RAPID DETERMINATION OF AMMONIA AND VOLATILE AMINES IN BEEF EXPOSED TO LOW LEVELS OF AMMONIA USING AMMONIA ION SELECTIVE ELECTRODE (ISE)

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Abstract__method to determine ammonia and volatile amines content of ammonia contaminated meat using ion selective electrode (ISE) specified for ammonia is described. Ground samples of ammonia contaminated meat were mixed with distilled water and ionic strength adjustor (ISA) to release ammonia from the tissue. Ammonia was determined as a mV reading, then converted to parts per million concentration using standard curve that has excellent linearity with correlation coefficients (R^2) ranging from 0.9984 to 0.9999 and recovery percentages ranging from 95.5 to 100.36. Compared to the traditional ammoniacal nitrogen determination method (AOAC # 990.03), ammonia ISE gave faster and more reliable determination of ammonia and volatile amines contents of meat.

Keywords: beef, ammonia exposure, ammonia ion selective electrode, ammonia content

I. INTRODUCTION

Ammonia effects, when leaked in refrigeration system, on the quality of meat and meat products has been characterized as increases in the pH, darkening in the color, changing in the flavor profile, and increases in the water holding capacity (Ireland 1988, Shaw et al., 1992; Hagyard et al., 1993; Al-Sahal, 1995). To determine the ammonia content of meat after ammonia contamination, AOAC total nitrogen determination method has been employed (Goodfellow et al., 1978). Kassem (1965) concluded that the pH of ammonia contaminated meat is not a reliable indicator of amount of absorbed ammonia after contamination due to the buffering capacity of meat that tends to hide ammonia effect. Also, Al-Sahal (1995) indicated that the method of AOAC was not capable to quantify an increase in the total nitrogen even with 50,000 ppm ammonia treatment that caused more than 2-3 pH unit increase. The direct ammonia analysis was recommended.

Direct methods available to determine ammonia in meat and meat products such as HPLC, enzyme assay method, and total volatile basic nitrogen determination (Brooks & Ammerman, 1978; Parris, 1984; Bonne et al., 1993) required proficient analysts, complete laboratory with expensive equipment, may induce potential safety risks, and more importantly take long time to be completed. However, in case of ammonia contaminated meat, the time of the analysis procedure is of importance when a corrective action to be taken in a HACCP program.

Ammonia ion selective electrode (ISE) has been employed successfully in determining ammoniacal content of water, juices/wine, soil, plants, plasma, waste water and blood (Davidson & Jennings, 1980; Mills, 1980; Cooke & Jensen, 1983; Chapin et al., 1994; Turbow et al., 2002). Furthermore, ammonia ion selective electrode was effective in screening of seafood (fish and shrimp) quality and decomposition as a rapid and simple method (Ward et al., 1979; Pivarnik et al., 1998; Ellis et al., 2000; Pivarnik et al., 2001). The only research available using ammonia ISE in determining ammonia content of red meat was using alcohol extraction method that deteriorates the electrode's membrane faster and required more time for the contact between the extract and the membrane in order to give the mV read (Parris & Foglia 1983).

No information has been published on the subject of using the ammonia ion selective electrode to determine ammonia in red meat or meat products using water extraction. Furthermore, the need is imperative to establish rapid and reliable analysis to measure ammonia content of fresh meat and other meat products following ammonia leak and contamination in warehouse storage. Therefore, the main objective of this study was to demonstrate and establish a fast and reliable method to measure ammonia content of ammonia contaminated fresh meat using ammonia ion selective electrode.

II. MATERIALS AND METHODS

A. pH Meter: Fisher Scientific Accumet Basic with pH/mV readability and BNC connector (Fisher Scientific, Fairlawn, New Jersey, USA)

B. Ammonia Electrode: A Thermo Orion model 95-12 ammonia sensing electrode was used to measure ammonia in samples. The ammonia sensing membranes had a reported ammonia detection range of 0.01 to 17,000 ppm in aqueous solution (Thermo Orion, Beverly, MA, USA). The electrode internal filling/solutions and calibration standards were also obtained from Thermo Orion. A 0.1M NH_4Cl solution was used to fill the electrode prior to operation.

C. Standard Solutions: A commercially prepared 1000 ppm nitrogen standard solution NH_4Cl (Catalogue No. 951007) was diluted to proper calibration solutions (1, 10, and 100 ppm) used to construct the standard curves. All stock solutions were stored at room temperature and used as needed.

D. Ionic Strength Adjuster (ISA) Solution: The commercially prepared ISA (Catalogue No. 951211) contained 5 M NaOH, 0.05 M disodium ethylenediaminetetracetate (EDTA), and 10% methanol with color indicator was used to adjust the solution pH to the operating rang of the electrode (pH 11-14).
E. ISE Assembly, Checking Electrode Operation (Slope), Analytical procedure and Standard Curve Construction: According to the instruction manual (Thermo Orion 1997).
F. Nitrogen/Ammonia Conversion Factor: According to instruction manual, ammonia can be measured in parts per million (ppm) by converting the concentration of ammonia as nitrogen (ppm) to ppm of ammonia (each 1.4 ppm ammonia as nitrogen = 1.7 ppm ammonia) according to the following equation:

Ammonia Concentration (ppm) = [Ammonia as N Concentration (ppm) X 1.7] / 1.4

G. Meat Samples Preparation: Fresh semitendinosus beef steaks (0.9 cm thickness) that had been contaminated with ammonia (0, 500, 1,000, 2,500, and 5,000 ppm) for 0, 5, 10 and 20 min were ground twice with a 0.3 cm blade using a Hobart electric grinder (Hobart Corporation, Troy, Ohio, USA). Then 10 g of ground beef was placed in a 200 ml beaker along with 90 ml of distilled water (1:10 dilution) and homogenized using a Fisher Scientific PowerGen 35 hand homogenizer (Fisher Scientific, Fairlawn, New Jersey, USA). The homogenized solution was mixed with 2 ml of ISA inside the same beaker and measured according to the previously mentioned procedure. The mV readings were recorded and the concentration of nitrogen in meat samples was determined from the regression equation of the standard curve. The results were multiplied by the dilution factor (10) to get the total ammonia as nitrogen concentration in the original sample. The nitrogen concentration was converted to ammonia concentration by the above mentioned conversion equation. Three replications and four samples per replication were analyzed for each treatment.
H. Electrode Storage: According to the instruction manual (Thermo Orion 1997).
I. Spiking Method and Recovery: Ground beef (semitendinosus) was divided into three samples and spiked with 10 and 25 ppm ammonia as nitrogen solution or used as a control. In order to get 100 grams of 10 ppm spiked meat, 99 grams of meat was mixed with 1 ml of 1000 ppm ammonia nitrogen standard. In the same manner to get 25 ppm spiked meat, 97.5 grams of meat was mixed with 2.5 ml of 1000 ppm ammonia nitrogen standard. In order to calculate the percent recovery, the background content of ammonia in the control meat sample was subtracted from the ammonia content of both spiked samples. Two replicates were prepared and three samples per replicate were tested.
J. Total Nitrogen Analytical Procedure: Nitrogen content of all meat samples was determined by AOAC (2002) official method # 990.03 using a LECO FP-2000 (LECO Corporation, St Joseph, Michigan, USA
K. Statistical Analysis: The statistical model was a 4 X 5 structure with a completely randomized design for exposure times (0, 5, 10, and 20 min) and ammonia concentrations (0, 500, 1,000, 2,500, and 5,000 ppm). The experimental data were analyzed using the GLM procedure of SAS (2000). The model included ammonia concentrations, treatment times, and all interactions. Means were compared to determine significance difference when a significant F-ratio was obtained in the two-way analysis of variance.

III. RESULTS & DISCUSSION

Ammonia Nitrogen Standard Curve: The logarithmic standard curve of different concentrations of ammonia as nitrogen and corresponding millivolt readings are shown in Fig. 1. The results indicate excellent linearity with correlation coefficients (R^2) 0.9999.

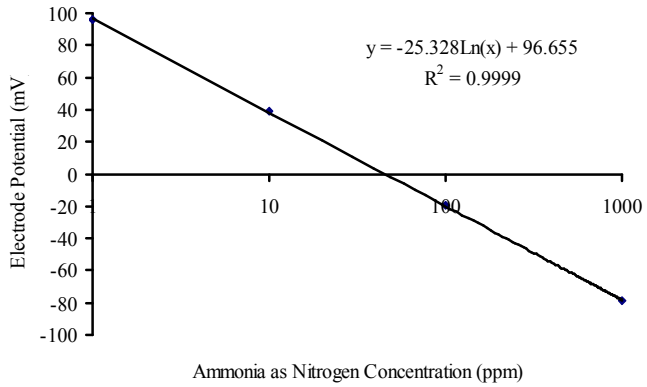

Figure 1. Ammonia as nitrogen logarithmic standard curve.

Percent Recovery of Ammonia: Recovery of ammonia was confirmed by spiking meat with 10 and 25 ppm standards. The results are displayed in Table 1. The percent ammonia recovery after spiking 10 ppm was in the range between 88 to

107% (Average 95.5%). For 25 ppm ammonia spiking, the average percent recovery was 100.4%. The results indicated excellent extraction of the spiked ammonia at high and lower concentrations.

Electrode Slope: The operation of the electrode was checked before use. The slope of the electrode was -58.11 ± 1.03 (SD, n=8). According to the electrode operation manual (Thermo Orion, 1997), these results were within the allowable range of -54 to -60 which indicates that the electrode was operating properly.

Table 1. The recovery percentage of ammonia spiked samples (n=6).

Ammonia as nitrogen (ppm)		
Spiked	Found	% Recovery
10	9.55	95.5
25	25.09	100.4

Ammonia Content- ISE Method: There were significant interactions between the times of exposure (0, 5, 10, and 20 min) and the concentrations of ammonia (0, 500, 1,000, 2,500, and 5,000 ppm) combination treatments (Table 2). In general, the ammonia contents of all ammonia treated samples were higher ($P < 0.05$) compared to the control (untreated) over all exposure times. At 500 ppm ammonia, the exposure for 5 and 10 min showed similar ammonia content to the control; however, 20 min of exposure was effective in significantly increasing the ammonia content of meat samples. Starting from 1000 ppm ammonia treatments, when the concentration of ammonia and time increased, the ammonia content of the meat increased. The increase in the amount of ammonia is the cause of the increase in the alkalinity of meat (pH) (Kassem, 1965; Anil, 1971; Al-Sahal, 1995) and the effects on the quality traits (Shaw et al., 1992; Hagyard et al., 1993; Al-Sahal, 1995).

Table 2. ISE determination of ammonia content of beef chops when exposed to different concentrations of ammonia for different times. Each mean represents the average of 12 samples (n=12).

	Ammonia Concentration (ppm)				
Exposure Time (min)	0	500	1000	2500	5000
0	73.3^{g}	61.4^{g}	72.6^{g}	76.2^{g}	85.9^{fg}
5	76.1^{g}	105^{fg}	186^{ef}	262^{d}	377^{c}
10	76.1^{g}	111^{fg}	242^{d}	320^{c}	548^{b}
20	72.0^{g}	135^{f}	235^{de}	361^{c}	683^{a}

^{abcdefg} **Means with different superscript letters differ ($P < 0.05$). Standard Error (SE)= 19.08.**

Ammonia Content -AOAC Method: Figure. 2 and 3 present the main effect data of ammonia concentration treatments and exposure time treatments. The statistical results indicated that there were neither significant interactions nor differences ($P > 0.05$) in nitrogen contents of all meat samples over the main treatments. These findings point out the lack of the ability to determine any increase in ammonia content of meat after ammonia contamination using the AOAC % nitrogen determination method.

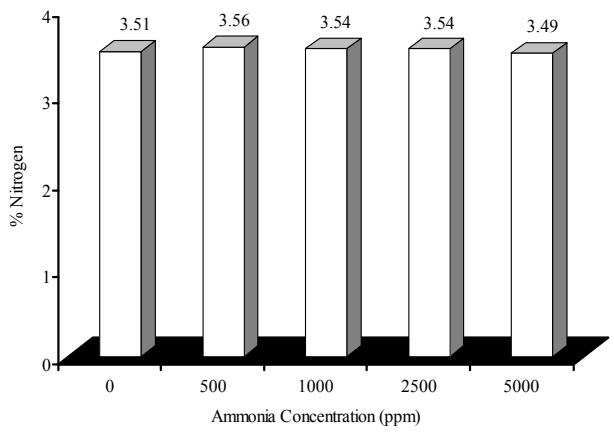

Figure 2. The change in ammonia content of beef chops when exposed to different concentration of ammonia (AOAC method).

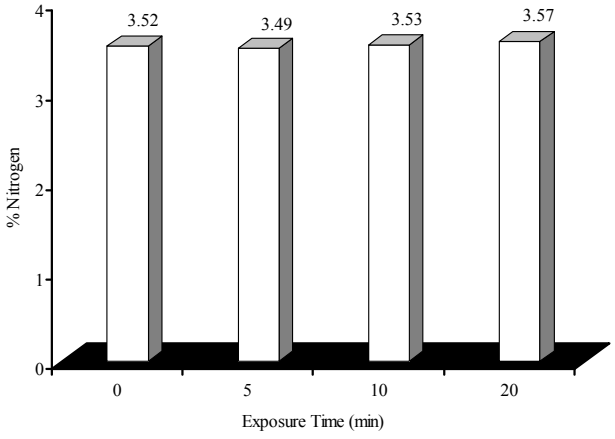

Figure 3. The change in ammonia content of beef chops when exposed to ammonia for different exposure time (AOAC method).

The linearity, reliability, and reproducibility of the ammonia ISE method showed that the technique can provide reasonable accuracy and precision for ammonia determination in beef and likely other red meat products. The majority of

the samples gave a response within 1 minute and less of contact between the electrode tip and the sample extract. The ISE method effectively detected and monitored ammonia content of shrimp (Ward et al., 1979). Similarly, Pivarnik et al. (1998) used ammonia ISE with water extraction in fish and concluded that only ISE and trimethylamine methods were correlated with the total volatile base method in indicating spoilage. Both Ward et al. (1979) and Pivarnik et al. (1998) concluded that the ISE method had great potential for screening fish and shrimp quality and decomposition. In this study, ammonia content of meat increased 1-10 fold after ammonia exposure. The increases in the amount of ammonia in contaminated samples as ammonia concentration increases over time was an indication of the ammonia absorbability of the meat and the accumulation of ammonia over time. Because of water content of the meat (75%), ammonia reaction with meat can be continuous until saturation. The time needed to reach that saturation point is unknown, but it is more than 20 min as indicated in this study. The extraction of ammonia with water was efficient due to the hydrophilic nature of ammonia.

The ammonia content of beef obtained by this study was in the range of 61-86 ppm, however, Parris and Foglia (1983) found ammonia content of 100 ppm when using the ammonia ISE method and 88 ppm with the enzyme method. Bonne et al. (1993) reported ammonia level of 111 ppm using the total volatile basic nitrogen method, while Parris (1984) indicated ammonia content of 221 ppm using the HPLC method and 121 ppm using the enzyme method. ISE coupled with water extraction method was more conservative in determining ammonia in red meat compared to others. Repeated determination of ammonia with ISE on beef showed excellent precision similar to what was indicated with fish (Ellis et al., 2000). This method could be applied in industry to evaluate ammonia content of red meat following ammonia leak.

IV. CONCLUSION

ISE method was rapid, simple, repeatable and precise to determine ammonia content of meat after ammonia leak, spill, or contamination. This method can be a very important tool in meat industry to enhance the quality control and a corrective action of a HACCP program.

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