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AMMONIA ION SELECTIVE ELECTRODE AND INDOPHENOL METHODS CAN BE USED SUCCESSFULLY TO EVALUATE MEAT CONTAMINATED BY AMMONIA

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Introduction

Anhydrous ammonia is used as a refrigerant in large warehouses for cooling meats, fruits, vegetables, milk, and other products. Ammonia offers several advantages over other refrigerants; it does not harm the ozone layer and is a very efficient heat transfer agent. However, cold storage facilities sometimes have ammonia leaks. When this happens, products are held for an indeterminate period or are condemned because there is no official method to evaluate the degree of product contamination. In one case, a warehouse owner discarded a product because he could not prove that it was safe. His insurance company would not compensate him because he failed to prove that the product was not safe for human consumption. Over the last several years, many owners of refrigeration warehouses have experienced this problem.

Foodborne illness outbreaks caused by ammonia have been reported twice in the United States. On October 30, 1985, a foodborne outbreak was reported in two elementary school children in Wisconsin. The children suffered from burning of the mouth and throat, as well as nausea, within one hour of drinking milk packaged in half-pint containers. Analysis of the remaining containers revealed that the milk was contaminated with ammonia at levels ranging from 530 ppm to 1,524 ppm. The pH levels of the contaminated milk ranged from 9.1 to 10.0, while normal milk pH ranges from 6.7-6.9. This was the first reported incident of acute ammonia poisoning by the Centers for Disease Control and Prevention. On November 25, 2002, another outbreak was reported in several dozen school children in Illinois. The children suffered from stomachache, nausea, and headache within one hour of eating chicken tenders. A laboratory investigation by the U.S. Department of Agriculture's Food Safety Inspection Service (FSIS) showed that the chicken tenders were contaminated with ammonia at levels ranging from 552 ppm to 2,468 ppm. Assessment of ammonia damage to determine whether food is fit for human consumption is based on tentative methods because published information is limited. According to the Food and Drug Administration (FDA), at least three different measurement methods should be used to assess contaminated products: ammoniacal nitrogen, sensory test, and pH measurement. The objective of this study was to evaluate assays for ammonia detection so that they could be used for rapid in-plant testing of meat contaminated by ammonia refrigerant leaks and to determine the ammonia background of different meat products using the ammonia ion selective electrode (ISE).

Experimental Procedures

Evaluations of ammonia ion selective electrode, indophenol, salicylate, and Reflectoquant[1]

[1]Reflectoquant is a registered trademark of Gallade Chemical Co., Santa Ana, CA.

test strip methods were done using ground eye of round beef spiked with ammonium chloride as standard. Beef samples were spiked with 25, 50, 100, or 200 ppm ammonia as nitrogen (N) and the amounts recovered were background corrected, depending on the background determined on the day of analysis. The ammonia-electrode assay was performed on aqueous homogenate and on a perchloric supernate. Meat protein was precipitated using perchloric acid, trichloroacetic acid, or tungstic acid and an aliquot of the filtrate was tested with indophenol, salicylate, or Reflectoquant test strips. To determine ammonia concentration in perchloric acid supernate from beef by ion selective electrode, meat protein was precipitated with perchloric acid. Ammonia liberated in the supernate upon alkalization was then measured with the ion selective electrode.

After evaluation of various ammonia assays, beef, pork, and chicken products were obtained from local stores and analyzed with the ion selective electrode to determine the normal ammonia background in these products. The ion selective electrode was chosen because it is fast, sensitive, and has a broad dynamic range.

Results and Discussion

The precision of the Reflectoquant test strips was evaluated by measuring a known ammonium chloride standard. The coefficient of variation was 11.6%, which means that this method is not precise (data are not shown in this paper). The recovery of ammonia from spiked beef samples by the Reflectoquant method ranged from 77.4% to 96.9% and the standard deviation (SD) was higher than 14% at all spiked levels (Table 1). The reaction of salicylate with ground eye of round beef was slow due to the interference of protein fragments. The recovery of ammonia from spiked beef samples by the salicylate methods was low (Table 2 and 3) when samples were spiked with low ammonia levels (25 and 50 ppm ammonia as nitrogen).

Recovery of the indophenol method was better than that of the salicylate and the Reflectoquant test strips, especially when perchloric acid was used to precipitate meat protein. Recovery of ammonia by indophenol method ranged from 95.4% to 113% and the SD was lower than 8.3% (Table 2).

The recovery of ammonia from the spiked beef filtrate by ion selective electrode ranged from 98.3% to 100% and the SD was less than 2%, while the recovery of ammonia from spiked beef samples by ion selective electrode-perchloric acid method, developed in our lab, ranged from 90% to110% and the SD was less than 7.6% (Table 3). This new method offers many advantages. It decreases the response time of the membrane, prevents any drift in the electrode potential, increases the useable life of the membrane, and gives excellent recovery.

The ammonia backgrounds of different meat products, analyzed with ammonia-ion selective electrode by direct homogenization, are shown in Table 3. These backgrounds are important because the FDA recommends analysis of similar foods that have not been exposed to ammonia to determine the normal ammonia background. According to the FDA, the product can be released if its ammonia content does not exceed the normal value by 1%. Our values (Table3) are lower than those obtained using the official ammoniacal nitrogen method.

Implication

Both ammonia-ion selective electrode and indophenol methods are precise and accurate. Rapid methods that can be used for in-plant testing of muscle food products potentially contaminated by ammonia refrigerant leaks.

Table 1. Summary of the Average Percent Recoveries of Ammonia Spikes in Ground Eye of Round Beef

Spiked Level	Indophenol	Salicylate	Reflectoquant Test Strips	Ion Selective Electrode	Number of Samples
Background ppm ammonia as (N)	78.4 ± 3.1	103.7 ± 10.1	93.3 ± 15.5	74.8 ± 1.4	10
25 ppm ammonia as (N)	78.0 ± 25.3	63.3 ± 32.2	96.9 ± 34.4	100 ± 1.8	10
50 ppm ammonia as (N)	85.8 ± 11.4	81.5 ± 11.1	81.0 ± 32.7	99.6 ±1.6	10
100 ppm ammonia as (N)	79.9 ± 5.4	98.0 ± 16	92.2 ± 14.2	99.2 ± 1.1	10
200 ppm ammonia as (N)	82.5 ± 5.0	99.3 ± 13.4	77.4 ± 15.0	98.3 ± 0.9	10

*Meat protein was precipitated with 10% sodium tungstate and 1 N sulfuric acid in the indophenol, salicylate, and Reflectoquant methods. Ammonia was extracted using distilled water in the ion selective electrode method, and the sample extract was spiked after recording the background reading.

Table 2. Summary of the Average Percent Recoveries of Ammonia Spikes in Ground Eye of Round Beef using 0.3 M Perchloric Acid as a Deproteinizing Agent

Spiked Level	Indophenol	Salicylate	Ion Selective Electrode	Numnber of Samples
Background ppm ammonia as (N)	93.9 ± 4.0	109 ± 6.5	103 ± 1.5	5
25 ppm ammonia as (N)	113 ± 8.3	34.7 ± 21.6	89.9 ± 6.1	5
50 ppm ammonia as (N)	109 ± 3.6	44.5 ± 11.3	93.5 ± 7.6	5
100 ppm ammonia as (N)	98.6 ± 5.8	118 ± 9.7	110 ± 3.1	5
200 ppm ammonia as (N)	95.4 ± 4.4	100 ± 3.0	102 ± 1.0	5

Table 3.Ammonia Background in Different Commercial Meat Products Analyzed by Direct Homogenization Using the Ion Selective Electrode

Type of Product	Ammonia Background (ppm)	Number of Samples	SD
Ground Chuck (80:20)	99.8	6	4.2
Beef eye of round (90:10)	134	6	5.3
Top loin beef	120	6	6.7
Turkey thigh	149	5	18
Chicken breast	166	6	10.6
Chicken thigh	150	6	21.1
Top loin pork	136	6	13.5
Pork leg (steak)	141	6	10.4
Breakfast sausage (turkey)	113	6	11.8
Chicken nuggets	87.2	6	8.6
Turkey franks	87.0	6	9.6