Executive Summary

Title: Evaluation and Analysis of Meat Products Contaminated by Low Levels of Ammonia

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Objectives:

The overall goal of this project was to develop a practical method for measuring ammonia contamination levels in meat products. The method was then used to study the uptake of ammonia by meat, and the effectiveness of methods designed to decrease contamination levels.

Conclusions:

A relatively rapid and simple method for measuring ammonia contamination levels in meat exposed to ammonia refrigerant leaks has been developed. This method uses an ion selective electrode measurement, and should be suitable for use as a quality control procedure in meat processing facilities. This method was used to investigate the uptake of ammonia by fresh meat exposed to air containing 200 ppm ammonia. Fresh meat rapidly absorbs and binds ammonia, achieving ammonia concentrations in the product that exceed the levels of ammonia in the surrounding air. The rate of ammonia uptake by frozen meat is much slower. Potential simple methods for removing ammonia from exposed product were evaluated, including air flushing, vacuum treatment, and acid rinsing; however, none of these simple procedures were effective in providing substantial reduction in the ammonia levels.

Deliverable:

A positive impact of this study is the availability of a practical method for measuring ammonia contamination in meat using ion selective electrode technology. This method should be applicable as a quality assurance tool in meat processing facilities. The results also provide better understanding of the levels of contamination that can occur when meat is exposed to ammonia contaminated air. A negative result is that the simple techniques explored for remediation of contaminated product, including air flushing, vacuum treatment, and acid rising, were not effective in providing meaningful reductions in ammonia levels.

Technical Abstract:

A successful ion selective electrode (ISE) method for quantifying ammonia in meat exposed to ammonia refrigerant leaks has been developed. Ammonia is extracted into 0.01 M phosphate buffer at pH 6 and quantified with an ammonia selective electrode. Recoveries >90% were achieved over a range of 10 to 200 ppm ammonia in spiked samples, while coefficients of variation of 5% or less were obtained for ammonia levels ≥ 50 ppm. The ISE method was equivalent in performance to ion chromatography. When the ISE method was used to evaluate the ammonia concentration in meat samples exposed to air containing 200 ppm ammonia, it was found that the rate of ammonia uptake in fresh meat increased linearly up to nine hours ($r^2 = 0.99$), with a rate of uptake of 58 $\pm$ 7 ppm per hour. After nine hours, the rate of uptake declined, indicating saturation was occurring. The concentration of ammonia in exposed samples was substantially higher than the concentration of ammonia in the surrounding air, indicating that proteins or other components in meat actively bind ammonia, and it is not a simple diffusion process that is occurring. The rate of uptake by frozen meat was much slower than the rate of uptake by fresh meat. Simple methods that could reduce the ammonia content of contaminated meat, including air flushing, vacuum treatment, and acid rinsing, were evaluated and found to be mostly ineffective at reducing ammonia levels.

Publications:

A manuscript is under preparation for submission to the Journal of Food Science. The manuscript can be formatted for another journal, if publication elsewhere is preferred by the AMIF.

Goals and Objectives:

The overall goal of this project was to develop a practical method for measuring ammonia contamination levels in meat products. The method was then used to study the uptake of ammonia by meat, and the effectiveness of methods designed to decrease contamination levels. Specific objectives of the project were:

1) To optimize ion selective electrode and ion chromatography methods for determining ammonia in meat, and compare those methods for accuracy and reproducibility.

2) To monitor the rate of ammonia uptake by fresh and frozen meats exposed to ammonia gas at selected temperatures, times and concentrations.

3) To investigate selected techniques for lowering ammonia levels in contaminated meats, including air flushing, vacuum treatment, and rinsing with dilute organic acid solutions.

Materials and Methods:

Meat Samples:

Meat samples used for this experiment were all top round steak purchased at a local supermarket (HyVee). Only lean meat samples were used for this experiment, as excessive fat could foul the hydrophobic membrane of the electrode. Recovery of ammonia was tested by spiking 10 gram meat samples with a 1000 ppm ammonia standard solution, so that the meat contained 10, 20, 50, 100, 150, or 200 ppm ammonia. For exposure to ammonia contaminated air, the top round was cut into 2.5 X 2.5 X 1 inch (6.35 X 6.35 X 2.5 cm) pieces.

Determination of Ammonia in Meat using an Ion Selective Electrode (ISE):

The ammonia selective electrode used in this experiment was an Orion 9512HPBNWP from Thermo Scientific, connected to an Orion model 420A pH meter. The electrode was maintained and stored according to the user guide manual provided by the manufacturer. All solutions and buffers were prepared using filtered nanopure water to avoid any ammonia contamination from the water source.

The procedure used to extract ammonia from meat samples was adapted from the recent publication by Hijaz et al (2007). Both vortexing and blending extraction procedures and several solvents were tested for their efficiency in extracting ammonia from meat samples. Solvents tested included nanopure water without pH adjustment; nanopure water with pH adjustment (2.0, 3.0, 4.0, 5.0, and 6.0) using HCl/$HClO_4$/NaOH; potassium phosphate buffer at different concentrations (0.1M and 0.01M) and pH (5.8, 6.0, 6.2, 6.5, 6.8, 7.0, and 7.2). Each sample was prepared by blending 10g of meat with 90mL of solvent at high speed for 30 seconds using a Waring blender. Intact muscle was ground three times using a table top Oster food grinder before blending. The blended meat solution was then centrifuged using an IEC Centra CL2 centrifuge for 10 minutes at 3300 rpm. This step helps to spin down meat particles, or solid fat and insoluble protein. Further removal of fat, insoluble proteins and other particles not spun down during the centrifugation step was achieved by filtering the supernatant through Whatman No. 4 filter paper. The volume of filtered supernatant was adjusted to 100mL, and its temperature equilibrated to room temperature for 60 minutes before ISE measurement to prevent any fluctuation of mV readings due to different temperatures of the supernatants.

ISE measurement of ammonia concentration in a sample extract was conducted according to the manufacturer's instructions by adjusting the ionic strength and pH of the extract using 1.6 ml of Ionic Strength Adjusting (ISA) solution. During measurement with the ISE, the extracts were stirred with a magnetic stirrer at constant moderate speed.

The levels of ammonia in meat samples were then determined by using a regression equation obtained from a set of ammonia standards. The standards used were either a set of meat samples spiked with different levels of ammonia, or a set of diluted aqueous ammonia standards. When ammonia was determined using diluted ammonia standards, the concentration of ammonia in an unspiked meat sample was subtracted from each sample concentration. On the other hand, concentrations of unknown samples determined using ammonia spiked meat as standards were reported without subtracting a background value or blank.

Determination of Ammonia in Meat Using Ion Chromatography (IC):

The sample preparation procedure was similar to the ISE procedure; however, prior to injection, samples were filtered through a nylon membrane with 0.45μm pore diameter to remove particles from solution.

Ammonia concentration in meat sample extracts was determined with a Dionex ICS-3000 chromatograph with dual pumps connected to a conductivity detector. The column used was an IonPac CS 12 analytical column (4 X 250mm) from Dionex. The IonPac CS12 column was packed with 8μm diameter macroporous particles consisting of an ethylvinylbenzene-divinylbenzene copolymer, and carboxylic acid as the functional group of the cation-exchanger. An IonPac CG 12 guard column (4 X 50mm) was used to

protect the analytical column. The columns were maintained at 30º C. Cations were suppressed with a Dionex CSRS 300 4 mm ion suppressor. Sample injections of 25µL were made with a Dionex AS-100 autosampler held at 4 °C. The mobile phase used for this application was a mixture of 20mM methanesulfonic acid (MSA) and nanopure water with a flow rate of 1 mL/minute. Gradient elution was used to optimize the separation, with the ratio of water decreasing from 90% to 18% over 30 minutes.

Exposure of Meat Samples to Ammonia Contaminated Air:

Cylinders of nitrogen gas certified to contain 200 ppm ammonia were obtained from Linweld (Lincoln, NE), a division of Matheson Tri-Gas. Cylinder outlet pressure was regulated at 4 psi, and a rotameter flow meter was used to control the rate of gas flow. A meat sample was suspended from a hook inside a polystyrene desiccator cabinet (18 liter volume, Bel-Art Scientific) that was used as the sample chamber for exposing the samples to the ammonia-containing gas. The desiccator cabinet was equipped with ports for gas inlet and outlet, and a door with a silicone rubber seal that allowed samples to be added and removed. Tygon tubing was used to connect the cabinet to the flow meter. An Eagle^{TM} ammonia monitor was connected to the outlet port of the cabinet so that the concentration of ammonia in the gas being exhausted from the sample chamber could be monitored. The desiccator cabinet used as the sample chamber was small enough that it could be placed inside either a laboratory refrigerator or freezer, as needed, to control the temperature at which the sample was held during exposure.

The protocol used for exposing the samples to ammonia involved flushing the chamber for four minutes at maximum flow rate after a sample was placed inside, in

order to exhaust the ambient air. The flow rate was then adjusted with the rotameter to 25 ml/sec and at that point timing was started, with flow maintained at that rate for the duration of the treatment. The ammonia content of the exhausted gas was monitored with the Eagle™ gas monitor to insure that a steady-state condition was maintained. Samples were exposed for the following combinations of times and temperatures:

Ambient temperature (approximately 22º C) for 1, 2, 3, 4, 5, and 6 hours

Refrigerator temperature (3- 5° C) for 1, 2, 4, 6, 9 and 12 hours

Freezer temperature (-13º C) for 1, 2, 4, 6, 9, and 12 hours

Each temperature/time combination was replicated three times. For each replicate, an unexposed sample of the same top round was measured for background ammonia content, and that value subtracted from the total ammonia content of the exposed sample, in order to obtain an accurate measure of the ammonia absorbed from the air.

Treatment of Meat Samples to Remove Ammonia Contamination:

Air Flushing:

A sample of top round was treated with 200 ppm ammonia for four hours at refrigeration temperature. The sample was subdivided, one portion was analyzed for ammonia content by the ISE method, and the other portion was returned to the sample chamber and flushed with clean air. Compressed air was introduced into the chamber at a rate of six liters per minute, such that ten complete exchanges of air occurred inside the cabinet every thirty minutes. The sample was held at refrigeration temperature during the air flush, and the air was bubbled through water prior to entering the chamber, in order to saturate the air with moisture and minimize surface dehydration of the sample. After the

air flush treatment, the ammonia concentration in the sample was measured by the ISE method. Samples were flushed with air for up to 2 hours, with 3X replication of each treatment time.

Vacuum Treatment:

A sample of top round was treated with 200 ppm ammonia for four hours at refrigeration temperature. The sample was subdivided, one portion was analyzed for ammonia content by ISE, and the other portion was placed in a vacuum desiccator. The vacuum desiccator containing the sample was held at refrigeration temperature while pulling a vacuum equal to 75 mm Hg on the sample. After the vacuum treatment, the ammonia concentration in the sample was measured by the ISE method. Samples were vacuum treated for up to 2 hours, with 3X replication of each treatment time.

Acid washing:

A sample of top round was treated with 200 ppm ammonia for four hours at refrigeration temperatures. The sample was subdivided, one portion was analyzed for ammonia content by ISE, and the other was rinsed twice with a 2% solution of acetic acid. The acetic acid rinse was applied to the sample using a polyethylene wash bottle, and sufficient solution was applied so that excess solution dripped from the meat sample. Each sample was rinsed twice with the acid solution, after which the sample was analyzed for ammonia content by the ISE method. The acid wash treatment was replicated three times.

Results:

Analytical method:

A comparison of the recovery values for the different extraction solvents evaluated showed that a 0.01 M phosphate buffer adjusted to pH 6 gave maximum recovery of ammonia. Also, the reproducibility of the ion chromatography method was found to be no better than the ISE method. Therefore, samples in this study were analyzed primarily using the ISE method, due to its greater speed and simplicity. Recovery results and reproducibility of the optimized ISE method are shown in Table 1.

Table 1. Recovery of ammonia from spiked top round samples.

AMMONIA CONCENTRATION IN MEAT (PPM)	AVERAGE RECOVERY (%)	CV(%)
10	91.2	14.0
20	91.0	8.1
50	93.7	5.4
100	94.5	4.5
150	94.5	4.0
200	95.1	3.7

Recoveries were >90% for all ammonia levels evaluated, with recoveries approaching 95% at levels of 100 ppm and above. The CV's obtained were rather high at the lowest ammonia concentrations, but at concentrations of 50 ppm and greater, CV's of 5% or less were achieved. We found these results acceptable, and because of the high recoveries obtained, aqueous ammonia standards were used for calibrating the electrode rather than

extracts of spiked meat samples.

Results of ammonia exposure:

Ammonia concentrations of samples exposed to 200 ppm ammonia for different times at ambient and refrigeration temperatures are shown in Figures 1 and 2, respectively.

Figure 1. Rate of ammonia uptake of fresh top round exposed to 200 ppm ammonia at ambient temperature.

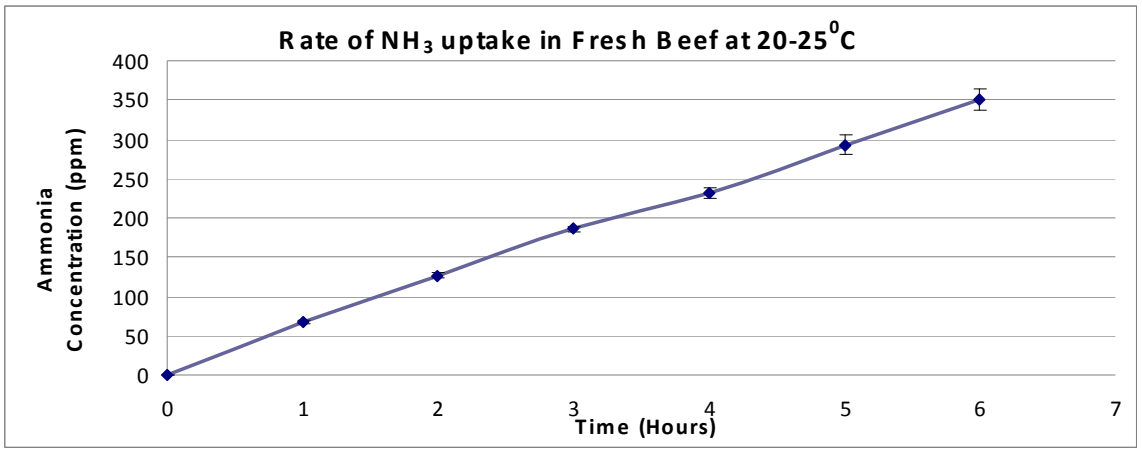
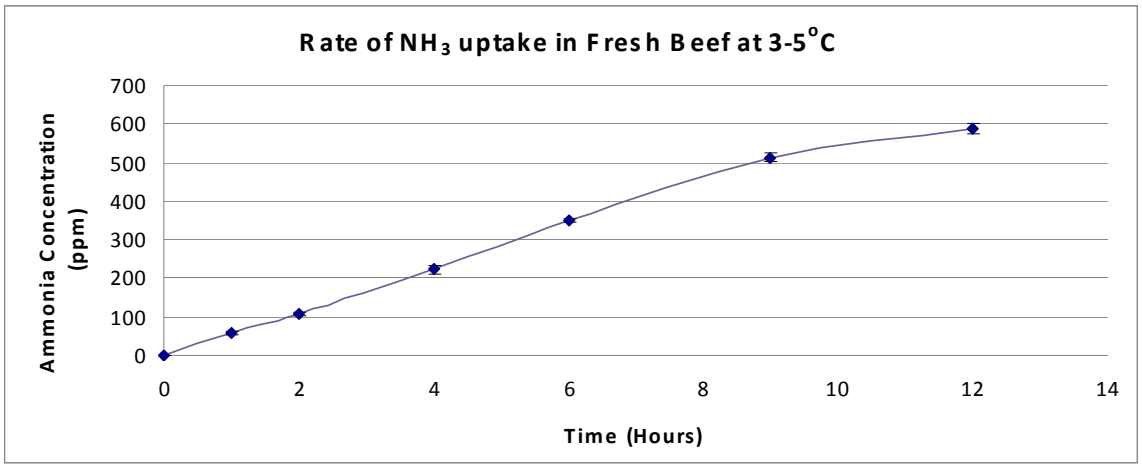


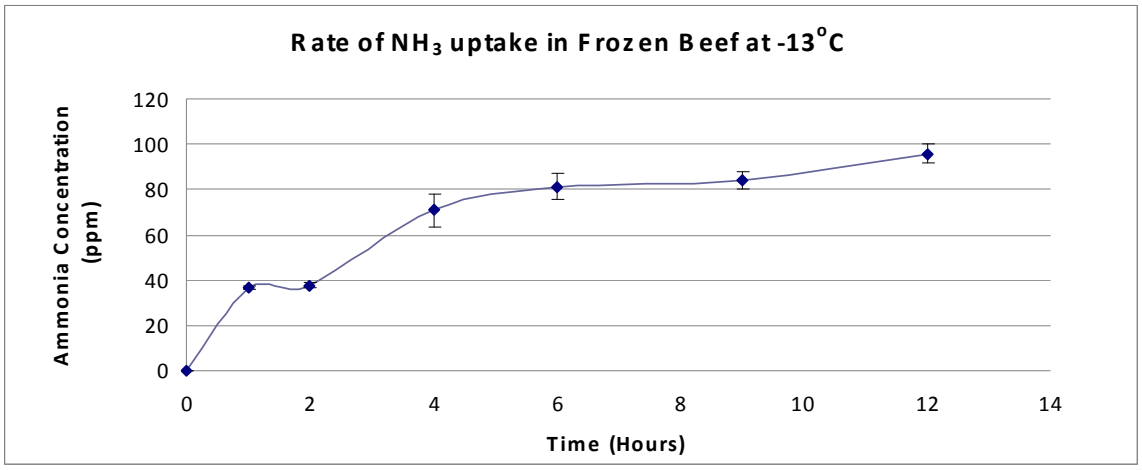
Figure 2. Rate of ammonia uptake of fresh top round exposed to 200 ppm ammonia at refrigeration temperature.



The rate of ammonia uptake was linear up to six hours at room temperature, and up to nine hours at refrigeration temperature, with r^2 values >0.99 for both temperatures. The rate of increase in ammonia concentration for both temperatures was identical at 58 $\pm$ 7 ppm/hour. Exposure times greater than six hours were not evaluated for the room temperature, due to the fact that microbial and enzymatic changes would begin to contribute to the ammonia levels at longer times. For samples exposed at refrigeration temperatures, evidence of saturation begins to appear above nine hours, as the rate of increase in ammonia concentration begins to decline. It should also be noted that at both temperatures, the concentration of ammonia in the meat increases above the 200 ppm level to which it was exposed. This implies that proteins or others substances in the meat are actively absorbing and binding the ammonia, and that more than a simple diffusion process is occurring.

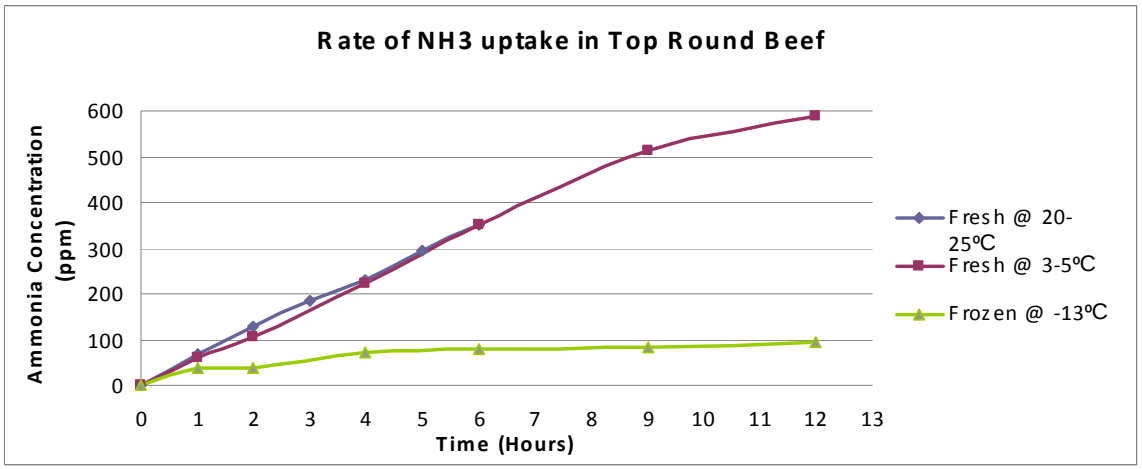
The pattern of ammonia absorption by frozen samples is quite different from absorption by fresh meat samples. Ammonia levels of frozen samples at different exposure times are shown in Figure 3.

Figure 3. Rate of uptake of frozen top round exposed to 200 ppm ammonia.



The rate of uptake in frozen samples is not linear, and the concentration of ammonia achieved after twelve hours is much lower. In fact, additional increases after four hours exposure time are minimal. Results of exposure at all three temperatures are summarized in Figure 4.

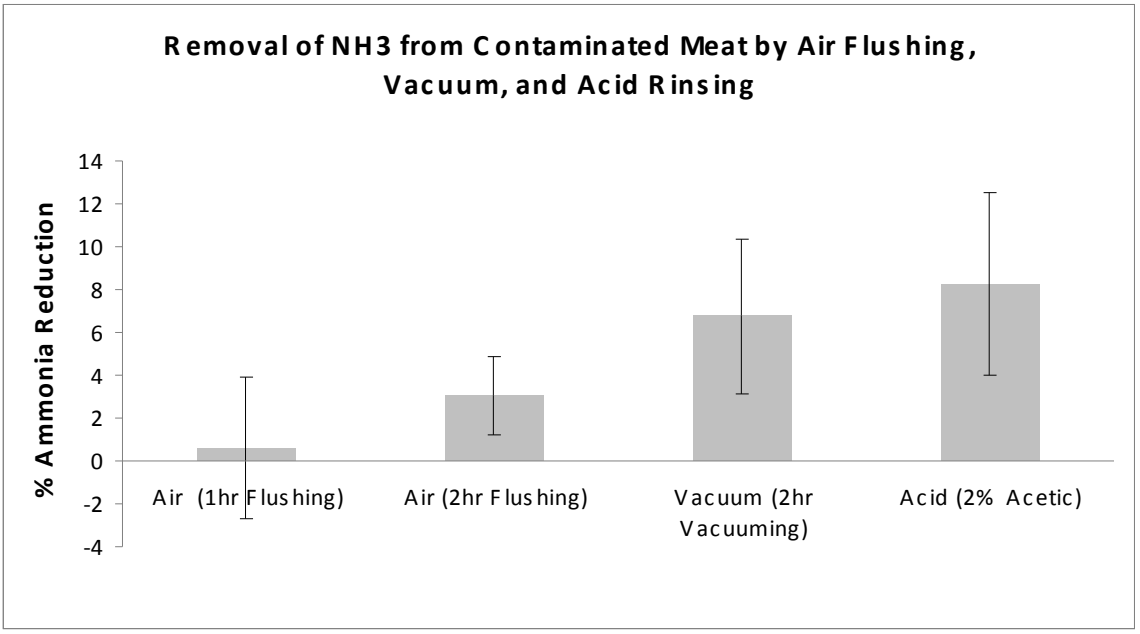
Figure 4. Summary of exposure of top round to 200 ppm ammonia at different temperatures.



Treatment of Meat Samples to Remove Ammonia Contamination:

All three treatments evaluated for removing ammonia from contaminated samples had minimal effectiveness (Figure 5). When starting from an ammonia concentration of 250 – 300 ppm, air flushing after two hours (equivalent to 40 complete exchanges of air) resulted in an average reduction of only 3%. Vacuum treatment of samples for 2 hours gave somewhat more variable results, but the average reduction achieved was under 10% and unlikely to be of commercial value. Acid washing also proved to be relatively ineffective, resulting in reductions of less than 10% from starting values.

Figure 5. Summary of ammonia reduction by air flushing, vacuum and acid rinsing treatments.



Conclusions:

The following conclusions can be made as a result of this study:

1) The ion selective electrode method is a relatively simple and rapid procedure that can accurately measure ammonia concentration in contaminated meat. The method should be applicable as a quality assurance procedure in commercial meat processing facilities.

2) Fresh meat at both room and refrigeration temperatures have similar linear rates of ammonia uptake. Saturation does begin to happen after longer (12 hour) exposure times.

3) The level of ammonia contamination in fresh meat can be greater than the concentration of ammonia in the surrounding air. Meat components appear to actively absorb and bind ammonia in what appears to be more than a simple diffusion process.

4) Freezing substantially slows the rate of ammonia uptake in meat.

5) Simple methods for treating ammonia contaminated meat (air flushing, vacuum treatment, and acid washing) were not successful in substantially reducing the level of ammonia contamination.

Recommendations for Future Research:

We are currently evaluating the effectiveness of barrier packaging films on the rate of ammonia uptake by fresh beef at refrigeration temperatures. Because of delays in receiving our last shipment of ammonia-in-nitrogen gas, we have not finished evaluating the data in time to include in this report. We will submit a supplementary report with our results as soon as our analysis is complete.

Additionally, we find it significant that the ammonia concentration in meat can exceed the concentration in the surrounding air. It would be of benefit to understand which proteins or other components in muscle are primarily responsible for binding ammonia. Also, the need for effective remediation processes remains, as the simple procedures investigated in this study were mostly ineffective.

Literature Cited:

Hijaz, F., Smith, J.S., and Kastner, C.L. Evaluation of various ammonia assays for testing of contaminated muscle food products. *J. Food Sci.* **2007**, 72, C253-257.