

MEAT AND MEAT PRODUCTS

Effect of Residual Ascorbate on Determination of Nitrite in Commercial Cured Meat Products

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Residual ascorbate in cured meat slurries results in different amounts of pigment being produced from different Griess reagent combinations. The phenomenon was used to study residual ascorbate in commercial cured meat products which had a variety of textures, acidities, moisture and meat content, fat, homogeneity, initial nitrite, and processing conditions. Diluting and heating the samples according to the AOAC procedure did not completely eliminate the ascorbate interference, but making the sample alkaline did. Determining nitrite separately in supernate and precipitate from the first dilution showed the effect of heating to be the elimination of interferences and solubilization or extraction of nitrite from the precipitate.

During a study of the kinetics of the Griess reaction, it was observed that the presence of ascorbate caused relatively more pigment to be produced from the reagent combination sulfanilic acid/*N*-(1-naphthyl)ethylenediamine (SAA/NED) than from the combination of sulfanilamide/1-naphthylamine (SAN/1-NA) (1). While 1-NA has been classified as a carcinogen (2), the combination of SAN/1-NA gave the best results in nitrite determination and gave the most disparate values when compared with the SAA/NED combination. In tests on heated meat slurries with and without added nitrite and various levels of ascorbate, it was found that the differential pigment production could be used as a test for residual ascorbate (3, 4). The meat slurries were uniform and the residual nitrite levels were relatively high. Commercial meat products present a more varied substrate, including variations in pH, moisture content, meat sources, tissue disruption, fat, homogeneity, initial nitrite content, and processing conditions. Because residual nitrite and ascorbate values vary greatly under these conditions, we determined nitrite by both colorimetric reagent combinations to see if the results from the slurry studies also applied to the more varied conditions of commercial meat products.

Experimental

Samples and Preparation

Commercial cured meat products were purchased at local markets. For the preliminary studies, 4 types of products were chosen: ham as an intact tissue product; bacon, because of its high fat content; frankfurters, representing emulsified products; and Lebanon bologna, a fermented (acid) comminuted meat product. The AOAC (5) procedure calls for finely mincing the sample and then dispersing it in hot water with a glass rod. We found that blending the sample gives more uniform results and is faster because it combines the comminution and dispersion steps. Therefore, the initial extracts were made by blending meat with an equal volume of water. A 2 mL portion of the resulting slurry was diluted to ca 80 mL with warm water and heated 2 h at 80°C, cooled, and centrifuged. Two departures from the AOAC procedure are noted, blending and centrifugation, but these do not appear to change the essence of the procedure.

Nitrite Determination

Three colorimetric reagent combinations were used: (1) the AOAC procedure of adding sulfanilamide to the sample, waiting 5 min, then adding *N*-(1-naphthyl)ethylenediamine (SAN/5 min/NED); and the 2 combinations of (2) sulfanilamide with 1-naphthylamine (SAN/1-NA) (2), and (3) sulfanilic acid-*N*-(1-naphthyl)ethylenediamine (SAA/NED), with both pairs of reagents premixed. Ascorbate has been shown to interfere strongly in the AOAC colorimetric determination procedure (6), and the differential production of pigment from the latter 2 reagent combinations is a measure of the residual ascorbate in cooked meat slurries containing nitrite (3). For the sake of brevity, we shall refer to the comparison as differential colorimetric analysis. Chemiluminescent detection (CLD) of nitric oxide produced from nitrite is a sensitive (2.5 ppb) method for nitrite determination (7) and was used to confirm the results of colorimetric analysis.

Procedure Modification

Because nitrite is labile in meat, there can be no absolute measure of determinable nitrite. In a previous study, we used a consensus approach, using the results of methods that gave the maximal and most consistent results with a minimum of interference (4). These were CLD determination, making AOAC samples alkaline before heating, and charcoal addition. Therefore, portions of the AOAC samples were adjusted to pH 8.0 (alkaline AOAC) before heating. Other portions of the 1:1 meat:water slurries were diluted to 1:10 meat:water, 0.5 g charcoal was added, the samples were shaken for ½ h and then centrifuged according to the procedure of Adriaanse and Robbers (8). To determine nitrite partitioning between supernate and precipitate in the initial extracts, cold water-extracted samples were diluted 1:100 meat:water and a portion was centrifuged immediately to precipitate the water-insoluble proteins, mitochondria, connective tissue, etc. The precipitate was resuspended in a volume of water equal to the supernate, and the initial extract, supernate, and suspended precipitate were heated according to the AOAC procedure.

The charcoal, alkaline AOAC, and separated component samples were turbid after centrifugation or filtration. Turbidity is most commonly eliminated by heavy metal addition, usually iron, zinc, or mercury. Iron is commonly added as the ferricyanide (known as Carrez I) but it interferes in pigment production (4). Mercury is effective, but it also interferes (4) and is expensive and a pollutant. One molar zinc sulfate (Carrez II) added at a ratio of 1 to 20 parts sample before centrifugation gave clear solutions and had no demonstrable effect on pigment formation.

CLD is 20 times as sensitive as colorimetric determination, which allowed us to determine the precision and accuracy of the latter method by comparison with the former. To test recovery, nitrite solution was added to the samples to make them 5 and 80 ppm nitrite before nitrite determination. Five ppm NaNO₂ diluted 1:100 and determined with SAN/1-NA gives an absorbance at 525 nm of about 0.04 AU, about the threshold of colorimetric measurement. Comparisons were

Table 1. Percent recovery of nitrite added to commercial products immediately before sample preparation

Sample	Spike, ppm	Recovery, %			
		CLD	AOAC	SAN	SAA
Bacon	5	86	86	93	108
	80	101	92	100	93
Frankfurters	5	95	103	117	129
	80	101	98	114	115
Ham	5	91	86	89	92
	80	99	98	101	95
Lebanon bologna	5	52	10	50	48
	80	90	83	97	104
Water	5	—	90	97	85
	80	—	95	100	83
\bar{x}		94.71	92.33	100.89	100.44
σ		5.91	6.69	9.13	14.98
CV, %		6.24	7.25	9.05	14.91

made between CLD (Antek Model 720 digital nitrogen detector) and Griess colorimetric determinations (GCD) (Cary 14 spectrophotometer) using techniques described previously (7). We prepared only 10 mL colored solution, but the reagent concentrations were those of the AOAC procedure. From the comparison, we were able to develop an estimate of the reliability of the colorimetric determinations, especially important because of the low nitrite levels encountered in cured meat products.

Results

Nitrite Recovery

The results of the nitrite recovery experiments are shown in Table 1. The low recovery value for the 5 ppm spiked Lebanon bologna samples was apparently due to sample preparation error because both nitrite determination methods

gave the same low value. When samples were reanalyzed, recovery values were 113% and 132% by CLD and SAN, respectively. Statistical analyses of the data were made without the 5 ppm Lebanon bologna data, because the latter were found to be outliers (9). The analysis showed that the percent recovery values for GCD were not significantly different from 100% or CLD, except for the AOAC reagent combination which was low ($P \leq 0.05$). The coefficients of variation (CV) values are comparable to the pooled CV value of 4.4% (4) obtained for replicates in the slurry studies. Subgroup analyses were made of the data, for example, 5 vs 80 ppm nitrite, CLD vs colorimetric, but there were no significant differences. Since the precision of the CLD determination is 5.5% (CV) (6), from which the coefficients of variation of the colorimetric determinations do not differ significantly ($< 4.56 \times CV_{CLD}$) (4), we conclude that the indicated values are close to the true precision and the accuracy of the colorimetric determinations. Since the residual nitrite values were subtracted from the total measured nitrite in the samples, the precision refers only to the spike and may not necessarily apply to residual nitrite in the products.

Supernate and Precipitate

Nitrite was determined in the supernate and precipitate before and after heating. Immediately after resuspension of the precipitate and before heating, there was no measurable nitrite in the precipitate. After heating, the average increases for 9 samples were 2 ppm nitrite in the supernate, 5.8 ppm in the precipitate, and 5.5 ppm in the whole extract. The sum of the increases in supernate and precipitate values (7.8 ppm) is larger than that for the whole sample, but not significantly. The values for measured nitrite in the supernates and precipitates of the 9 samples after heating are shown in Table 2. There was no significant difference between the sum of the parts (precipitate + supernate) and the whole extract. The

Table 2. Nitrite determination by CLD and SAN/1-NA in samples prepared by AOAC method

Sample	Nitrite determination method											
	CLD					SAN/1-NA						
	Spt	+	Ppt	=	Sum	Whole	Spt	+	Ppt	=	Sum	Whole
Bacon I	6.05		3.13		9.18	6.74	7.9		4.4		12.3	9.1
II	8.19		1.92		10.11	10.11	8.9		4.6		13.5	9.1
III	4.73		2.71		7.44	6.98	7.7		5.3		13.0	9.2
III (OH)	5.07		4.13		9.20	8.80	7.7		6.8		14.5	12.0
+ Carrez II			4.20			7.72			8.1			10.5
Frankfurters I	13.22		2.11		15.33	16.00	14.1		4.0		18.1	16.3
II	0.65		4.26		4.91	4.65	0.0		4.8		4.8	5.3
III	1.22		4.86		6.08	5.65	0.0		7.3		7.3	6.3
III (OH)	1.93		8.09		10.02	8.13	3.6		7.0		10.6	10.0
Ham I	2.20		1.62		3.82	4.99	4.4		2.2		6.6	4.6
II	0.63		3.16		3.79	4.46	1.6		4.0		5.6	6.1
III	0.90		3.46		4.36	3.56	1.8		6.1		7.9	8.1
III (OH)	1.35		5.77		7.12	7.00	2.8		8.3		11.1	9.2
+ Carrez II			5.00			5.68			11.1			9.6
Lebanon bologna I	3.24		4.90		8.14	8.58	4.8		6.1		10.9	10.5
II	1.03		5.59		6.62	8.60	2.6		4.8		7.4	8.1
III	1.57		5.83		7.40	8.55	1.0		10.0		11.0	10.6
III (OH)	2.52		11.81		14.33	12.84	1.7		14.8		16.5	14.9
Carrez II			11.23			12.76			13.2			14.9
Paired variate analysis												
Pair	\bar{d} (ppm) ^a	<i>P</i>	Pair	\bar{d} (ppm)	<i>P</i>							
Sum/whole: CLD	0.0	NS	0/+ alkali:spt	1.0	0.05							
SAN	1.3	0.005	:ppt	2.7	<0.01							
SAN/CLD: whole	1.6	<0.001	:sum	3.6	<0.001							
0/+ Zinc: all	0.2	NS	:whole	3.0	<0.001							

^a \bar{d} is average of differences of the first member of pair minus the second.

Table 3. Comparison of 3 methods of sample preparation with initial extract by CLD and 3 Griess reagent combinations

Sample	Preparation methods												
	Initial			AOAC				AOAC-OH			Charcoal		
	AOAC ^a	SAN	SAA	CLD	AOAC	SAN	SAA	AOAC	SAN	SAA	AOAC	SAN	SAA
Bacon	4.3	21.0	53.0	55.8	44.2	54.8	62.6	63.2	60.1	61.1	69.5	61.3	73.0
Corned beef	9.1	12.6	14.3	14.5	11.2	14.3	15.7	22.2	26.6	31.1	14.1	14.7	16.0
Frankfurter	2.9	19.4	55.0	60.5	49.2	57.4	66.4	61.0	65.0	69.8	69.0	60.7	72.3
Frankfurter, chicken	0.3	0.2	1.2	10.8	7.0	8.7	9.7	16.1	18.4	24.6	5.0	4.5	5.0
Genoa salami	0.6	1.2	1.9	5.2	3.4	5.1	4.7	10.2	12.1	12.2	5.8	6.1	6.7
Pepperoni	1.0	1.6	2.6	8.3	5.9	7.9	8.5	11.0	12.1	9.4	7.1	7.1	8.6
Pork butt	6.4	22.0	51.9	70.4	52.1	63.9	80.5	68.2	71.2	75.1	69.6	64.9	83.3
Smoky link	10.2	15.6	18.2	17.0	11.9	16.1	16.1	22.6	26.6	34.7	18.0	17.9	17.8

Prepn methods	Nitrite detn	\bar{d} (%)	S _d	P	Prepn methods	Nitrite detn	\bar{d} (%)	S _d	P
AOAC	CLD/AOAC	38	4.1	<0.001	AOAC	SAN/AOAC	29.1	3.6	<0.001
	CLD/SAN	4.5	1.2	<0.01		SAA/SAN	8.2	3.1	0.05
	CLD/SAA	-2.7	3.5	NS					
AOAC-OH/AOAC	AOAC	88	10.6	<0.001	AOAC-OH	SAN/AOAC	10.8	3.0	<0.02
	SAN	61	17.2	<0.02		SAA/SAN	9.3	6.3	NS
	SAA	67	25.7	0.05					
AOAC-OH/charcoal	AOAC	51.8	27.0	NS	charcoal	AOAC/SAN	4.6	2.7	NS
	SAN	77.8	35.5	NS		SAA/SAN	14.7	3.1	<0.005
	SAA	80.4	47.6	NS		SAA/AOAC	9.8	3.1	0.02

^aAbbreviations used in this row are for the following methods of nitrite determination: AOAC, addition of sulfanilamide (SAN), wait 5 min, add *N*-(1-naphthyl)ethylenediamine (NED); SAN, combination of SAN and 1-naphthylamine; SAA, combination of sulfanilic acid and NED; CLD, chemiluminescent detection.

Griess reagent combination SAN/1-NA measured more nitrite than did CLD, but the difference was small (1.5 ppm). The addition of zinc (Carrez II) to the precipitate and whole samples removed the faint but visible turbidity that was present, but made no difference in the amount of nitrite determined. Finally, the adjustment of the extracts to pH 8.0 increased the measured nitrite 1.0 ppm in the supernates, 1.9 ppm in the precipitates, and 3.0 ppm in the whole samples.

Sample Preparation Procedures

Table 3 shows the results of nitrite determination by the 3 Griess reagent combinations in samples of the initial extract and after 3 sample preparation procedures. CLD was used to determine nitrite in the AOAC-prepared samples as a control. The nitrite concentrations in the initial extracts as measured by the AOAC (SAN/5 min/NED), SAN/1-NA, and SAA/NED reagent combinations show the effect of residual ascorbate in all of the cured meats, that is, the ordering of the determinations in terms of pigment formed was AOAC < SAN < SAA (4). A summary of the statistically significant differences between the preparation procedures and nitrite determination reagents is shown below the data. Because of the wide range of nitrite concentrations in the samples, the data were analyzed as percent changes between the 2 sets of data being compared. This treatment of the data is indicated because the differences between sample preparation procedures are the result of improving a given determination rather than adding a fixed amount to each nitrite concentration. The statistical analysis of the results of the 3 colorimetric preparation procedures and the CLD determination are shown in the first 3 sets of data, left side of Table 3. The \bar{d} values are the average differences in percent increase or decrease of the first-indicated preparation method compared with the second. For example (first row), the CLD method gave 38% higher average nitrite values than did the AOAC colorimetric determination in the same AOAC preparation, indicating some

residual ascorbate activity. The SAN/1-NA values averaged 4.5% lower than the CLD, and the SAA/NED 2.7% higher, again indicative of residual ascorbate. However, the differences between the SAA/NED and CLD values were more variable than the differences between the SAN/1-NA and CLD values, 3.5% and 1.2%. If we take CLD as the reference, the lower variation in the differences means SAN is giving more reliable values than SAA.

Referring to the second set, AOAC-OH/AOAC, making the samples alkaline before heating resulted in a significant increase in measured nitrite over the standard AOAC procedure (61–88%). Since one effect of alkalization is to reduce the ascorbate interference, the greatest increase was in the AOAC reagent determinations, although there was also a significant increase in the SAN/1-NA and SAA/NED values. The AOAC-OH procedure also gave higher measured nitrite values than did the charcoal treatment (third set), but the differences between the treatments were so variable that the increase (fourth column) was not significant (see next section).

The differences in nitrite measured by the 3 colorimetric reagent combinations on each of the 3 preparation methods are shown in the right hand sets of data (Table 3). The 29.1% difference between the AOAC and SAN determinations confirms that the AOAC preparation procedure is incompletely removing ascorbate. The difference of 8% between SAN/1-NA and SAA/NED in both the AOAC and AOAC-OH preparations is a minimum figure due to chloride in the samples (3). The order of increasing nitrite was AOAC/SAN/SAA for both heating procedures, but for the charcoal treatment it was SAN/AOAC/SAA (second set), which suggests that the 2 preparation procedures are not equivalent. The charcoal treatment eliminates the ascorbate effect since the AOAC and SAN/1-NA reagents are almost equivalent. The SAA/NED values are higher, but the combination is sensitive to Cl⁻ (3, 10), and ions from the charcoal may cause a false increase in the amount of pigment produced.

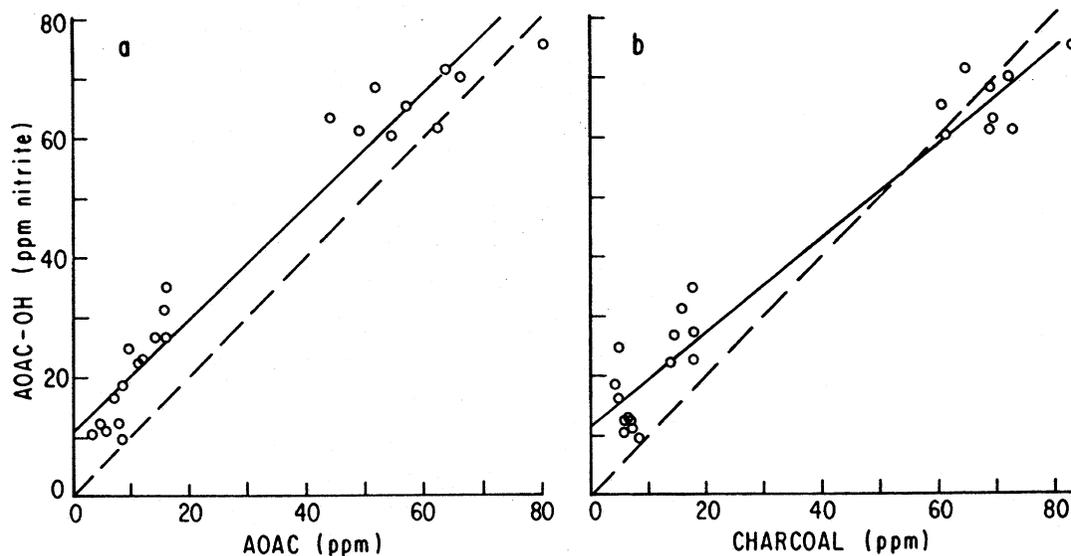


Figure 1. Nitrite as determined by 3 preparation methods. 1a—Covariant plot of nitrite concentrations as determined by sulfanilamide/1-naphthylamine in samples prepared by AOAC procedure, with and without alkalization. 1b—Same reagent, samples prepared by AOAC-OH and charcoal addition.

Comparison of AOAC-OH with AOAC and Charcoal Addition Preparation Methods

The plots for nitrite determined by SAN are shown in Figure 1, in which the AOAC-OH data are plotted as functions of the AOAC (1a) and charcoal addition (1b) methods. The dashed line is the expected regression if both sample preparation methods gave the same amount of measured nitrite for each sample. Similar to the slurry studies, making the samples alkaline improved recovery (Figure 1a) by about 10 ppm nitrite at all levels. In contrast, charcoal addition was as effective as AOAC-OH preparation at high nitrite concentrations, but yielded less nitrite by about 10 ppm at the lower nitrite levels. This explains the high average difference and standard deviation observed between the AOAC-OH and the other 2 preparation methods (second 2 sets of data, lower left side, Table 3).

Nitrite Determination in Different Products

As a final test of the different nitrite determination procedures, 16 different kinds of cured meat products were tested, including bacon (3 pork, 1 beef, and 1 Canadian), 2 corned beef, frankfurters (2 pork and beef, 2 chicken, and 1 turkey), 1 ham, 2 pepperoni, 1 pork butt, 2 Lebanon bologna, salami (1 hard and 2 Genoa), 1 alternative bacon product, 1 smoky link, and 1 beef strip. The results of the 3 colorimetric determinations are shown as a function of the reference method CLD in Figure 2. The regression equations are

$$\text{AOAC} = 0.803 \text{ CLD} - 1.37 S_{yx} = 1.22$$

$$\text{SAN} = 0.938 \text{ CLD} + 0.77 S_{yx} = 2.56$$

$$\text{SAA} = 0.996 \text{ CLD} + 4.98 S_{yx} = 3.80$$

The intercepts of the AOAC and SAA plots are significantly different from zero ($P = 0.005$ and 0.002 , respectively), but the SAN intercept is not. The solid line is the perfect correlation (slope = 1, intercept = 0), and it is apparent that over the entire range the SAN/1-NA combination is the closest to the ideal. The slope of the SAA/NED:CLD plot is closer to 1, but the offset (4.98 ppm) and the variability ($S_{yx} = 3.8$) are indicative of interference by residual ascorbate and chloride.

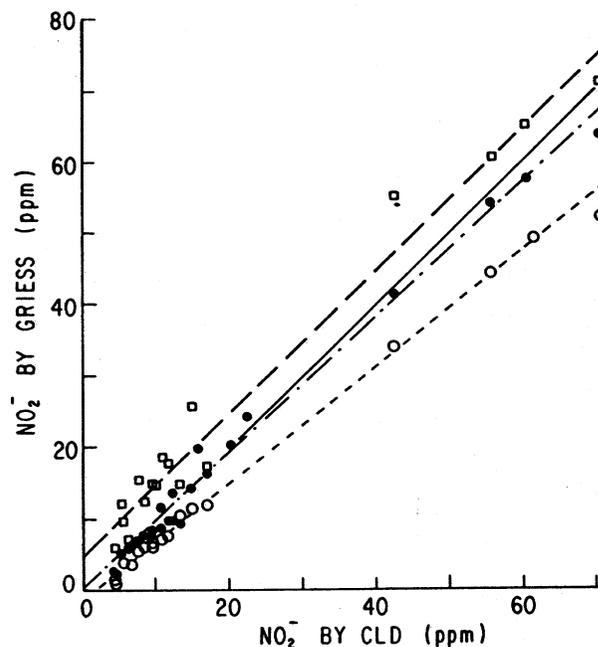


Figure 2. Nitrite in samples prepared by AOAC procedure as determined by chemiluminescent detection technique and 3 reagent combinations. O, add sulfanilamide, wait 5 min, add *N*-(1-naphthyl)ethylenediamine (AOAC). ●, Sulfanilamide/1-naphthylamine. □, Sulfanilic acid/*N*-(1-naphthyl)ethylenediamine.

Discussion

Magnitude of the Differences: Precision

The average differences between the various preparation techniques and reagent combinations tend to be quite small and in some cases negligible. For example, heating for 2 h resulted in an average increase of 5 ppm nitrite, partly by eliminating the ascorbate interference and partly by freeing nitrite from the precipitate; alkalization of the samples resulted in an average increase of 3 ppm in one set of values, in another set, 10 ppm. These differences do not at first appear to justify the use of one procedure or reagent over another, yet alkal-

ization is more effective in eliminating ascorbate interference, and because residual ascorbate, like nitrite, is variable, more uniform and accurate nitrite determinations result (4).

The same may be said for reagent combinations. By comparison with the reference method CLD, the reagent combination of SAN/1-NA, which is less sensitive to ascorbate, gave more precise and accurate values than did SAA/NED. Both the spike recovery figures and the covariance of the CLD and Griess nitrite measurements suggest an increasing variability in pigment formation in the order, AOAC < SAN < SAA. The CV of spike recovery increased in the same order, $7.25 < 9.05 < 14.91\%$, as did the S_{yx} of the CLD/Griess reagent covariant plots, $1.22 < 2.56 < 3.80$. This coincidence led us to examine the data from previous studies and it was found indeed that the SAA/NED values showed a higher variation than did the SAN/1-NA values. It is safe, therefore, to conclude that the SAA/NED combination is inherently more variable (less precise) than the SAN/1-NA combination. The explanation is that the nitrosation rate of both the nitrosated species, SAA, and the coupling reagent, NED, are about equal ($k_{1st} = 0.180$ and 0.186 min^{-1} , respectively). Because of the molar excess of SAA over NED and the partial reversibility of the nitrosation of the latter, the reaction goes principally to the formation of pigment. Nevertheless, where the rates of nitrosation are approximately equal, any slight variation affecting the 2 rates differently will result in quite different amounts of pigment being formed. Slight variations in temperature, mixing rates, absolute reagent concentrations, other ions, etc., can cause the difference. For example, chloride affects the rate at which the pigment is formed (9). If the ratios of the reaction rates with SAA and NED are different for the 2 nitrosating species, nitrogen trioxide and nitrosyl chloride, the amount of pigment formed will be different with and without chloride. To counteract this effect, Sen and Donaldson (11) proposed adding excess chloride, which shifts the nitrosating species to nitrosyl chloride. However, the variability observed in the previous study was within isochloric sample groupings, and therefore could not be due to chloride variation. To decrease the variability, either faster reacting nitrosated species or slower reacting coupling reagents may be used, for example, SAN/1-NA, or the nitrosated species may be prereacted as in the AOAC reagent combination/procedure.

Spikes, Slurries, and Commercial Samples

The results obtained for the determination of nitrite in the spiked samples of this study and the slurries of the previous 2 studies (3, 4) illustrate the flaw in such procedures: It is too easy to get good results. Nitrite recovery from the spikes was very good, even for the AOAC colorimetric determination which is sensitive to residual ascorbate. Slurries, while useful for establishing mathematical parameters, are too uniform to serve as a test of the ruggedness of a determination procedure. The residual nitrite in the slurries was high and relatively uniform. Charcoal addition to the slurries resulted in nitrite concentration in measurements equivalent to the alkaline AOAC or CLD techniques (4), but in commercial samples, charcoal addition failed at low nitrite levels. Again, the necessity of testing procedures of both sample preparation and nitrite determination in a wide variety of products is confirmed. This does not imply that comparing techniques in model systems and then applying the technique of choice to commercial samples is sufficient. Rather, a formal testing of all techniques of interest in commercial products is required.

Nitrite Partitioning

The forms in which nitrite occurs in cured meat have not been fully defined. Part may occur as nitric oxide in the pigment (12), part perhaps as nitrosothiols (13), and part as some as yet unidentified small molecules (14). Nitrite, in the form of nitrosothiols or specifically bound to proteins, will be in the precipitate because most of the protein is the water-insoluble actomyosin, which contains the greater part of the free sulfhydryl groups of meat. Nitrite bound or reacted with the reducing compounds, primarily coenzymes or added ascorbate, will be in the supernate. The first step in analyzing sample preparation procedures is determining the partitioning of nitrite between the fractions. Mirna (15) and Olsman and van Leeuwen (13), in a study of the effect of mercuric chloride on nitrite in the precipitate, concluded that the nitrite was in the form of nitrosothiols, but they did not determine total nitrite nor did they study any other sample preparation procedure. We find that on average the nitrite partitions equally between the supernate and precipitate, and that the AOAC heating procedure is effective in releasing the protein-bound nitrite. In this regard, Rougie et al. (16) found that the nitrite could be freed from the precipitate simply by repeated extractions. The effect of heating is therefore twofold: to eliminate ascorbate and other reduced substances that interfere in the Griess reaction, and to free protein-bound nitrite.

Summary

Differential colorimetric analysis of a variety of commercial cured meats demonstrates that residual ascorbate is the major factor in reduced pigment formation in nitrite determination of these products. While the AOAC procedure of sample preparation is fairly effective in eliminating the interference, making the sample alkaline before heating improved yields and reduced ascorbate interference, regardless of the kind or initial acidity of the products. In the previous study on meat slurries, charcoal addition provided results equal to the alkaline-AOAC or CLD procedures, except that at lower nitrite concentrations in cured meats, it gave lower measured nitrite values. Nitrite determination by the AOAC reagent combination/procedure is highly sensitive to residual ascorbate and while the alkaline-AOAC sample preparation apparently did not completely eliminate the interference, the 3 reagent combinations gave values acceptably close to each other. As a result of these studies it is evident that the AOAC sample preparation procedure is acceptable but could be improved by making the samples alkaline before heating, which may necessitate addition of Zn^{++} ions to clarify the solutions. The nitrite determination procedure of the AOAC method is not a good technique because it gives low values for measured nitrite. It should be changed, preferably to the simultaneous addition of sulfanilamide and 1-naphthylamine, although the latter reagent may not be acceptable because it is a potential carcinogen.

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