

Comparison of Chemiluminescent and AOAC Methods for Determining Nitrite in Commercial Cured Meat Products

WALTER FIDDLER, ROBERT C. DOERR, ROBERT A. GATES, and JAY B. FOX, JR
U.S. Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center, Philadelphia, PA 19118

A chemiluminescent detector was used to measure nitrite in a variety of commercial cured meat products, using the AOAC sample preparation procedure, 24.041. A comparison of the NaNO_2 values obtained by using sulfanilamide/*N*-(1-naphthyl)ethylenediamine (SAN/NED) reagent, sulfanilamide/1-naphthylamine reagent, and chemiluminescent detection (CLD) revealed no significant differences between the latter 2 detection methods. The AOAC SAN/NED reagent combination gave an average of 24.6% lower NaNO_2 results than CLD. Examination of the sample preparation extraction and heating steps indicated that the procedure could not be made more rapid because of the need to destroy residual reductants and release "bound-complexed-reacted" nitrite from the meat samples.

Although other investigators have determined nitrite with chemiluminescent detection (CLD) (1, 2), this type of detection method has not been widely applied to food products. Chemiluminescence has been used to measure nitrite in freeze-dried cod fish (3) and in nonfat dried milk powder extracts (4), and was compared with other nitrite methods in cured meat slurries (5, 6). However, a thermal energy analyzer chemiluminescent detector, normally used for determining nitrosamines, was used in these latter investigations. The primary objective of the current study was to evaluate the accuracy of a moderately priced nitric oxide-ozone chemiluminescent detector for determining nitrite in a variety of comminuted and noncomminuted commercial cured meat products by comparative analysis with AOAC method 24.041 (7). Another objective was to make nitrite detection methods, including CLD, more rapid by simplifying the sample preparation step of the current procedure. The results are reported herein.

Experimental

Sample Preparation

Cured meat products used for this study were obtained from local retail stores. A thoroughly ground sample, 5.0 g, and ca 50 mL hot water (80°C) were added to a 250 mL Virtis homogenizer flask and blended at medium speed for 5 min. This and the ensuing steps of AOAC method 24.041 (7) and its modifications are shown in Figure 1. For the experiments designed to simplify sample preparation, either hot or cold (room temperature) water extraction, and either heating on a

steam bath for 2 h or no heating treatments, were used and are noted with an asterisk in Figure 1.

Colorimetric Analysis

Two 8 mL aliquots of sample filtrate were added to different 10 mL volumetric flasks. Two different colorimetric reagent sets were added, one to each flask: (1) 1 mL premixed 1.0mM sulfanilamide (SAN)/0.2mM 1-naphthylamine (1-NA) in 15% acetic acid; (2) 0.5 mL 2mM sulfanilamide, allowing the solution to stand 5 min, then adding 0.5 mL 0.4mM *N*-(1-naphthyl)ethylenediamine (NED), both in 15% acetic acid. This is subsequently designated as the AOAC Griess reagent combination (SAN/NED). The above solutions were diluted to volume and allowed to stand 15 min for color development, then read at 525 nm in a Cary 14 spectrophotometer. The results were compared with results for freshly prepared NaNO_2 standard solutions.

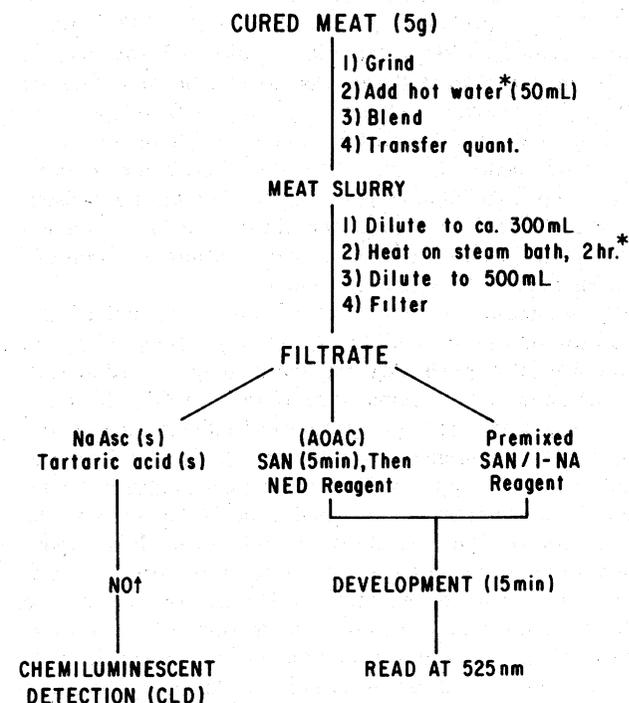


Figure 1. Schematic diagram for sample preparation and detection methods for determination of nitrite in cured meat products.

Received April 8, 1983. Accepted September 2, 1983.

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.

Chemiluminescent Analysis

Twenty mL nitrite-containing filtrate was injected by syringe into a 100 mL 3-neck flask containing ca 170 mg each of sodium ascorbate (NaAsc) and tartaric acid and a few drops of Dow Corning silicone defoamer. The nitric oxide generated from the stirred solution was expanded into an evacuated 8 mL internal volume sample tube made of Teflon tubing. The details of the procedure and equipment have been reported elsewhere (5), except an Antek Model 720 digital nitrogen detector and a vacuum pump, operated at 0.5 mm Hg, were connected to the system through the 6-port minivalve. The oxygen flow to the detector was 20 mL/min, and the optimum helium carrier flow rate was 560 mL/min. The concentration of nitric oxide was determined, in triplicate, by comparing the digital read-out numbers with those on a curve obtained from standard NaNO₂ solutions that were analyzed daily.

Statistical Analysis

Statistical analysis was carried out according to the methods of Snedecor and Cochran (8).

Results and Discussion

Chemiluminescence Characteristics

The NaNO₂ response of the chemiluminescent detector was linear ($r > 0.999$) over the range 5–10 000 ppb, equivalent to 0.5–1000 ppm with respect to meat, using 20 mL sample filtrate from a 1/100 dilution.

For the recovery experiments, 4 different types of cured meat products were spiked at 5 and 80 ppm NaNO₂. The appropriate fortification level was added to the ground meat sample and mixed before extraction. The representative product types were bacon, a high fat content product; ham, a high lean content noncomminuted product; frankfurter, a comminuted emulsified product; and Lebanon bologna, a comminuted fermented product. The product pH values were 5.4, 5.6, 5.6, and 4.7, respectively. The mean recovery values for the 5 and 80 ppm fortified samples were $96.3 \pm 11.8\%$ and $97.8 \pm 5.3\%$, respectively, for CLD and $91.7 \pm 9.8\%$ and $92.8 \pm 7.1\%$, respectively, for the AOAC (SAN/NED) method. Statistical analysis of the individual results by 2-way analysis of variance indicated there was no significant difference between the recoveries obtained by using CLD and the SAN/NED methods at either fortification level. There was, however, a significant difference ($P < 0.05$) between recoveries for samples fortified at 80 ppm NaNO₂ that was not present for the 5 ppm samples. This difference may be due to sample-method interactions probably caused by different amounts of ascorbate/erythorbate in each sample.

The minimum detection level for NaNO₂, assuming a 2:1 signal-to-noise ratio, was 40 ng/20 mL (equivalent to 2 ppb in solution and 0.2 ppm in meat with the standard 1/100 dilution). This detection level compared favorably with the detection limit (25 ng/10 mL) previously reported for the thermal energy analyzer chemiluminescent detector (5). From a practical point of view, 0.5 ppm NaNO₂ in the meat sample was considered the minimum level of reliable measurement, whereas the detection limit for the AOAC colorimetric method is about 1 ppm in meat, using the same 5:1 signal-to-noise ratio criterion. The minimum detectable level for CLD can be lowered either by using a larger volume sample loop or by increasing the size of the sample filtrate aliquot, so that more nitric oxide is available for detection. Alternatively, degassing the entire sample of nitric oxide as described by others is feasible to lower the detection level (1–3). The detection level cannot be lowered by taking a larger aliquot for the colorimetric

analysis because the NaNO₂ is measured in nondiluted filtrate and the filtrate concentration is not changed; therefore, no additional pigment is formed. The minimum detectable level for both the colorimetric method and CLD may be lowered by increasing the initial sample size, depending on the amount of ascorbate/erythorbate present in these types of samples, which can cause significant loss of nitrite. The 1/100 dilution in the AOAC sample preparation method minimizes this effect (9).

For the determination of CLD instrument repeatability, 3 levels of NaNO₂ standards (70, 350, and 700 ppb in the sample solution) were each determined 16 times over several weeks; the results are shown in Table 1. Analysis of variance of results obtained by duplicate analyses of 25 assorted cured meat products yielded an overall repeatability and coefficient of variation for the entire procedure, including sample preparation and detection. Because the variances were not homogeneous over the range of sample determinations, the samples were split into 3 ranges of NaNO₂ designated low, medium, and high, then re-analyzed statistically. The results in Table 1 indicate that for cured meats containing low (<10 ppm) and high (>40 ppm) NaNO₂, the CV values for repeatability were 2.4 and 2.3%, respectively. For the samples in the medium, 10–40 ppm NaNO₂, range, the CV value was 3.3%. The precision of the method was superior to that reported previously with the thermal energy analyzer, which gave a CV value of 6.5% for 8 samples of cured meats containing varying amounts of nitrite (5).

Since consistent NaNO₂ results have been obtained with the premixed SAN/1-NA reagent combination (6), we decided to use this system in addition to the AOAC SAN/NED reagent. The SAN/NED system is susceptible to erroneous results from residual ascorbate during the 5 min incubation with SAN before addition of NED (10). The final chemiluminescent measurement is not subject to reductant interference because sodium ascorbate is used as a reactant to stoichiometrically generate the nitric oxide that is detected. However, both detection methods measure reduced nitrite levels as a result of reductant reaction during the heating step of the sample preparation.

Twenty-one assorted cured meat samples were analyzed for NaNO₂ by using these 3 methods. The raw data are shown in Table 2. The overall mean values for CLD, and the SAN/NED and SAN/1-NA methods were 19.3, 14.6, and 18.1 ppm NaNO₂, respectively. The results of a 2-way analysis of variance are shown in Table 3. A highly significant ($P < 0.01$) difference was found among the samples, as expected, because a wide range of sample types were analyzed. Differences among methods were also highly significant, and further investigation was required to isolate the differences using individual contrasts between pairs of methods. Only the CLD-SAN/1-NA pair demonstrated a nonsignificant difference. The AOAC method gave an average of 24.6% lower NaNO₂

Table 1. Chemiluminescence repeatability

	NaNO ₂ , ppb	SD, ppb	CV, %	
Instrument (Std solns)	70	5.1	7.3	
	350	9.1	2.6	
	700	21.6	3.1	
	NaNO ₂ , ppm	n	SD, ppm	CV, %
Method overall (Cured meats) ^a	<10	10	0.17	2.4
	10–40	11	0.49	3.3
	>40	4	1.33	2.3

^appm NaNO₂ (meat) = ppb (std soln) × 100 (diln factor) × 10⁻³ ppm/ppb.

Table 2. Sodium nitrite in commercial cured meat products determined by 3 detection methods

Product	Sodium nitrite, ppm		
	CLD	(AOAC) SAN/NED	SAN/1-NA
Bacon (regular)	55.8, 13.1, 11.3	44.2, 10.1, 10.2	54.9, 9.2, 11.9
Beef strips	6.6, 4.5	3.6, 0.5	5.9, 1.5
Pork strips (formed)	9.8	6.3	8.5
Ham	4.5, 8.7, 70.4	1.4, 6.8, 52.1	2.6, 7.9, 63.9
Corned beef	15.0	11.2	14.3
Frankfurter	60.5, 21.7	49.2, 19.2	57.4, 22.2
Chicken frankfurter	10.9, 42.4	7.0, 34.0	8.7, 41.3
Smokey link	17.1	11.9	16.1
Hard salami	5.2, 7.7	3.4, 5.2	5.1, 6.5
Pepperoni	8.3	5.9	7.9
Lebanon bologna	11.7, 9.5, 11.1	7.6, 6.7, 9.3	14.5, 7.2, 12.1

Table 3. Statistical results (2-way analysis of variance)

Source	df	SS	Mean square	F-ratio
Samples	20	19889.2	994.5	178.5**
Methods	2	256.1	128.0	23.0**
Error	40	222.9	5.6	
Total	62	20368.2		

(Individual contrasts)

Source	Ratio
CLD-(AOAC)SAN/NED	42.7**
CLD-SAN/1-NA	2.9
SAN/NED-SAN/1-NA	23.3**

**P<0.01.

values than CLD. Eight additional samples were analyzed only by CLD and SAN/1-NA. A paired *t*-test also indicated no significant difference (*df* = 7, *t* = -1.37).

The dilution and heat treatment steps in the AOAC sample preparation procedure are thought to be essential to help clarify the solution for colorimetric analysis and to maximize measurable nitrite by reducing interferences caused by endogenous or added reductants (10). While many analysts use variations of these treatments and other reagents, including clarification agents, it is questionable whether all are necessary (6). Obviously, simplification of the entire procedure is desirable regardless of the detection method. We were particularly interested in devising a simple sample preparation for CLD, so we studied the effect on NaNO₂ quantitation of combinations of sample extractions by using cold (room temperature) or hot (80°C) water, and heating the sample on a steam bath for 2 h vs no heating. Cold water was used so as not to expose some samples to any heat treatment. The nitrite results obtained from bacon, ham, frankfurter, and Lebanon bologna are presented in Table 4 and the statistical results are shown in Table 5. Paired 2-tailed *t*-tests indicated that all the comparisons were significant at the *P*<0.01 level. Hot water extraction yielded a higher overall mean before heating, with a difference (*d*) of 1.21 ppm NaNO₂ compared

with the cold extraction. The range of before and after heating differences for the hot/cold water extraction was 0.4–16.3 ppm NaNO₂. After heating, CLD and the SAN/1-NA reagent combination gave a mean difference of approximately 4 and 5 ppm higher than before heating, respectively, and the difference between the hot and cold extractions disappeared. After heating, the AOAC SAN/NED combination mean NaNO₂ level was significantly lower than those obtained by the other 2 methods, indicating the ascorbate or other interferences were not completely removed by the heating step. If the ascorbate concentration is variable in different products, then the difference in NaNO₂ values will be more variable with the AOAC detection system. Despite this, we tentatively conclude that the precision of the nitrite measurement and the accuracy are improved by the heating step. Of more significance is that heating on the steam bath destroys the reductants, probably destroys some nitrite, but also liberates more overall nitrite as evidenced by the higher NaNO₂ values after heating. While many refer to this as “bound” nitrite, particularly that obtained from heating the extract in the presence of heavy metal ions, we wish to coin the term “bound-complexed-reacted” or BCR nitrite to more accurately designate the nitrite not readily available, except possibly through exhaustive extraction. Bound implies a loose type of bonding. The C and R portions of BCR include that nitrite which is complexed, as in nitrosylmetmyoglobin, and reacted nitrite as represented by nitrosothiols in free amino acids, peptides, and proteins or in other, unknown species. Paired *t*-tests showed no significant difference between CLD and SAN/1-NA determinations either before (*df* = 7, *d* = 0.93 ppm, *t* = 0.922) or after (*df* = 7, *d* = -0.10 ppm, *t* = 0.244) heating. However, significant differences were found both before and after heating between both the CLD-SAN/NED pair and the SAN/NED-SAN/1-NA pair. These results support our contention that SAN/1-NA reagent combination is less prone to interferences compared with the AOAC combination of SAN/NED. Clearly, a change in the AOAC method is warranted on the basis of the data reported in this paper.

Table 4. Effect of extraction temperature and heat treatment on measured sodium nitrite, ppm

Product	Extn	CLD		SAN/1-NA		(AOAC) SAN/NED	
		Heating treatment					
		Before	After	Before	After	Before	After
Bacon	cold	9.4	10.5	7.7	10.1	1.8	9.5
	hot	10.3	11.3	7.1	11.9	2.3	10.2
Ham	cold	5.1	7.9	5.1	8.9	4.2	6.8
	hot	5.3	8.7	7.5	7.9	4.7	6.8
Frankfurter	cold	16.2	22.0	12.1	19.8	2.0	18.3
	hot	17.6	21.7	13.5	22.2	3.4	19.2
Lebanon bologna	cold	2.9	10.4	3.0	11.5	2.0	8.8
	hot	5.1	11.1	8.5	12.1	7.4	9.3

Table 5. Statistical results (paired 2-tailed t-test)

Treatment	Method	df	Mean diff., ppm	t-statistic
Hot/cold extrn	Overall	23	1.21	3.80**
	CLD	7	3.96	4.77*
Heat/nonheating	SAN/1-NA	7	4.99	4.64**
	(AOAC)SAN/NED	7	7.64	3.77**

** $P < 0.01$.

While the SAN/1-NA combination gives good results, 1-NA has been classified as a toxic and hazardous substance (11), and is not readily available commercially. However, making the extract alkaline may be a good alternative because it permits the use of the SAN/NED system in measuring nitrite without the error associated with reductants (6).

Because CLD also requires the heating step for maximal nitrite production, there is no advantage in shortening and simplifying the sample preparation step. Therefore, CLD must stand on its merits as a nitrite determination procedure. CLD, as described in this paper, offers little advantage for analyzing large numbers of samples, including cured meats. Each sample has to be handled individually rather than in large numbers, as in the case for the colorimetric method in which the reagents can be added before nitrite determination. CLD requires purging the reaction flask with helium to remove air, addition of the filtrate to the solid reactants, equilibration, and subsequent measurement for each determination. Without a manifold device, the entire system must be disconnected and these steps must be repeated for each sample. However, because of the excellent accuracy and precision of CLD, it can serve as a good reference method for colorimetric pro-

cedures since the extent of reductant interference could not be measured previously. CLD also has the potential for measuring low nitrite concentrations in small samples of biological origin, in which nitrite cannot be measured by other means. In addition, CLD could also be useful for measuring low levels of nitrite in samples that cause problems with colorimetric procedures because of cloudy, colloidal suspensions of protein and/or lipids.

Acknowledgment

The authors gratefully acknowledge the loan of the digital nitrogen detector, Model 720, from Antek Instruments, Inc., Houston, TX.

REFERENCES

- (1) Cox, R. D. (1980) *Anal. Chem.* **52**, 332-335
- (2) Cox, R. D., & Frank, C. W. (1982) *J. Anal. Toxicol.* **6**, 148-152
- (3) Walters, C. L., Downes, M. J., Hart, R. J., Perse, S., & Smith, P. L. R. (1978) *Z. Lebensm. Unters. Forsch.* **167**, 229-232
- (4) Doerr, R. C., Gates, R. A., & Fiddler, W. (1982) *J. Assoc. Off. Anal. Chem.* **65**, 616-618
- (5) Doerr, R. C., Fox, J. B., Jr, Lakritz, L., & Fiddler, W. (1981) *Anal. Chem.* **53**, 381-384
- (6) Fox, J. B., Jr, Doerr, R. C., & Lakritz, L. (1982) *J. Assoc. Off. Anal. Chem.* **65**, 690-695
- (7) *Official Methods of Analysis* (1980) 13th Ed., AOAC, Arlington, VA, sec. **24.041**
- (8) Snedecor, G. W., & Cochran, W. G. (1967) *Statistical Methods*, 6th Ed., Iowa State University Press, Ames, IA
- (9) Nicholas, R. A., & Fox, J. B., Jr (1973) *J. Assoc. Off. Anal. Chem.* **56**, 922-925
- (10) Sen, N. P., Lee, Y. C., & McPherson, M. (1979) *J. Assoc. Off. Anal. Chem.* **62**, 1186-1188
- (11) *Code of Federal Regulations* (1976) **29**, 1910.1004