dry for 15 min before dichloromethane elution, NPYR values varied 15% from those obtained when columns were run normally.

Sodium nitrite was determined in all samples of dry-cured bacon before frying. After frying, 16 dry-cured bacon samples were analyzed in duplicate for NPYR by each of 3 methods: mineral oil distillation (MOD), multidetection (MD), and the ERRC dry column procedure that is designated "ERRC." The results averaged over 2 determinations are shown in Table 1. Individual NPYR values ranged from 0.62 to 12.78 ppb for MOD, none detected (ND) to 9.62 ppb for the MD method, and ND to 13.21 ppb for the ERRC procedure. The minimum detectable level for MOD and MD was 0.2 ppb and for ERRC 0.5 ppb. This is because MOD and MD methods use a 25 g sample and the ERRC method uses only 10 g. Mean recoveries of the internal standards were 93.7, 94.4, and 97.9% for the MOD, MD, and ERRC methods, respectively.

Contrary to reports of correlations in cure-pumped bacon (13), no significant correlation ($P < 0.05$) was found between residual nitrite and NPYR values ($r = 0.354, 0.285, \text{ and } 0.496$ for the MOD, MD, and ERRC methods, respectively). The number of variables involved and the heterogeneous nature of the dry-cured bacon may preclude any simple linear correlations.

Repeatability (within-laboratory variation) of NPYR determinations obtained from a 1-way analysis of variance (Table 2) is 0.62 ppb for the MOD and MD methods and 0.43 ppb for the ERRC method. The repeatability of the internal standard recoveries was 6.5, 6.2, and 5.4% for the MOD, MD, and ERRC procedures, respectively.

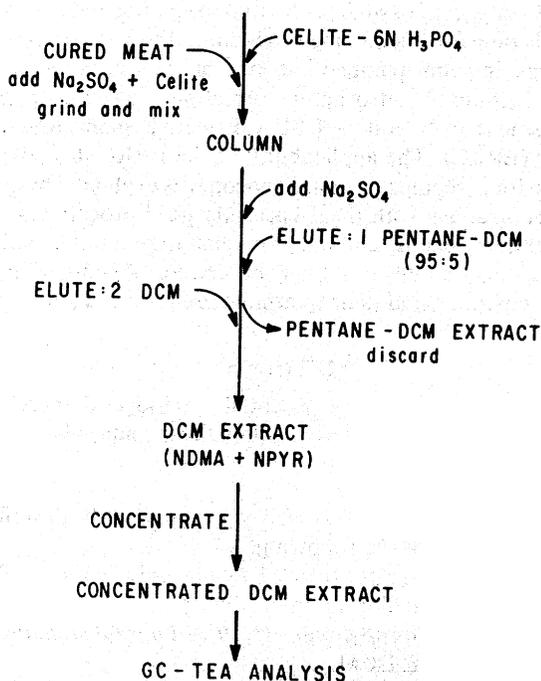


Figure 1. Diagram of ERRC dry column method for determining *N*-nitrosopyrrolidine in fried bacon.

Table 1. Determination of *N*-nitrosopyrrolidine in fried dry-cured bacon by 3 methods^a

NaNO ₂ , ppm	Mineral oil		Multidetection		ERRC	
	NPYR, ppb	NDPA, % rec.	NPYR, ppb	NDPA, % rec.	NPYR, ppb	NAZET, % rec.
ND ^b	2.85	72.9	3.76	87.5	1.10	100.4
8	1.33	104	ND	103.7	ND	85.7
8	4.84	95.2	4.62	94.6	3.79	107.3
10	1.78	75.6	1.37	98.1	1.64	94.1
14	2.15	103.3	1.45	88.8	1.84	97.3
24	0.67	101.6	ND	90.3	ND	97.8
24	9.69	87.2	9.53	89.7	6.32	91.2
28	4.25	96.6	3.26	107.5	3.10	103.6
38	3.73	95.0	4.03	86.8	3.41	96.6
40	6.15	88.8	5.40	83.6	4.38	94.1
40	10.28	82.3	6.97	90.7	6.86	96.6
46	10.94	93.6	7.27	101.0	5.77	112.2
76	3.28	98.8	2.52	98.9	3.36	88.2
90	6.21	107.5	5.23	94.3	4.88	113.9
— ^c	9.44	100.2	5.96	100.5	8.44	95.5
—	12.35	96.7	8.71	95.0	12.50	92.5
Mean	5.62	93.7	4.38	94.4	4.21	97.9

^aResults are averages of duplicate determinations.

^bND = none detected.

^cNitrite analysis not performed.

Table 2. Statistical analysis for repeatability of 3 methods for determining N-nitrosopyrrolidine in fried dry-cured bacon

Source	DF	Mineral oil			Multidetetection			ERRC		
		SS	MS	F	SS	MS	F	SS	MS	F
ANOVA of Corrected NPYR Values										
NPYR	15	431.92	28.79	75.89**	251.77	16.78	43.48**	322.37	21.49	116.72**
Error	16	6.07	0.379	—	6.18	0.386	—	2.95	0.184	—
Total	31	437.99	—	—	257.94	—	—	325.31	—	—
Repeatability ^a (ppb)			0.62			0.62			0.43	
ANOVA of Internal Standard Recovery Values										
Internal std	15	1997.4	199.8	4.65**	1356.0	90.4	2.32	1888.2	124.9	4.39**
Error	16	686.9	42.9	—	623.0	38.9	—	458.6	28.7	—
Total	31	3684.2	—	—	1979.0	—	—	2346.8	—	—
Repeatability (%)			6.5			6.2			5.4	

^aRepeatability = \sqrt{MS} error.

**P < 0.01.

Two-way ANOVA of the NPYR values, assuming random samples, is shown in Table 3. Differences among samples are highly significant ($F = 196.90$, $P < 0.01$) as is to be expected from random sampling of different manufacturers. Differences among methods ($F = 7.98$, $P < 0.01$) and interactions between samples and methods ($F = 7.50$, $P < 0.01$) were also highly significant, possibly due to artifactual nitrosamine formation.

Further investigation into the differences between procedures, using the least significant difference (LSD) between NPYR means, yielded significant differences ($>LSD = 0.786$ ppb, $P < 0.05$) between the MOD, MD pair and between the MOD, ERRC pair. No significant difference was found between the MD, ERRC pair.

NPYR values tend to be higher with the mineral oil distillation method than with either of the other 2 methods. This may be due to artifactual formation of NPYR during analyses. To determine whether NPYR would form artifactually in the ERRC procedure, Pensabene et al. (8, 9) added up to 100 ppm sodium nitrite to fried nitrate-free bacon and up to 10 ppm morpholine, an easily nitrosatable amine, to fried nitrite-free and nitrite-cure-pumped bacon. Neither NPYR nor N-nitrosomorpholine (NMOR) were detected in any of the samples. In more recent studies, sodium nitrite, sodium nitrate, sodium chloride, and morpholine were added to fried nitrite-free bacon in various combinations; nitrosamines were not detected except in those samples in which sodium nitrite and morpholine were added simultaneously prior to analysis. (J. W. Pensabene, USDA, ARS, ERRC, 1982, unpublished data).

A further study of artifactual NPYR formation was conducted using fried dry-cured bacon with normally incurred levels of sodium nitrite and sodium nitrate. Adding 10 ppm morpholine to the pentane wash solvent of the ERRC procedure resulted in no detectable NMOR in the samples. When morpholine was added directly to the meat, rapid nitrosation occurred. Morpholine added to the solvent simulates an envi-

ronment rich in amines available for nitrosation on the column. No NMOR was produced from solvent-added morpholine, indicating little chance of artifactual nitrosamine formation. Mineral oil distillation was performed on the identical samples as above to which the same level of morpholine was added to the mineral oil. High amounts of NMOR, from 10 to 24 ppb, were found in the mineral oil distillation extracts, suggesting that artifactual formation was probable in samples containing residual nitrite after cooking. Artifactual nitrosamine formation has been observed by several laboratories using the MOD method. As a result, a few laboratories add ascorbate and α -tocopherol inhibitors. While this is effective for most cured meat products, where relatively high levels of nitrite may be present, this is not the case for all samples of fried dry-cured bacon. FSIS has recently found evidence of artifactual NPYR formation in 8 of 37 samples tested (A. J. Malanoski, USDA, FSIS, personal communication).

In conclusion, the ERRC dry column method is particularly applicable to screening dry-cured bacon for NPYR. Using the ERRC procedure, which requires a minimal amount of equipment and solvents, a single analyst in our laboratory has analyzed 18 samples in a single work day, limited only by the sample input rate of the GC-TEA system. Compared with the MOD procedure, the more rapid ERRC method is much less susceptible to artifactual nitrosamine formation, which can occur in fried dry-cured bacon with high residual nitrite levels. In addition, the ERRC method is quantitatively comparable to the lengthy multidetection procedure. It is proposed that the ERRC method replace the MOD method as the screening procedure for NPYR in both fried cure-pumped and dry-cured bacon.

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Table 3. Analysis of variance among 3 methods for determining N-nitrosopyrrolidine in fried dry-cured bacon

Source	DF	SS	MS	F
Method	2	37.86	18.93	7.98**
Samples	15	934.87	62.32	196.90**
Interaction	30	71.18	2.37	7.50**
Error	48	15.19	0.317	
Total	95	1059.10		

**P < 0.01.

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