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Development of Protocol for Measuring Ammonia Evolution from Meat during Spoilage

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The purpose of this project was to determine a procedure for monitoring beef spoilage with commercially available sensors to serve as a point of comparison to sensors I hope to develop in the lab. The process of spoilage in beef samples was monitored over the course of 10-days with an ammonia gas sensor, carbon dioxide sensor, and oxygen sensor. The influence of two main factors, temperature, and size were observed. For temperature, beef samples were held at ambient, cold, and freezing temperatures. To determine how sample size influenced spoilage, samples were prepared between 5.0-grams and 15.0-grams. The experiment's results showed the spoilage was observed with the 5.0-gram samples stored at ambient temperature. Here, the conditions corroborated the relationship between carbon dioxide, oxygen, and ammonia.

Studying meat spoilage is of interest throughout the scientific community in order try to prevent food poisoning. Food poisonings or food related illnesses are an ongoing issue in the United States. Over the past 20 years, there have been 8 outbreaks related to food-related illnesses [1]. Yearly, the United States encounters millions of cases for food related illnesses per year, where the majority resulting from ground beef consumption. In 2006, electronic nose instrumentation [2] was used to detect odors but not concentrations of certain chemicals. The limits for this technology were in its reproductivity, sensitivity, and selectivity [3]. However, even with these advancements, there is still further exploration needed in this area. Recent studies have been directed towards obtaining better methods to detect the spoilage process. At the University of Texas, a research group investigated the detection of ammonia via "polymer-based" [4] gas sensors. These sensors were paired with a communication circuit smartphone to detect the presence of cadaverine and putrescine and other "biogenic amine gases" [4].

The focus of this research reported here was to study meat spoilage from first-hand experimentation to corroborate the factors involved in the process of beef decomposition and determine an appropriate apparatus for making measurements. The components used to monitor the spoilage process were carbon dioxide (CO_2), oxygen (O_2), and more importantly ammonia gas (NH_3). NH_3 gas accumulation is an indicator the decaying process is occurring [5]. These gases were measured for samples stored in varying environments, such as ambient, cold, and freezing temperatures [6]. The application of this information could be used to detect meat spoilage with sensors made with composites of polypyrrrole and molybdenum oxide. Ammonia is an important factor to detect the contamination of a meat. In this project, following the spoilage process with multiple commercial sensors, results suggest that it usually takes 4 to 6 days for ammonia to become detectable, while carbon dioxide and oxygen decrease over the course of the spoilage process. This is consistent with other work [7].

In this experiment, CO_2, O_2, and NH_3 were monitored on beef samples of approximately 5.0 grams, 10.0 grams and 15.0 grams over the course of 10-days that were stored in ambient, cool, and freezing temperatures. The beef was obtained from a White Plains Stop & Shop and weighed approximately 707 grams, after which the external surface was removed to eliminate possible error. Each sample was placed into a labeled BioChamber 250 Vernier bottle. The ten samples

were cut into cubes and labeled from 1-10. The distribution of samples in the three conditions can be seen below.

Table 1. Distribution of Samples

Sample #	Estimated Mass	Actual Mass	Environment	Temperature Range (℃)
1	5.0g	5.041g	Ambient	24.0-26.0
2	10.0g	10.349g	Ambient	24.0-26.0
3	15.0g	14.965g	Ambient	24.0-26.0
4	5.0g	5.067g	Ambient	24.0-26.0
5	10.0g	10.101g	Ambient	24.0-26.0
6	15.0g	14.974g	Ambient	24.0-26.0
7	10.0g	10.245g	Freezer	2.1 to 2.3
8	10.0g	10.228g	Freezer	2.1 to 2.3
9	10.0g	9.756g	Refrigerator	8.5 to 8.7
10	10.0g	10.291g	Refrigerator	8.5 to 8.7

The sensor set-up was configured as shown in Figure 1. For each set up, it was ensured the samples did not touch any of the sensors.

Figure 1. Apparatus set-up for detection of CO_2, O_2, and NH_3 for Beef Samples

A total of 21 readings were acquired for each sample for 10 minutes for 10 consecutive days. To gather the data, Vernier software and manual logging was used to collect the data and each data set was entered into a MS Excel spreadsheet. Graphs of the raw data were prepared showing gas concentration vs time, where time was measured in minutes and concentration in parts per million (ppm). A second set of graphs was constructed, plotting only measurements acquired between minutes eight and ten. The graphs were taken and compared to one another to identify which samples showed the most valuable information (See Figure 2). Especially, the already validated trend between carbon dioxide, oxygen, and ammonia, seen in other studies [5][6], were seen here.

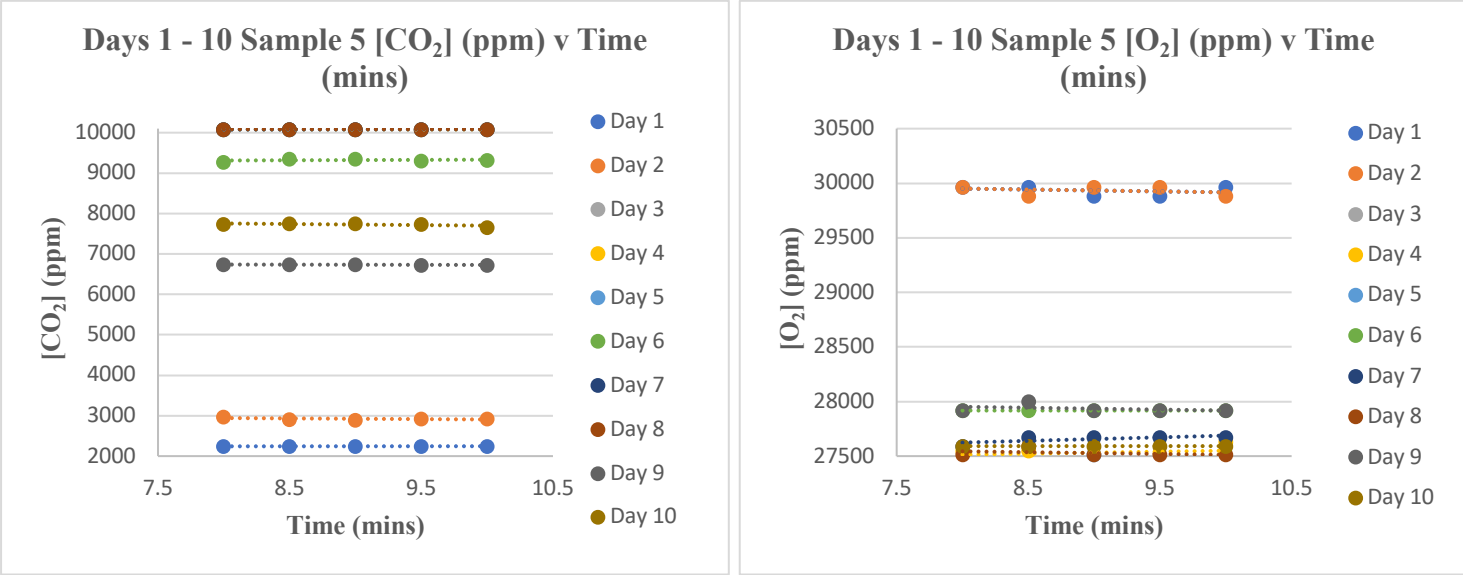

Figure 2. Sample 5: Relationship between Concentration(ppm) and Time(min) for CO_2 and O_2

Table 2. Data Collected for Ammonia Concentration(ppm) for Sample 5 over 10-Day period

Sample 5	Ammonia Gas (ppm)									
Time (mins)	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
0.0	0	0	0	0	0	0	0	0	1	0
0.5	0	0	0	0	0	0	1	0	1	1
1.0	0	0	0	0	0	0	1	0	1	1
1.5	0	0	0	0	0	0	1	0	1	1
2.0	0	0	0	0	0	0	1	1	1	1
2.5	0	0	0	0	0	0	1	1	2	2
3.0	0	0	0	0	0	1	1	1	2	2
3.5	0	0	0	0	0	1	1	1	2	2
4.0	0	0	0	0	0	1	1	1	2	2
4.5	0	0	0	0	0	1	1	1	3	2
5.0	0	0	0	0	0	1	1	1	3	2
5.5	0	0	0	0	0	1	1	1	3	3
6.0	0	0	0	0	0	1	1	1	3	3
6.5	0	0	0	0	0	1	1	1	4	3
7.0	0	0	0	0	0	1	1	2	4	3
7.5	0	0	0	0	0	1	1	2	4	3
8.0	0	0	0	0	0	1	1	2	4	3
8.5	0	0	0	0	0	1	1	2	4	4
9.0	0	0	0	0	0	1	2	2	5	4
9.5	0	0	0	0	0	1	2	2	5	4
10.0	0	0	0	0	0	1	2	2	5	4

In the end, ammonia evolution was observed in samples stored at ambient temperature between days 1 through 9, for samples 1 through 6. For example, with Sample 5, day 10 showed a negative relationship that was not expected which can be seen in the graphs and table above. The numbers did not reach the established amount from the previous day, showing a negative correlation. For days like this, throughout samples 1 through 6, CO_2 and O_2 followed the expected pattern. However, samples in freezing and cold temperatures did not show any evolution of ammonia.

The error observed in days 9 and 10 can be attributed to the ammonia device's sensitivity. It is likely with further exposure to the sample in addition to the ten minutes of exposure from the original procedure, more information could have been obtained. Since there was an accumulation area for the ammonia sensor, instead of a direct reading, this could have caused a lag time in data recording. Nevertheless, the raw data were mainly observed between the 8 to 10-minute mark to take this lag time into account. However, it is possible more time was necessary for samples after the 9^{th} day which resulted in skewed data.

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