



# Investigation of the Effects of Oxygen and Other Considerations on the Shelf-Life of Craft Beer

A Major Qualifying Project Report

Submitted to the faculty of

Worcester Polytechnic Institute

In partial fulfillment of the requirements for the

Degree of Bachelor of Science

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## **Abstract**

The goal of this project was to determine the effects of dissolved oxygen (DO) and other phenomena on the shelf-life of Purgatory Beer Company's Two-Car Garage Double IPA, maintained at two separate storage temperatures. Indigo carmine titration and oxygen analyzing equipment were used to identify the changing profile of DO, while sensory analysis and Fourier-transform infrared spectroscopy were used to measure variations in physical qualities and chemical composition. Data from these tests indicate that the DO reactions were most likely diffusion-limited. Recommendations of a 7-week shelf-life and refrigerated storage were made to Purgatory Beer Company after analysis.

## **Acknowledgements**

Our team would like to acknowledge several people and organizations that aided greatly in the success of our project, to whom we are indebted:

Professor Stephen Kmiotek, PhD, PE, our advisor, for his steadfast commitment to guiding our team through the entirety of our project; his intellect and unwavering sense of humor were essential to the success, spirit, and continued education of our team.

Purgatory Beer Company, for providing us the opportunity necessary to expand our knowledge and experiences as Chemical Engineers. A special thanks to Brian Distefano and Kevin Mulvehill for their constant sacrifices of time and resources to further our project and introduce us to the world of craft beer.

Iron Heart Canning Company, for volunteering their time to collect dissolved oxygen data when our own equipment could not. These data significantly improved the quality of our findings.

Professor Drew Brodeur, PhD, for the helpful organic advice, as well as the several months spent running sizable samples on complex equipment.

Professors Michael Timko, PhD, Andrew Teixeira, PhD, John Bergendahl, PhD, and Eric Young, PhD, for their many consultations that substantially aided our analyses.

Tom Partington, for sharing his vast knowledge of laboratory equipment and procedures with our team and for answering all our impromptu questions.

Avery Brown, for the days of laboratory training and access he gave to us, as well as his unrivaled devotion to meeting attendance.

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## Introduction

Breweries, as well as canning companies, acknowledge the negative effect that dissolved oxygen within beer has on the stability of the taste. In some cases, if the oxygen concentration is measured at an unacceptably high value prior to canning, such as 100 ppb, the product life will not be adequate, and the company will not proceed with the canning. Over time, the dissolved oxygen can react with organic molecules in the beer to negatively affect, among other things, the product's taste, color, and turbidity, which may affect the customer's perception of the beer. Furthermore, despite many studies to determine the shelf-life and analyze what reactions are likely to occur, no full conclusion exists because of the large variability of organic compounds available due to differences in style and raw material.

This ambiguity has led small, local breweries, such as Purgatory Beer Company of Massachusetts, to develop independent methods to approximate shelf-life. A common heuristic used is known as the "3-30-300 Rule" which was developed from a study by MillerCoors and states that a beer ages the same amount in 3 days at 90°F as it does in 30 days at 71°F and 300 days at 33°F.<sup>1</sup> However, this rule specifically applies to filtered, pasteurized beer, and as Purgatory Beer Company brews and sells unfiltered, unpasteurized beer, this rule does not necessarily provide an adequate approximation required for the retail sale of canned beer.

On behalf of Purgatory Beer Company, we developed and performed an experiment to determine the shelf-life of one of their signature beers, "Two-Car Garage DIPA." During this time period, we quantified changes in the oxygen concentration, chemical composition, and the physical qualities of the beer, such as color and taste. The beer was held at two different storage temperatures and tested over a range of 10 weeks. We utilized a modified titration method to measure the change in the dissolved oxygen, Fourier-transform infrared spectroscopy (FTIR) and gas chromatography/mass spectroscopy (GC/MS) to determine the chemical composition, and a sensory analysis survey to quantify the changes in the physical characteristics of taste, aroma, and color. The beer samples were divided into two groups, one of which was stored in a refrigerated and the other at room temperature to investigate the difference in the aging process under two likely temperature storage conditions. According to the "3-30-300 Rule" and the available literature, we hypothesized that the reactions responsible for the taste degradation of beer would likely be thermodynamically controlled, resulting in the faster depletion of dissolved oxygen and shorter shelf-life in the room temperature (warmer) beer samples.

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<sup>1</sup> Martin, A. n.d.

## Background

The brewing and canning processes of beer have become standardized regardless of the size of the brewery and the type and uniqueness of the beers produced, due to the long and enjoyable history of brewing beer. Over time, these processes have been optimized to minimize oxygen contamination, which is acknowledged to cause several reactions that contribute to the aging and taste degradation of the beer. Beers experience many additional reactions, often based on their unique chemical compositions, and a full catalogue of every possible reaction is therefore not available from the literature.

### 1.1 Operation Processes

Oxygen primarily enters the beer solution through the brewing and canning processes. Over time, these processes have been optimized and improved to minimize the contact between oxygen and the beer.

#### 1.1.1 *Brewing Process.*

Over the centuries-long existence of beer, the brewing process has been standardized to a general practice carried out by both large and small breweries. Therefore, while Purgatory Beer Company constantly invents novel varieties of beer with unique ingredients and proportions, the types of ingredients remain relatively consistent with those found in previously studied beers.<sup>2</sup> The standard process involves, in addition to yeast, the combination of water, grain (barley or wheat), and hops.<sup>3</sup> This combination of ingredients ensures that, dominantly, the chemical compounds found in beer are long-chain hydrocarbons, usually from 8 to 24 carbons long, such as eicosane.<sup>4</sup> Moreover, this hydrocarbon mixture contains both alkanes and alkenes from the saturated starches in the grains and the unsaturated fatty oils in the hops, respectively. Additionally, some brews may contain organic compounds with ester or sulfuric functional groups due to specialty hops and other ingredients added to create a desired taste.<sup>5</sup>

All brewing processes begin with the heating, mashing, and mixing of the grain in order to breakdown the starches into simple sugars. This substance is now referred to as “wort.” The wort is then combined with the hops, and any additional flavorants, which will be boiled. The hops contain a variety of fatty oils that are essential in giving each style of beer a unique, distinct taste.<sup>6</sup> After boiling, the liquid portion of the mixture is combined with yeast and allowed to ferment for a set period. Purgatory Beer Company typically allows its beer to ferment for several weeks. During or after the fermentation process, more hops can be added to the beer without further heating; this step is called “dry hopping”. In small batch brewing processes, oxygen can easily be

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<sup>2</sup> Bamforth, C.W. (ed.) **2009**.

<sup>3</sup> Sileoni, V., Marconi, O, Perretti, G. **2012**.

<sup>4</sup> Roberts, M.T., Dufour, J-P., Lewis, A.C. **2003**.

<sup>5</sup> Pickett, J. Berdahl, D. 2019.

<sup>6</sup> Bamforth, C.W. (ed.) **2009**.

introduced at any of the combination or transfer steps.<sup>7</sup> Therefore, brewers attempt to minimize the oxygen by purging their equipment (particularly the fermenter) with carbon dioxide before use.<sup>8</sup>

### 1.1.2 Canning Process

The canning process employed by Iron Heart Canning Company was designed to minimize the amount of oxygen that can diffuse into the beer. The open-topped cans were loaded onto the conveyer belt and tipped upside down, where they were doused with sanitizer. They were then brought back into the upright position and purged with carbon dioxide. Meanwhile, the filler spigots were connected directly to the fermentation tanks via pumping tubes. The cans were filled quickly to reduce the amount of oxygen, until a small head of foam surpassed the top of the can. This head was scraped off and the cans were given a final squirt of carbon dioxide; the lid was then dropped on, squeezed down, and sealed. Iron Heart Canning Company guarantees under 100 ppb of dissolved oxygen in the finished, canned product, with a preference for under 50 ppb.<sup>9</sup>

## 1.2 New England-Style IPA

New England-Style IPAs (NEIPA) are relatively new to the beer brewing industry; although the style is believed to have originated in the mid-1990s at The Alchemist in Vermont, it has risen to national popularity within the last ten years.<sup>10</sup> NEIPA was recognized just last year by the Brewer's Association in their 2018 Beer Style Guidelines through the following trio of categories: "Juicy or Hazy Pale Ale," "Juicy or Hazy IPA," and "Juicy or Hazy Double IPA."<sup>11</sup>

These IPAs have a relatively low level of bitterness, high hop aroma and flavor, and soft texture.<sup>12</sup> Many are described as "ripe," "juicy," and "fruity."<sup>13</sup> They have a "straw to deep gold" color, with noticeable levels of cloudiness, and an alcohol-by-volume (ABV) of 7.6-10.6%.<sup>14</sup> The haze of the IPA is often due to yeast, hop, and protein in the beer that has not been removed through centrifuging or filtering – most are dry-hopped near or at the end of fermentation.<sup>15</sup> Because of the lack of filtering, the storage of NEIPAs is key; when stored in warm conditions, yeast still in the beer may reactivate and continue the fermentation process, causing a build-up of pressure within the container.<sup>16</sup> Additionally, organic compounds may begin to degrade within the container, creating off-flavors.<sup>16</sup>

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<sup>7</sup> Bamforth, C.W. (ed.) **2009**.

<sup>8</sup> Hach Company 2014.

<sup>9</sup> Schaeffer, Z.; Caruso, A. 2019.

<sup>10</sup> Bernot, K. 2018, Moorhead, J. 2017.

<sup>11</sup> Brewers Association Releases **2018**, Beer Style Guidelines **2015**.

<sup>12</sup> Sparhawk, A 2018.

<sup>13</sup> Moorhead, J. 2017, Fisher, N. 2017.

<sup>14</sup> Brewers Association Beer Style Guidelines. **2015**.

<sup>15</sup> Sparhawk, A 2018, Brewer Association Beer Style Guidelines. 2018, Fisher, N. 2017.

<sup>16</sup> Bernstein, J. **2017**.

### 1.3 Two-Car Garage Double IPA (DIPA)

Our studies were conducted on Purgatory Beer Company's Two-Car Garage DIPA, which is a New England-Style IPA. The beer has an ABV of 8%. A combination of five different grains are used as the base of the beer, of which the largest portion is Pilsner. The beer is hopped with Centennial, Citra, and Mosaic hops before the yeast is added, and later dry-hopped with Citra and Mosaic. Its characteristic taste profile is a grapefruit/orange taste, a fruity, citrusy aroma, and a smooth finish. The beer itself can be cloudy due to hops and yeast that remain in suspension even after they are given time to settle out.

Two-Car Garage DIPA is a popular beer at Purgatory, requiring it to be brewed frequently to maintain stock. Due to its popularity, it was one of two beers Purgatory chose to can after contracting their first professional canning company, Iron Heart Canning Company. Before this study, the shelf-life for this beer was estimated as one month.

### 1.4 Expected Reactions in Aging Beer

According to the available literature, between the wort, hops, and yeast used in the brewing process, there can be as many as 600-700 compounds present in beer that can contribute to its overall flavor.<sup>17</sup> As reactions take place in the beer, these compounds can rise above or dip below their flavor thresholds, resulting in a change in flavor.<sup>17</sup> Although no two beers age identically, beers generally tend to decrease in bitterness, fruity and floral notes, and can be perceived as harsh.<sup>17</sup> In addition to a decreased intensity of positive flavors, the beer develops stale flavors simultaneously, leading to an overall decline in taste.<sup>18</sup> In general, pale and dry-hopped beers are more susceptible to aging than dark and kettle-hopped beers.<sup>19</sup>

The most well-documented reactions occurring in aging beer are due to reactive oxygen species (ROS). These are products of the dissolved oxygen in the beer, which can be activated to form radical and non-radical oxidizing agents, such as hydronium ( $\text{OH}^+$ ), oxide ions ( $\text{O}_2^-$ ), hydrogen peroxide ions ( $\text{HO}_2^-$ ), and hydrogen peroxide ( $\text{H}_2\text{O}_2$ ).<sup>20</sup> These ROS are integral to the formation of compounds that produce distinct off-flavors after reaching their flavor thresholds.<sup>20</sup>

Some of the most common compounds created through ROS reactions are Strecker aldehydes, ketones and ethyl esters, cyclic acetals, heterocyclic compounds, and more.<sup>21</sup> Hop bitter acids and polyphenols can also degrade in the presence of ROS.<sup>21</sup>

In terms of the effects on shelf-life, a MillerCoors study on beer storage developed the "3-30-300 rule," where beer ages the same amount in 3 days at 90°F as it does in 30 days at 71°F and 300

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<sup>17</sup> Bamforth; Lentini. **2009**.

<sup>18</sup> Bamforth. **1999** and Whitear; Carr; Crabb; & Jacques. **1979** as reported in Vanderhaegen, Neven, Verachtert, Derdelinckx. **2006**

<sup>19</sup> Aron. 2014.

<sup>20</sup> Wietstock; Kunz; Methner. **2016**.

<sup>21</sup> Vanderhaegen et al. **2006**

days at 33°F. This study indicates a strong temperature dependence of the staling reactions.<sup>22</sup> Similarly, other studies have found that beer stored at 20°C exhibited stale characteristics at approximately 90 days, while beer stored at refrigeration temperatures (about 4-5°C) went stale after approximately 325 days.<sup>23</sup> However, these studies were done using filtered, pasteurized beer, which has been treated to remove impurities and degrade compounds that are likely to react. Craft beers, which are typically unfiltered and unpasteurized, experience loss of flavor, flatness, and staleness much faster than pasteurized beers.<sup>22</sup>

## Methodology

To quantify the changing levels of dissolved oxygen in the beer, as well as any corresponding changes to the physical and chemical characteristics of the beer, a set of experiments were designed and improved by the project team over the course of several months. The following section provides an overview of the methods used to collect the data used in analysis. For a detailed description of all procedures, successful and discarded, as well as the rationale behind their development, please see Appendix A.

### 2.1 Sample Grouping

On January 14, 2019, the day of canning, 24 cans of Two-Car Garage DIPA were acquired from Purgatory Beer Company. Half of this sample group became the “refrigerated” group; these samples were placed in a refrigerator, kept at approximately 5°C. The other half became the “room temperature” group, stored on a lab bench at an average temperature of 20°C. The samples remained in their respective locations for the 10-week duration of testing.

### 2.2 Quantitative Determination of Dissolved Oxygen Content

To determine the amount of dissolved oxygen in the beer over time, a weekly titration was carried out on each sample (refrigerated and room temperature). The titration reacted a temperature-treated indigo carmine solution with the dissolved oxygen in the beer. This resulted in a color change; the final shade of the dyed beer solution, as well as the initial color of the beer, was recorded by photograph. To standardize the photographs, the samples were placed in a special-made photograph apparatus. The color analysis software *ImageJ* was later used to quantitatively determine the color change over time captured in the photographs.

The exact content of dissolved oxygen in both sample groups was measured by a Hach Orbisphere dissolved oxygen sensor on three separate occasions: the day of canning, mid-way through the testing period, and at the end of experimentation. These measurements were provided through the

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<sup>22</sup> Martin, A. n.d.

<sup>23</sup> Bamforth; Lentini. 2009.

courtesy of Iron Heart Canning Company, as the equipment available at WPI was not capable of reliably or accurately measuring dissolved oxygen contents below 1 ppm.

### **2.3 Sensory Analysis**

To determine the effects of dissolved oxygen on the physical characteristics of the beer, a sensory analysis test was developed. The goal of this analysis was to quantitatively measure changes in the following characteristics of beer: aroma, appearance (which includes color and clarity), flavor (which includes alcohol and bitterness), and mouthfeel (which includes carbonation and smoothness)<sup>24</sup>.

The final analysis consisted of a four-page rating survey, which can be seen in Appendix B. The survey and samples from each group beer were brought to Purgatory Beer Company weekly for internal blind sensory testing. To mask the storage conditions of each sample and remove bias, the refrigerated and room temperature samples were randomly labelled “A” or “B” each week.

### **2.4 Liquid-Liquid Extraction for FTIR and GC/MS**

To prepare the samples of beer for both Fourier-transform infrared spectroscopy and gas chromatography-mass spectroscopy, it was necessary to separate the organic component of the samples from the aqueous solution; this was done on a weekly basis to ensure both instruments were only reading the component of the beer relevant to analysis. To separate out organic compounds, di-chloromethane (DCM), an organic solvent, was added to samples of the beer. On each testing day, two samples were prepared from the refrigerated beer, and two samples were prepared from the room temperature beer. In later tests, pulverized calcium chloride salt was added to the beer solution to attain a greater separation. This added two more samples to the weekly batch. All samples were then shaken and centrifuged to assist separation. The organic layers of the separated solutions were pipetted into filter vials and sent to each instrument to be read. The entirety of this procedure was conducted at room temperature.

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<sup>24</sup> Beer Style Guidelines 2015.

## Data and Analysis

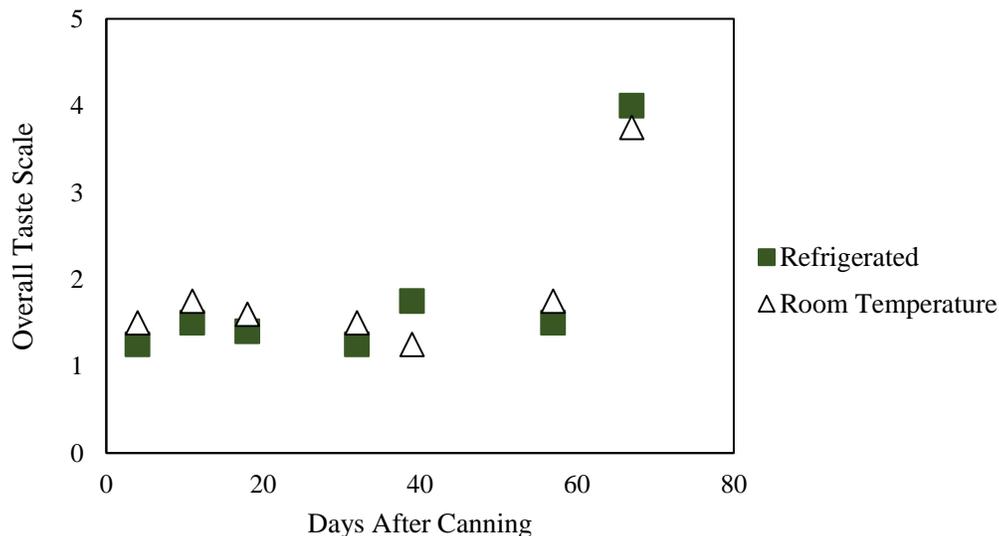
The data were collected over a span of 10 weeks on 2-Car Garage DIPA canned on January 14, 2019. Beer from a further canning run on March 4, 2019, was also acquired, but proved too different from the January canning run to be included in the analysis. This section contains analysis of the sensory data, the clarity and foaminess of the beer, dissolved oxygen content, and FTIR spectra.

### 3.1 Sensory Data

We collected sensory data on several different aspects of the aroma, appearance, flavor, and mouthfeel of the beer. Several of these aspects displayed no noticeable correlations or trends over time. The data points from weeks 1 and 2 were collected before the survey was finalized and therefore have estimated values. Additionally, data from weeks 4 and 7 were removed from the below figures, as they were given by a taste-tester who had a different interpretation of the provided scales. The entire sensory analysis data set can be viewed in Appendix C.

#### 3.1.1 Overall Taste of the Beer

Data collected on the overall taste of the beer showed very little variance over the first 9 weeks (see Figure 1). The refrigerated samples were generally rated better than the room temperature samples. The refrigerated and room temperature samples were generally rated within 0.25-0.75 points of each other; however, the room temperature samples often displayed off-flavors or aftertastes that were noted qualitatively during the tasting sessions.



**Figure 1:** Overall taste of the beer samples according to sensory analysis data. The provided scale was: 1=very good, 2=pretty good, 3=decent, 4=not very good, 5=bad.

Over the first 9 weeks, the quality of taste of the samples fluctuated, appearing to either improve or decline marginally. This could potentially be due to several factors, such as the carbonation level of the sample and the starting oxygen levels of each sample.

When the samples were prepared, they were poured from the cans into the sample jars and then refrigerated to retard the oxidative reactions. The lids of the sample jars sometimes allowed pockets of air to be trapped with the sample, which could then decrease the carbonation level by allowing gases to come out of solution. Higher amounts of carbon dioxide retention can create a fuller taste experience for a beer drinker, which then impacts their impression of the overall taste of the beer.<sup>25</sup>

Additionally, as previously stated, there was variability between the starting oxygen levels of each can. Cans with slightly higher starting oxygen levels would likely have slightly more stale flavors, as the rates of oxidation reactions are dependent on the concentration of oxygen available in solution.

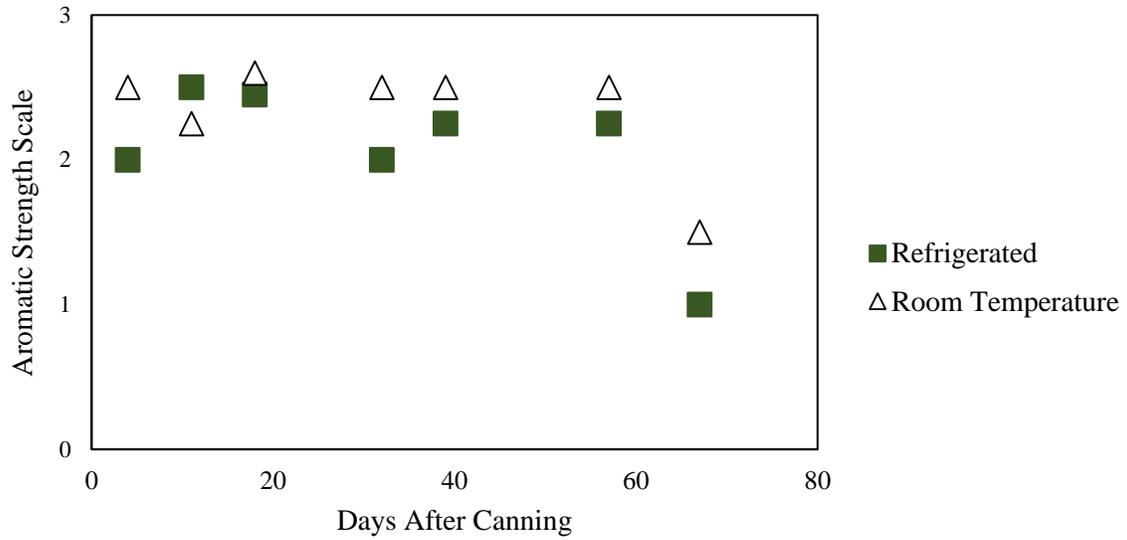
Both the refrigerated and the room temperature samples were described as having a “not very good taste” 67 days after canning, at which point they were deemed unsellable. It is likely that at this point, some stale or “off-flavor” compounds within the beer reached their designated taste threshold concentrations, giving the samples distinctly worse flavors than those tested previously. The heightened presence of these compounds was observed in the final week of FTIR testing, as described in Section 3.4.

### *3.1.2 Other Sensory Data*

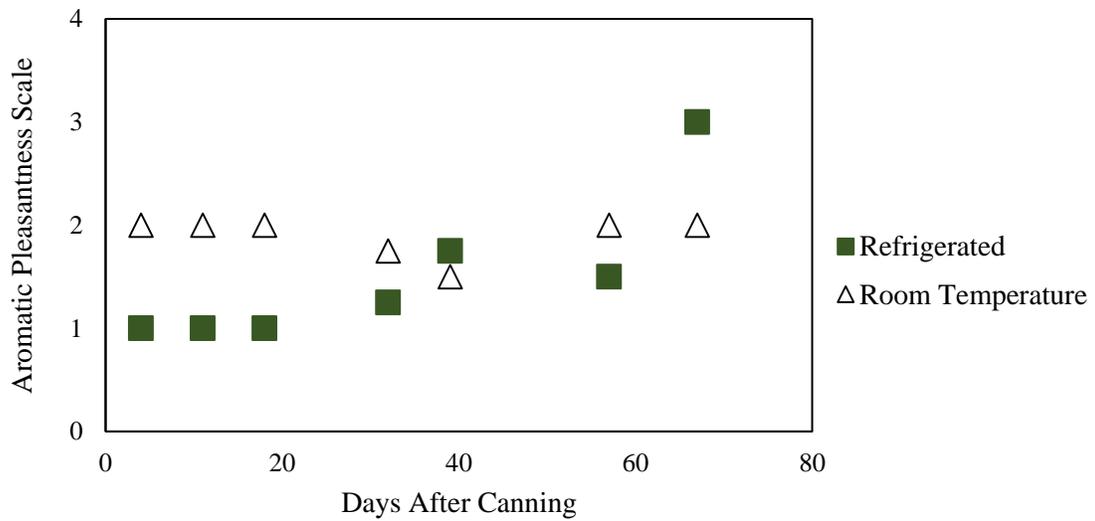
Other sensory aspects of the beer that displayed noticeable trends were the aromatic strength and pleasantness of the beer, as well as the strength of the citrus flavor. The aromatic strength and the citrus flavor of the beer declined over time, while the aromatic pleasantness went from “very pleasant” to “neutral” (Figures 2, 3, and 4). These trends support the change in composition identified by the overall taste data and other data collected during the testing period. The decline in citrus flavor is typical of aging beer, as noted by Bamforth & Lentini (2009).

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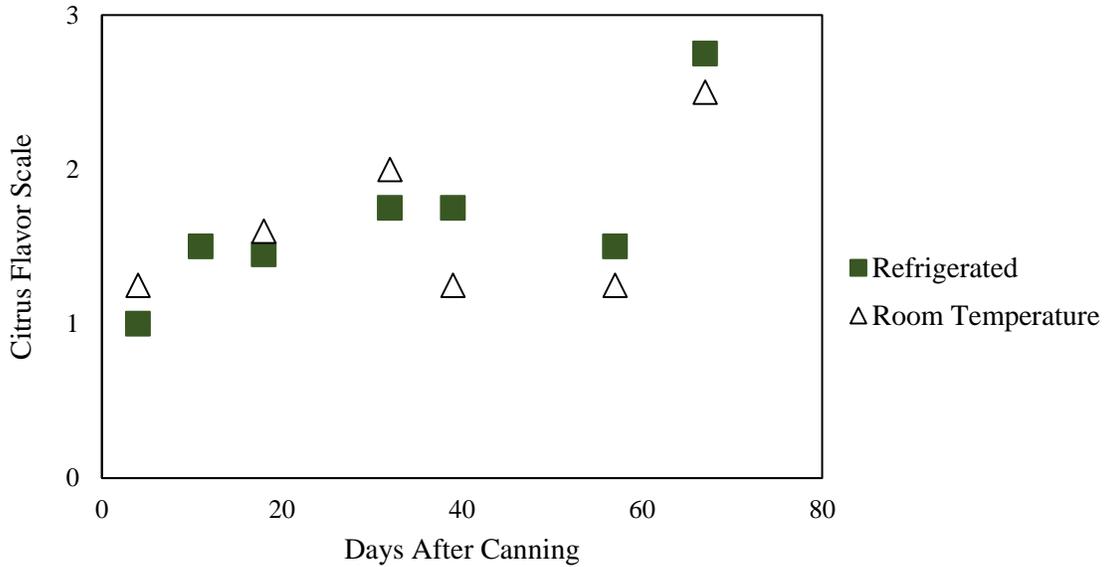
<sup>25</sup> Technical Committee of the Brewers Association 2017.



**Figure 2:** Aromatic strength according to sensory analysis data. The scale provided was: 1=very little smell, 2=some smell, 3=strong smell.

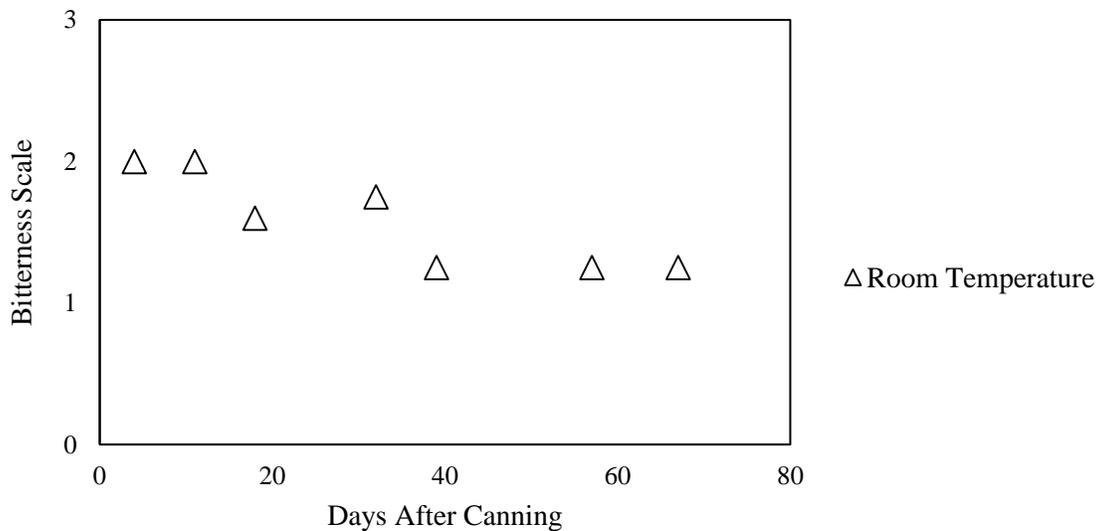


**Figure 3:** Aromatic Pleasantness according to sensory analysis data. The scale provided was: 1=very pleasant, 2=moderately pleasant, 3=neutral, 4=slightly unpleasant, 5=very unpleasant.



**Figure 4:** Citrus flavor characteristics in the beer according to sensory analysis data. The provided scale was: 1=strong citrus flavor, 2=some citrus flavor, 3=little/no citrus flavor.

Additionally, the room temperature beer showed a decline in bitterness over the aging period, as seen in Figure 5. The refrigerated beer also exhibited a net decline in bitterness over the testing period, but the data not fit to any reasonable trend line and was therefore not included in Figure 5. This decrease in the bitterness of the sample is likely due to the degradation of the hop bitter acids, as predicted by Bamforth & Lentini (2009) and Vanderhaegen et al. (2006). Interestingly, bitterness was also noted in several samples as an aftertaste, indicating that the bitterness quality shifted from existing in the body of the beer to only being noticeable in the aftertaste.

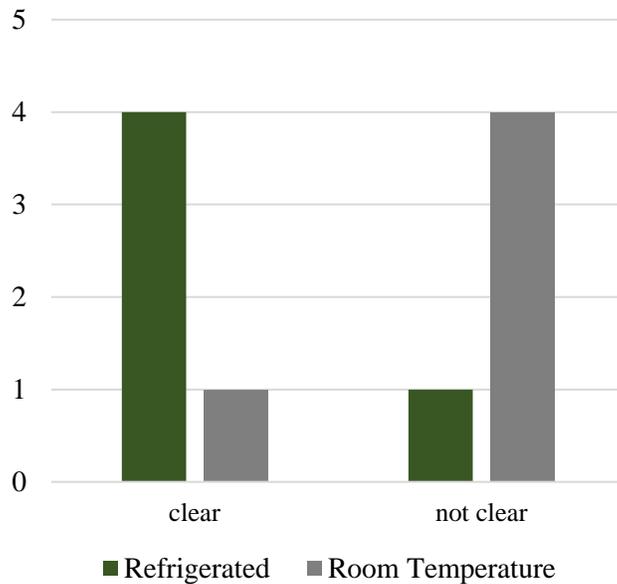


**Figure 5:** Bitterness of the room temperature beer according to sensory analysis data. The provided scale was: 1=not bitter, 2=slight bitterness, 3=moderate bitterness, 4=very bitter, 5=too bitter to drink.

### 3.2 Clarity and Foaminess

The clarity or level of haze in the beer was distinctly different between the room temperature and refrigerated samples. Generally, the room temperature samples were cloudy or hazy, while the refrigerated samples were clear (Figure 6). Some level of haze is expected in this type of beer, due to the lack of centrifuging and filtering in the brewing process. However, after being canned, particulates floating in the beer were given the chance to settle out, as seen in the case of the refrigerated beer. The room temperature beer most likely experienced reactions between polyphenols and polypeptides, which can bond covalently to form particles of sizes 1-10 $\mu\text{m}$ .<sup>26</sup> These reactions of molecules can cause a permanent haze, different from the haze naturally caused by hops.<sup>26</sup> Additionally, while performed the indigo carmine titration, it was observed that the room temperature samples appeared to hazier than the refrigerated sample over time (Appendix H, Figure 16).

The foaminess of the beer also appeared to be a direct result of the beer storage temperature. During the sample preparation process, the room temperature cans were noticeable more pressurized and contained more foam than the refrigerated cans. It is likely that some yeast remained in the fermentation tank after the majority was allowed to settle out, and was canned with the rest of the beer; the yeast was then able to reactivate at room temperature and continue the fermentation process, producing carbon dioxide gas and pressurizing the can. Noticeably, a can from the second canning run became so pressurized that it “popped” out of shape after a month of room temperature storage (see Figure 7).



**Figure 6:** Sample clarity according to sensory analysis data. 18 days after canning, the refrigerated sensory analysis sample was cloudier than the room temperature sample; on all other testing days, the refrigerated sample was clearer than the room temperature.

<sup>26</sup> Leiper; Meidl. 2009.



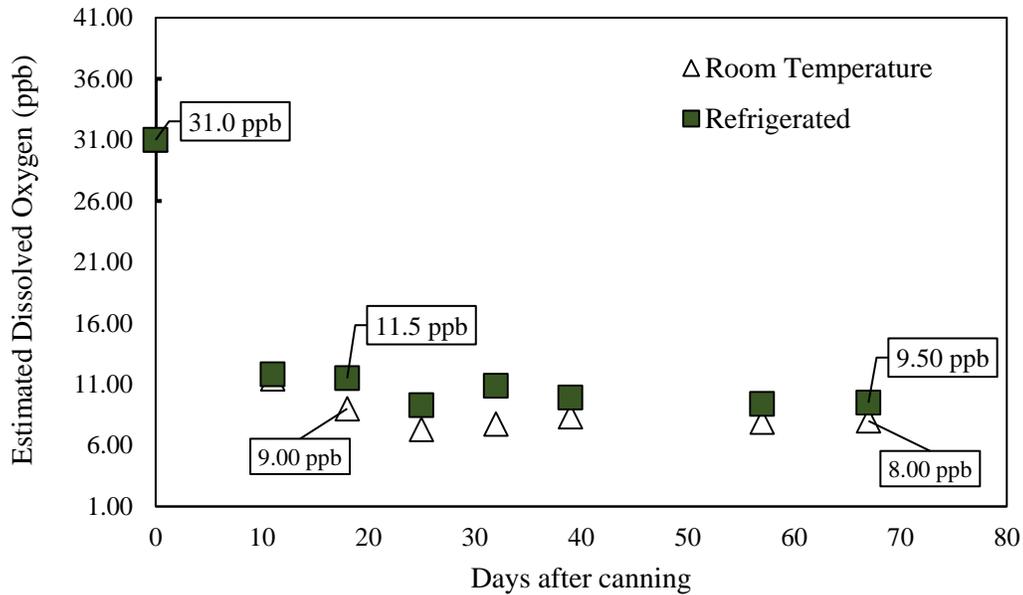
**Figure 7:** Can from the second canning batch that experienced over-pressurization. This can was held at room temperature.

### **3.3 Dissolved Oxygen Content**

Using the modified titration method with indigo carmine, we were able to measure the amounts of dissolved oxygen in the samples of beer under both refrigerated and room temperature conditions. The initial concentration of dissolved oxygen in the samples was  $30.3 \pm 5.0$  ppb, according to tests performed by Iron Heart Canning Company.<sup>27</sup> As expected, over a 67-day period, the dissolved oxygen content decreased in both the refrigerated and room temperature beers. (Figure 8). Based on the data we collected, we were able to estimate the lower limits of dissolved oxygen for each storage condition; Figure 8 shows that the room temperature sample group decreased to an apparent lower limit of approximately  $8.0 \pm 0.8$  ppb, which is comparative to the apparent lower limit of approximately  $9.5 \pm 0.8$  ppb for the refrigerated sample group. At all testing dates, the refrigerated samples had a higher concentration of dissolved oxygen than the room temperature samples.

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<sup>27</sup> Schaeffer, Z.; Caruso, A. 2019.



**Figure 8:** Estimated dissolved oxygen concentration over time for both room temperature and refrigerated storage conditions. The labelled data points are the assumed empirical values from the canning company. The initial starting value has an error of  $\pm 5.0$  ppb while the others have an undeterminable error.

The numerical values for dissolved oxygen were estimated by correlating the color of the indigo carmine-treated beer to the accurate dissolved oxygen values provided by the canning company. Of the three primary color components of the beer (Red, Green, and Blue), the red color showed the most definitive trend, increasing with time until a plateau was reached (Appendix E, Figure 12). This indicates that the indigo carmine reacted with less oxygen in the longer exposed samples, causing the beer solution to develop less of a blue-green color. Unfortunately, we cannot adequately calculate the error for the dissolved oxygen points outside of the first point; while the canning company provides a numerical error of  $\pm 0.8$  ppb from their equipment, we also introduced error inherent in the color analysis software and the procedure. The procedure error comes from the brief exposure of the beer to the air while being poured into sample vials, during which time oxygen was able to diffuse in it.

From the color values, an empirical relationship was drawn between the intensity of the red color and the dissolved oxygen value for the two time points (17 and 67 days) that it was determined. The relationship can be written as a power law depicted in Equation 1, in which the  $y$ -variable represents the estimated oxygen concentration, the  $x$ -variable denotes the intensity of the red color, and “ $n$ ” and “ $C$ ” are constants differing for each of the temperature conditions. With this model the power “ $n$ ” represents the relative rate of the decreasing dissolved oxygen.

$$y = C * x^{-n} \quad (\text{Eq. 1})$$

Table 1 shows fitted values of the constants in Equation 1 for both the room temperature and refrigerated sample groups. The power is 1.782 for the room temperature samples and 1.151 for the refrigerated samples (Table 1). This indicates that the beer stored at a higher temperature lost oxygen faster than that stored at a colder, refrigerated temperature, suggesting slightly quicker reactions and a slightly shorter shelf-life.

**Table 1:** Fitted values for the empirical relationship between titration color and oxygen content.

	<b>Room Temperature</b>	<b>Refrigerated</b>
<b>C</b>	19.716	15.134
<b>n</b>	1.782	1.151

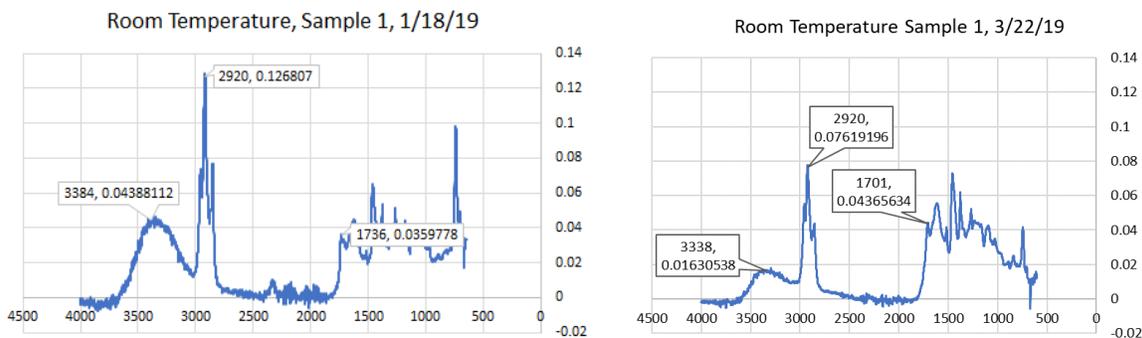
However, the existence of a lower, and similar valued, limit in each sample group suggests that while oxidation reactions are occurring within the beer, they are likely mass transfer limited instead of thermodynamically. At a starting point of 30.3 ppb, the rate of oxidation reactions is likely limited by the infrequency of molecular collisions caused by the low concentration. At higher concentrations, we would have likely seen a more significant difference in the reaction rate between the different storage groups with temperature becoming a more controlling variable.

### 3.4 Fourier-Transform Infrared Spectroscopy

After a preliminary analysis of the FTIR spectra, it was observed that a peak at  $1470\text{ cm}^{-1}$  was present and of similar shape for all spectra. This peak was used to normalize the data, creating the spectra used for the following analysis; a select portion of these normalized spectra can be seen in Appendix G.

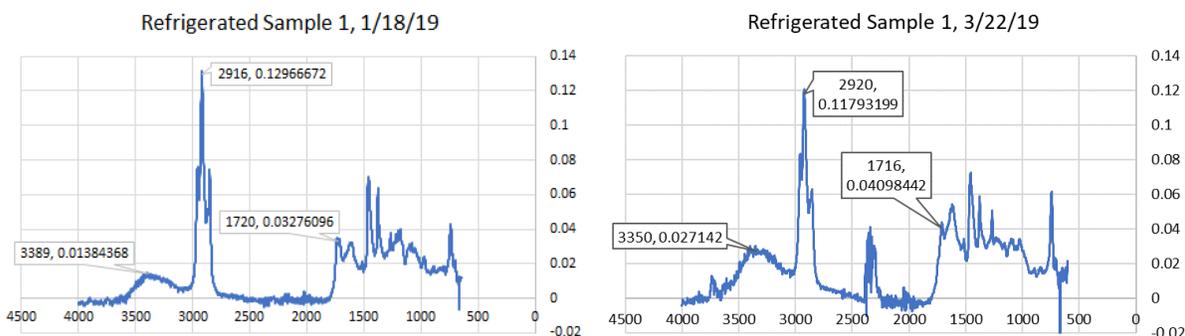
When analyzing the normalized spectra, a few key peaks were examined closely. These peaks were the carbonyl peaks around  $1700\text{ cm}^{-1}$ , the C-H bond peaks around  $2500\text{-}3000\text{ cm}^{-1}$ , and the O-H or amide stretch around  $3000\text{-}3500\text{ cm}^{-1}$ .

The carbonyl peak around  $1700\text{ cm}^{-1}$  corresponds to aldehydes, ketones, and esters, all of which are possible products of an oxidation reaction in beer. Over the course of the testing period, the carbonyl peak signal increases from 0.036 to 0.044 in the room temperature samples (Figure 9).



**Figure 9:** Infrared Spectra of the Room Temperature samples. On the left is the first test from January 18, 2019, while on the right is the last test from March 22, 2019

In the refrigerated samples, the increase in the peak is from 0.033 to 0.041 (Figure 10). This difference is small but, given the amount of oxygen that starts in the beer, this increase points to oxidation reactions in the beer.



**Figure 10:** Infrared Spectra of the Refrigerated samples. On the left is the first test from January 18, 2019, while on the right is the last test from March 22, 2019

Other changes seen in the beer are in the C-H bond region. The peaks near  $2920\text{ cm}^{-1}$  can indicate an aldehyde with a double bond on a nearby carbon. This region, however, also shows signals from any C-H bond. Changes seen in this region of the spectra showed no definite trends and are inconclusive for oxidation reaction studies.

The O-H stretch between  $3000\text{-}3500\text{ cm}^{-1}$  changes in height over the course of the testing period; additionally, the center of the peak shifts as well. The difference in height is most likely due to changes in hydrogen bonding in the sample, while peak shifting is due to the presence of carbonyl groups such as ketones and aldehydes in the sample.<sup>28</sup> Because we see a shift in the peak from  $3380\text{ cm}^{-1}$  to around  $3350\text{ cm}^{-1}$ , the theory that carbonyl groups are formed over the life of the beer is supported.

<sup>28</sup> Silverstein, R.M.; et al. 2015.

Over the entire testing period, the FTIR profile of both samples were not observed to change greatly. This is most likely due to the low level of dissolved oxygen initially present in the beer. The quantities of compounds formed from dissolved oxygen reactions would therefore be small, and not readily observable in an IR spectrum. However, one key observation is that the small changes in the heights of the peaks, as well as any significant peak shifting, occurred at the end of the testing period for both the refrigerated and room temperature samples. These changes occurred simultaneously in both types of sample, mostly likely because, as postulated in previous sections, the reactions occurring in the beer were primarily mass-transfer limited instead of thermodynamically limited. Additionally, the change in spectra seen in the final week of testing correlates with a portion of the sensory analysis; it was in the final week's round of taste-testing that the employees of Purgatory Beer Company determined the beer to be no longer sellable.

### **3.5 Second Batch Analysis**

A second batch of Two-Car Garage DIPA was canned on March 4<sup>th</sup>, 2019. Eight cans were acquired from this batch for testing purposes. These samples were tested using the same methods as the samples from the last batch. However, the results from this batch greatly differed from the results of the first batch and were therefore incomparable to the first batch. Additionally, no baseline dissolved oxygen measurement was taken on the day of the second batch canning, making it impossible to compare the initial levels of dissolved oxygen between batches.

Visible sediment was observed in samples taken from the second batch that was not observed in the first batch. Also, the samples from the second batch were much cloudier and of a slightly different color than the initial samples from the first batch. As mentioned in Section 3.2, one of the cans from the second batch “popped” out of shape after one month of room temperature storage, most likely due to yeast reactivation which generates carbon dioxide gas. This was not observed to happen to any of the first-batch cans over 10 weeks of testing. This indicates that there may have been more yeast in the second canning batch than the first, likely visible as part of the sediment.

FTIR data from the second batch samples differed greatly from the spectra gathered from the first batch (see Appendix G, Figure 15). IR spectra were gathered from the new samples on only two different dates, and as such the lack of additional data makes the IR results from the second batch inconclusive.

The inability to compare cans between batches and the extreme pressurization of the second batch points towards potential inconsistencies in the brewing process and/or canning process.

## Conclusions and Recommendations

Over the 67-day exposure, we found evidence of reactions and changing chemical composition within the Two-Car Garage DIPA samples. The refrigerated and room temperature beer both showed a similar decrease in the dissolved oxygen content, reaching lower limits of 9.5 and 8.0 ppb, respectively, after approximately 58 days. The FTIR spectroscopy analysis showed the formation of carbonyl containing functional groups with the growth of the peak at  $1700\text{cm}^{-1}$  and the shifting of a wide peak (around  $3000\text{ cm}^{-1}$ ) to lower wavenumbers, likely due to reactions of dissolved oxygen with organic compounds. Additionally, the overall color of the beer did not show any noticeable alterations over time for either storage temperature; this is most likely a result of the low oxygen concentration and thus low concentration of the reaction products.

The sensory analysis tests showed that the storage temperature had minimal effect on the taste of the beer. Both beers were deemed unsellable after 67 days of testing. The two beers were ranked similarly on overall taste during each tasting session, although the room temperature sample were more often noted to have aftertastes or off-flavors. This suggests that the reactions occurring, with and without oxygen, are primarily limited by the diffusion of reactive oxygen species rather than by temperature and thermodynamics. The similar decrease in dissolved oxygen and change in the IR spectrum for both temperature groups support this postulate. Further studies should be performed to provide evidence for or against this stated postulate.

The haze that formed in the room temperature samples indicates a separate, permanent joining of polypeptides and polyphenols to form visible particles. This haze formation, however, is not considered to affect the perceived quality of the beer, as NEIPAs are allowed and in some cases expected to have a level of cloudiness.<sup>29</sup> We do, however, consider the excessive amounts of foam present in the room temperature samples, likely due to the reactivation of dormant yeast, to affect the quality of the beer; the foaminess made pouring from the cans a difficult endeavor, resulting in lost beer.

Based upon the analysis of the results generated from studying this batch of Two-Car Garage DIPA, we recommend a conservative shelf-life of 1.5 months, or 7 weeks. The purpose of this timespan, shorter than period of salability identified by the sensory analysis, is to account of inconsistencies between batches. Further studies of other batches over a longer time span is recommended to develop a more accurate shelf life. This recommendation applies to the beer regardless of storage temperature. Additionally, to confirm the low level of oxygen found initially in the first canning batch, we suggest that Purgatory Beer Company request oxygen testing during at least one more canning session with Iron Heart Canning Company.

Although storage temperature had a minimal effect on taste, we do recommend that the cans are stored at refrigeration temperatures to limit excess foam production and over-pressurization, at both the vendor location and the location of those planning to consume the contents. This will result in a higher-quality product for the consumer. We recommend that the beer can labels say

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<sup>29</sup> Bernot, K. 2018.

“Keep Refrigerated,” to display the message to the consumer and promote storage at cool temperatures.

The preventative measures taken by Purgatory Beer Company and Iron Heart Canning Company, such as the carbon-dioxide purging of all containment equipment prior to brewing and each can prior to canning, were sufficient in minimizing the dissolved oxygen content in the studied batch of Two-Car Garage DIPA. We do not recommend additional steps be taken to minimize the oxygen contamination of beer.

Further studies with Purgatory Beer Company could include investigations into methods to extend the shelf-life of 2-Car Garage DIPA or the other beers regularly brewed there. A longer shelf-life could enable Purgatory Beer Company to increase their geographic range of product distribution, reaching a wider consumer audience.

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## Appendices

### Appendix A: Detailed Methods and Experimental Design

To determine the changing levels of dissolved oxygen in the beer, as well as the effects these changes had on the physical characteristics and chemical composition of the beer, a set of experiments were designed and improved by the project team over the course of several months. It is the purpose of this appendix to outline the rationale behind the design of each experiment, explain the discontinuation of certain methods, and to provide a detailed description of the procedure for each.

#### *Quantitative Determination of Dissolved Oxygen Content*

As measuring the amount of dissolved oxygen in the beer over time was integral to the objectives of the project, it became necessary to develop a reliable method for doing so. In the early stages of testing, it was believed that the dissolved oxygen probe on-hand at WPI would be capable of taking reliable and repeatable readings from the beer. After initial testing, however, it was found that this dissolved oxygen probe could not measure at an accuracy of  $\pm 1$  ppb, as was needed; oxygen content in most beers is known to be around 20 to 120 ppb, according to *Brewing and Beverage Industry International*. To determine the oxygen content, the indigo carmine titration method from “The Determination of Oxygen in Beer” (G. A. Howard and J. D. R. Mawer, **1977**) was researched and modified to fit the project’s resource constraints. The following procedures describe, in detail, the methods independently modified and practiced by the project team.

To transform the indigo carmine into a useful dissolved oxygen indicator, an indigo carmine dye solution was created of 0.3 grams of glucose, 0.3 grams of indigo carmine, and 50 mL of hot, distilled water. The mixture was stirred in a 200 mL beaker to break up and dissolve all solid matter. 50 mL of glycerol was then added and stirred. This solution was stored in a brown bottle at room temperature, to be used later in the experimentation. This procedure was carried out twice during the testing period, as the solution could be used for multiple weeks.

The beer samples for dissolved oxygen testing were prepared on a weekly basis. To do so, a 5-dram septum vial was filled with sample beer until it was overflowing. A septum cap was screwed tightly onto the opening of the dram vial such that very little air (and therefore oxygen) was trapped in the vial. The vial was then placed upside-down in a small mason jar. The jar was filled with sample beer until the vial was submerged entirely and then screwed shut. This mason jar acted as a beer-bath, ensuring there was no additional diffusion of air into the dram vial through the septum cap. This process was repeated for both the refrigerated and room temperatures samples of beer. The beer baths, containing one 5-dram sample vial each, were placed into the refrigerator, as the remainder of the experiment was set up.

For the indigo carmine dye solution to be used as an immediate indicator of dissolved oxygen, it had to be prepared immediately prior to its injection into the beer. This was done by first combining 10 mL of the indigo carmine dye solution (stored in a brown bottle, as described earlier) with 0.3 mL of a 12.47 molar solution of potassium hydroxide. Up to 1 mL of this solution was used to fill

an airtight syringe, the end of which was quickly capped with a rubber cork. This decreased the likelihood of oxygen from the air reacting with and ruining the solution. The corked syringe, needle facing downwards, was fully submerged in hot water (80 to 90°C), until the dye was reduced to a pale yellow-brown color, indicating it had transformed into its leuco form. This change usually occurred after approximately 5 minutes. The corked syringe was then allowed to chill to room temperature. At this point, the dye solution was ready to be injected into the beer sample.

The 5-dram sample vial was removed from its beer bath. With slight pressure maintained on the plunger of the now-uncorked airtight syringe, the needle was inserted into the beer sample through the septum cap. A second needle was also inserted into the cap, to allow beer to escape as the solution was added. 0.2 mL of the leuco solution was added into the beer sample, after which the second needle was removed, followed by the needle of the airtight syringe. The dram beer sample was mixed by repeated inversion for five minutes after no further color change was visible.

Once dyed, the solutions were placed in a photograph apparatus; this device consisted of a cardboard box, lined with white paper on the inside, and a mount for a cell-phone camera on the outside, designed to provide a constant lighting environment. This device was designed and constructed by the project team. Photographs of each dyed solution were immediately taken after each titration. Photographs of the 5-dram sample vials were also taken immediately prior to dying, to aid in an analysis of the changing color and clarity of the beer.

Initially, the changing color of each solution from these photographs was to be compared to a standard created earlier. This standard, made by adding different amounts of indigo carmine into a sample of beer, proved to be incomparable to the samples created by later titration. To use the data from the titration solution photographs, *ImageJ*, a color analysis software, was used to quantitatively determine the color change over time in the photographs. The curve developed from the color data was normalized with separate dissolved oxygen measurements taken from the beer samples; these measurements, taken on more accurate DO equipment, came courtesy of Iron Heart Canning Company, and were evenly spaced three times over the testing period.

### *Sensory Analysis*

As stated in the Methods section, a sensory analysis test was developed to determine the effects of dissolved oxygen on the physical characteristics of the beer. The goal of this analysis was to quantitatively measure changes in the five main characteristics of beer: aroma, appearance (which includes color and clarity), flavor (which includes alcohol and bitterness), mouthfeel (which includes carbonation and smoothness), and ingredients (which includes yeast and hops).

The final analysis consisted of a four-page ranking survey, which can be seen in Appendix B. The survey and samples of beer were brought to Purgatory Beer Company for internal sensory analysis testing. These taste samples of beer were prepared in clean mason jars the day of testing, temporarily stored in the refrigerator to reduce reactions, and kept on ice during transportation to Purgatory Beer Company.

### *Liquid-Liquid Organic Extraction*

In order to monitor the changing chemical composition of the beer, testing using FTIR and GC/MS equipment was necessary. To prepare the beer for these instruments, the organic compounds in the beer had to be separated from the aqueous solution. To do so, DCM was used as the extraction agent. The following procedures were independently developed by the project team, based on research and in-lab modification, and performed on a weekly basis over the testing period.

To prepare a sample, 8 mL of beer was placed by pipette into a 50 mL centrifuge vial. Two samples were prepared from the refrigerated beer, and two samples were prepared from the room temperature beer. DCM was then added to each of the four vials until the total volume in each vial reached 32 mL, creating an approximate 1:3 ratio. The vials were shaken vigorously by hand for 10 minutes, and then centrifuged, two at a time, for 10 minutes at 2,000 RPM.

The separated organic portion of each sample (observed to be the clear portion of liquid at the bottom of each vial) was pipetted into two 1 mL filter vials. One set of sample vials were sent to be tested by Fourier-transform infrared spectroscopy, and the other set were sent to be tested by gas chromatography/mass spectroscopy.

In later tests, pulverized calcium chloride salt was added to the beer solution with the intention of achieving greater separation. This was done by adding small amounts of the calcium chloride to the beer solution until either the solution was saturated, or a total of 5.5 g of salt was added. 24 mL of DCM was added to the solution and centrifuged, adding two additional samples to each round of testing. Some samples prepared in this manner emulsified instead of separating, making extraction impossible.

Mid-way through testing, an acid-based extraction was attempted. Hydrochloric acid (1 molar) was added to the centrifuge vial containing 8 mL of beer in 1 mL increments, until a significant decrease in pH was noted on litmus paper. DCM addition, shaking, and centrifuging was then completed for the sample, as described above. Unfortunately, this addition of acid was observed to drastically alter the spectra generated from the infrared spectroscopy. This method was determined ineffective by the project team, and not repeated after its pilot trial.

### *Fourier-Transform Infrared Spectroscopy*

To gain the IR spectrum of each sample, a Fourier-transform infrared spectrometer was used on a weekly basis. The separated organic portions of the beer/DCM solutions were pipetted into 1 mL filter vials, and were then transferred by pipette onto the crystal of the spectrometer. This required a surprising amount of finesse. To get an accurate spectrum, the engineer administering the sample had to blow on the drops of the sample as they were placed on the crystal; this ensured the DCM would volatilize, leaving only the organic portion of the beer to be read by the FTIR. As simple as this may seem, only one member of the MQP project team was capable of producing reliable spectra, despite all members attempting to do so.

### *Gas Chromatography/Mass Spectrometry*

On a weekly basis, 1 mL filter vials of the separated organic portion of each beer sample were sent off for testing on the gas chromatograph/mass spectrometer. For liability reasons, members of the project team were not allowed to operate the GC/MS. Instead, the samples were given to an authorized operator, who ran each sample. The time between sample delivery and sample analysis caused certain issues; for example, it was often the case that the DCM in the samples would evaporate before being tested, rendering the results useless. To combat this, samples were often re-vitalized with a later addition of DCM, with mixed results. During analysis of the GC/MS samples, the peaks shown by the spectra were found to be various hops oils, likely due to dry-hopping. These peaks were very consistent between tests and did not display any noticeable trends correlating to the reactions identified by other forms of testing. This might be due to the use of DCM as a solvent; other organic solvent might have produced better results.

### *Hydrometer Testing*

Several attempts were made by the project team to determine the changing ethyl alcohol content of the beer samples. Initially, a 100 mL graduated cylinder was filled with sample beer, whereupon a beer hydrometer was floated in the solution. Unfortunately, the hydrometer provided to the project team was unable to be reliably read, as it provided inconsistent and inaccurate readings (the readings were known to be inaccurate because tests run on the brand-new beer were well above its known alcohol content). Additionally, the volume of beer required to gain a weekly reading was often greater than the amount of beer at our disposal. Later, a digital density meter was used, but was unable to provide a reading on the samples of beer, most likely due to the carbonation.

### *Samples from the Second Canning*

Another batch of Two-Car Garage DIPA was canned on March 4<sup>th</sup>, 2019. The project team acquired eight cans from this batch. These samples were tested using the same methods as the samples from the last batch. However, the results from this batch greatly differed from the results of the first batch and were therefore incomparable (see Appendix G, Figure 15– the IR spectra from the second batch samples differed greatly from the first batch IR spectra).

## Appendix B: Sensory Analysis Template

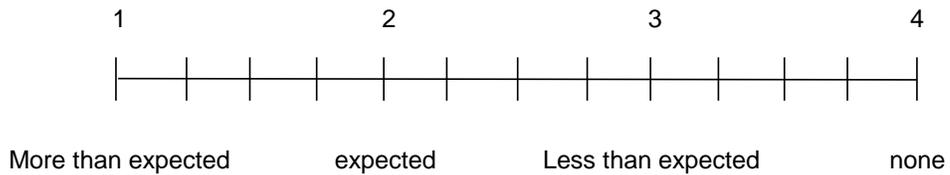
Sensory analysis data were collected on the following categories: visual characteristics, aromatic characteristics, basic taste characteristics, mouthfeel, and overall impression. The taste tester was provided with a scale on which to rank each sensory aspect, with corresponding descriptions. The full sensory analysis template is provided below. Additionally, the samples were labelled as “A” and “B” when given to the taste tester to prevent bias towards either storage method.

Beer Name: Two-Car Garage  
Canning Date:  
Tasting Date:  
Taster:

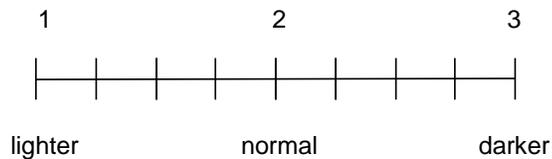
### Visual Analysis:

Clarity: **A:** Clear / Not Clear    **B:** Clear / Not Clear

#### Head when poured:

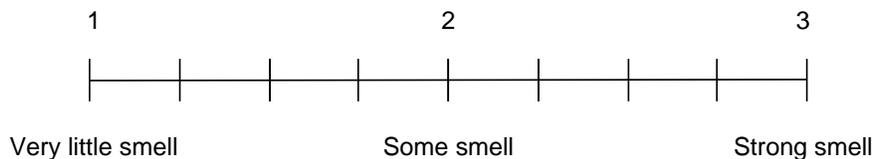


#### Color:

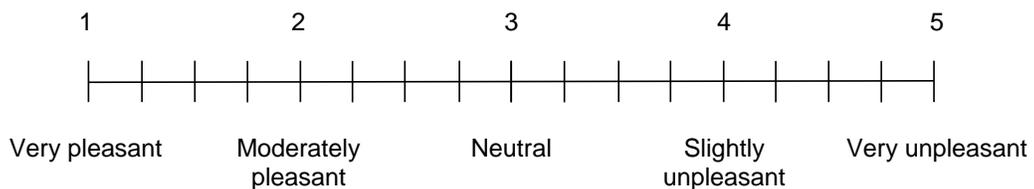


### Aromatic Analysis:

#### Smell (strength):

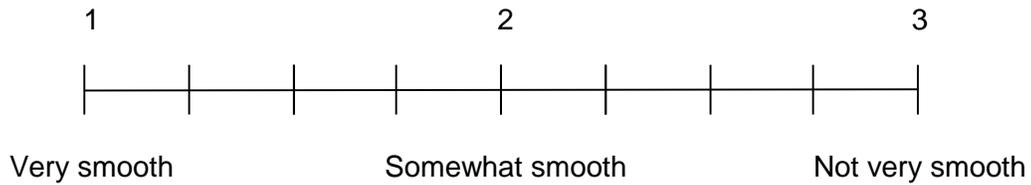


#### Smell (pleasantness):



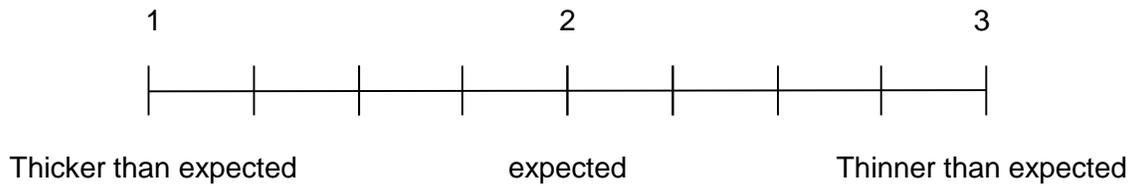


**Smoothness:**

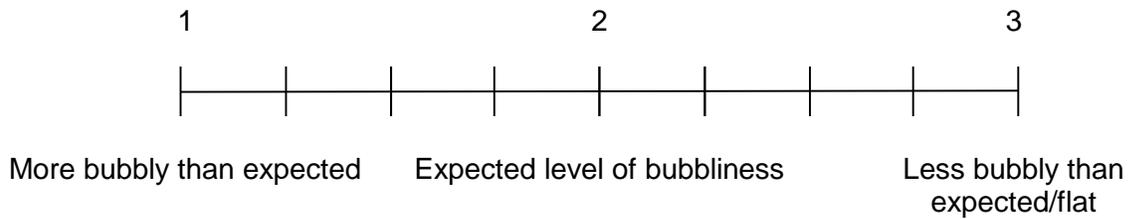


**Mouthfeel:**

**Texture:**



**Carbonation:**



**Any off flavors: A ( Y / N) B ( Y / N)**

If yes, describe:

**Aftertaste: A ( Y / N) B ( Y / N)**

If yes, describe:

**Summary:**

Palatable? A ( Y / N) B ( Y / N)

Sellable? A ( Y / N) B ( Y / N)

## Appendix C: Full Sensory Analysis Data

This appendix contains the full data collected during the sensory analysis. If a piece of datum was not available for a specific sample, it is notated with a dash.

**Table 2:** Sensory Analysis Sample Information. The column “Refrigeration Time” refers to the period of time the samples spent in the refrigerator after being poured from a fresh can for the day of testing.

Sample Name	Testing Date	Days After Canning	Taster	Refrigeration Time (hr)	Storage Conditions
01-18 Refrig	01/18/2019	4	B	7	Refrigerated
01-18 Room T	01/18/2019	4	B	7	Room Temperature
01-25 Refrig	01/25/2019	11	B	9	Refrigerated
01-25 Room T	01/25/2019	11	B	9	Room Temperature
02-01 Refrig	2/1/2019	18	B	1.5	Refrigerated
02-01 Room T	2/1/2019	18	B	1.5	Room Temperature
02-08 Refrig	2/8/2019	4	K	6	Refrigerated
02-08 Room T	2/8/2019	4	K	6	Room Temperature
02-15 Refrig	2/15/2019	32	B	8.5	Refrigerated
02-15 Room T	2/15/2019	32	B	8.5	Room Temperature
02-22 Refrig	2/22/2019	39	B	5	Refrigerated
02-22 Room T	2/22/2019	39	B	5	Room Temperature
03-01 Refrig	3/1/2019	7	K	6	Refrigerated
03-01 Room T	3/1/2019	7	K	6	Room Temperature
03-12 Refrig	3/12/2019	57	B	3	Refrigerated
03-12 Room T	3/12/2019	57	B	3	Room Temperature
03-22 Refrig	3/22/2019	67	B	4.5	Refrigerated
03-22 Room T	3/22/2019	67	B	4.5	Room Temperature

**Table 3:** Visual and Aromatic Sensory Analysis Data

<b>Sample Name</b>	<b>Clarity</b>	<b>Head when poured</b>	<b>Color</b>	<b>Smell (strength)</b>	<b>Smell (pleasantness)</b>
01-18 Refrig	-	-	-	2	1
01-18 Room T	-	-	-	2.5	2
01-25 Refrig	-	1.5	-	2.5	1
01-25 Room T	-	2.5	-	2.25	2
02-01 Refrig	less clear	2	1.9	2.45	1
02-01 Room T	more clear	3	2.1	2.6	2
02-08 Refrig	clear	2.25	2	2	1.5
02-08 Room T	clear	2.5	2.25	1.5	2.75
02-15 Refrig	clear	2.75	2	2	1.25
02-15 Room T	not clear	1	2	2.5	1.75
02-22 Refrig	clear	2.25	2	2.25	1.75
02-22 Room T	not clear	1.5	2	2.5	1.5
03-01 Refrig	clear	3	2.5	2	1.25
03-01 Room T	not as clear	3	2	2.25	3.25
03-12 Refrig	clear	1.5	2	2.25	1.5
03-12 Room T	not clear	2	2	2.5	2
03-22 Refrig	clear	3.25	2.25	1	3
03-22 Room T	not clear	3	2	1.5	2

**Table 4:** Basic Tastes Sensory Analysis Data

<b>Sample Name</b>	<b>Taste</b>	<b>Bitterness</b>	<b>Alcohol</b>	<b>Citrus Flavor</b>	<b>Smoothness</b>
01-18 Refrig	1.25	1.25	1	1	1
01-18 Room T	1.5	2	1	1.25	1.5
01-25 Refrig	1.5	1.25	1	1.5	1.5
01-25 Room T	1.75	2	1.5	-	-
02-01 Refrig	1.4	1.4	1.3	1.45	1.4
02-01 Room T	1.6	1.6	1.45	1.6	1.5
02-08 Refrig	1.75	2.5	1.25	2	1.25
02-08 Room T	2.5	3	1.75	1.75	1.5
02-15 Refrig	1.25	1.5	1	1.75	1.5
02-15 Room T	1.5	1.75	1.25	2	1.75
02-22 Refrig	1.75	1.75	1.5	1.75	1.75
02-22 Room T	1.25	1.25	1.25	1.25	1.25
03-01 Refrig	1.75	1.25	1	1.75	1.25
03-01 Room T	2.75	2	1.25	2.25	2
03-12 Refrig	1.5	1	1	1.5	1.25
03-12 Room T	1.75	1.25	1	1.25	1.5
03-22 Refrig	4	1.5	1	2.75	2
03-22 Room T	3.75	1.25	1	2.5	2

**Table 5:** Mouthfeel Sensory Analysis Data and Summary of Sample Impression.

<b>Sample Name</b>	<b>Texture</b>	<b>Carbonation</b>	<b>Off-flavors</b>	<b>Aftertaste</b>	<b>Palatable?</b>	<b>Sellable?</b>
01-18 Refrig	2	2	N	N	Y	Y
01-18 Room T	1.5	-	Y	Y	Y	Y
01-25 Refrig	2	2	N	N	Y	Y
01-25 Room T	-	2.5	Y	N	Y	Y
02-01 Refrig	2	2	N	Y	Y	Y
02-01 Room T	2	2	Y	Y	Y	Y
02-08 Refrig	2.5	2.5	N	N	Y	Y
02-08 Room T	2.5	2.5	N	Y	Y	Y
02-15 Refrig	2	2.25	N	Y	Y	Y
02-15 Room T	2	1.75	N	N	Y	Y
02-22 Refrig	2	2	Y	Y	Y	Y
02-22 Room T	2	2	N	N	Y	Y
03-01 Refrig	2	2.25	N	Y	Y	Y
03-01 Room T	2.25	2.5	Y	Y	Y	Y
03-12 Refrig	1.75	2	N	N	Y	Y
03-12 Room T	2	2	Y	Y	Y	Y
03-22 Refrig	2.75	2.75	Y	N	Y	N
03-22 Room T	2.5	2.5	Y	N	Y	N

**Table 6:** Compilation of off-flavors, aftertaste, and other comments.

<b>Sample Name</b>	<b>Off-flavors description</b>	<b>Aftertaste description:</b>	<b>Other comments:</b>
01-18 Room T	In the beginning, can taste an off-flavor	On back end of sip, a little too bitter	Still palatable, just more bitter than usual
01-25 Refrig	Can tell it isn't first day beer		
01-25 Room T	Tastes more "smelly" (a bit like yeast)		
02-01 Refrig			Slightly thin on the backend (watery)
02-01 Room T	Slight bitterness on back-end	See off-flavors	
02-08 Room T		Lingering bitterness; can taste hops	
02-15 Refrig		Slightly flat	
02-22 Refrig	A little rough	A little aftertaste	
03-01 Refrig		Slight bitter aftertaste	Sell this more so than the room temperature
03-01 Room T	A slight bitter flavor on the front		Some skunky smell
03-12 Room T	Slight maltiness	Slightly bitter on the back end	Sample was a little harsh, slightly malty (not enough to not sell)
03-22 Refrig	Flat, little aroma, and thin finish		Sample no longer sellable
03-22 Room T	Flat, little aroma, and thin finish		Sample no longer sellable

## Appendix D: Second Canning Sensory Analysis Data

Samples from the second canning on 03/04/2019 were tested with the original purpose of confirming sensory analysis trends observed in the first few weeks of testing. However, the sensory analysis deviated what we expected, most likely due to differences in the two canning runs. The sensory analysis data from the second canning was not used in the results and discussion.

**Table 7:** Sensory Analysis Samples Information: second canning.

Sample Name	Testing Date	Days After Canning	Taster	Refrigeration Time (hr)	Storage Conditions
01-15 Nrefrig	01/15/2019	11	B	5.5	Refrigerated
01-15 NRoom T	01/15/2019	11	B	5.5	Room Temperature
03-22 Nrefrig	03/22/2019	18	B	4.5	Refrigerated
03-22 NRoom T	03/22/2019	18	B	4.5	Room Temperature

**Table 8:** Visual and Aromatic Sensory Analysis Data: second canning.

Sample Name	Clarity	Head when poured	Color	Smell (strength)	Smell (pleasantness)
01-15 Nrefrig	clear	2.25	1.75	2.75	1.75
01-15 NRoom T	not clear	1.75	1.75	2.5	1.5
03-22 Nrefrig	not clear	1.5	2	2.25	1.5
03-22 NRoom T	not clear	2.25	1.75	2	1.75

**Table 9:** Basic Tastes Sensory Analysis Data: second canning.

Sample Name	Taste	Bitterness	Alcohol	Citrus Flavor	Smoothness
01-15 Nrefrig	2	1.5	1.25	1.75	2
01-15 NRoom T	1.25	1.25	1.25	1.5	1.5
03-22 Nrefrig	1.75	1.75	1	1.25	1.5
03-22 NRoom T	2	1.5	1	1.75	1.75

**Table 10:** Mouthfeel Sensory Analysis Data and Summary of Sample Impression: second canning.

Sample Name	Texture	Carbonation	Off-flavors	Aftertaste	Palatable?	Sellable?
01-15 Nrefrig	2.25	2	N	N	Y	Y
01-15 NRoom T	2	2	Y	Y	Y	Y
03-22 Nrefrig	2	1.75	N	N	Y	Y
03-22 NRoom T	2.25	2.25	Y	Y	Y	Y

**Table 11:** Compilation of off-flavors, aftertaste, and other comments: second canning.

<b>Sample Name</b>	<b>Off-flavors description</b>	<b>Aftertaste description:</b>	<b>Other comments:</b>
01-15 NRoom T	Slightly off aftertaste		
03-22 NRoom T	Slightly thin/slightly malty	Slightly watery	Noticeable amount of suspended particles in the beer - some settled out after being poured into testing samples

**Appendix E: Post-Titration Color Values from *ImageJ***

**Table 12.** Compilation of all post-titration RGB color values for the room temperature samples.

Room Temperature Samples											
Date	Days	Mean All RGB			Normalized			Average	Average	Average	
		Color	Mean Red	Mean Green	Mean Blue	Red	Green	Blue	Normalized Red	Normalized Green	Normalized Blue
1252019	11	77.49	106.49	111.49	14.48	1.37	1.44	0.19	1.36	1.44	0.21
	11	78.59	106.30	112.52	16.95	1.35	1.43	0.22			
	11	78.29	107.04	113.36	14.46	1.37	1.45	0.18			
	11	78.28	105.41	112.34	17.11	1.35	1.43	0.22			
	11	77.62	104.05	111.49	17.31	1.34	1.44	0.22			
2012019	18	78.28	121.96	105.95	6.91	1.56	1.35	0.09	1.55	1.35	0.09
	18	78.25	120.66	105.24	8.85	1.54	1.34	0.11			
	18	78.16	122.23	106.34	5.89	1.56	1.36	0.08			
	18	77.93	120.56	105.56	7.68	1.55	1.35	0.10			
2082019	25	89.20	156.47	110.13	1.00	1.75	1.23	0.01	1.74	1.24	0.02
	25	89.10	155.40	110.45	1.45	1.74	1.24	0.02			
	25	86.78	151.44	107.17	1.72	1.75	1.24	0.02			
	25	88.70	154.15	109.58	2.38	1.74	1.24	0.03			
	25	88.03	153.47	108.66	1.95	1.74	1.23	0.02			
	25	87.56	152.49	108.30	1.90	1.74	1.24	0.02			
2152019	32	84.35	143.81	107.50	1.75	1.70	1.27	0.02	1.69	1.28	0.03
	32	84.48	143.42	107.39	2.62	1.70	1.27	0.03			
	32	84.86	143.62	108.51	2.45	1.69	1.28	0.03			
	32	86.27	145.59	111.24	1.98	1.69	1.29	0.02			
	32	85.46	142.42	110.19	3.76	1.67	1.29	0.04			
2222019	39	95.59	154.19	118.36	14.23	1.61	1.24	0.15	1.62	1.24	0.14
	39	95.40	155.38	118.04	12.79	1.63	1.24	0.13			
3122019	57	90.00	151.49	112.39	6.12	1.68	1.25	0.07	1.67	1.24	0.09
	57	91.47	151.04	113.03	10.34	1.65	1.24	0.11			
	57	92.17	154.56	114.52	7.43	1.68	1.24	0.08			
3222019	67	91.81	151.18	115.04	9.20	1.65	1.25	0.10	1.66	1.25	0.09
	67	91.35	152.65	114.29	7.12	1.67	1.25	0.08			

**Table 13.** Compilation of all post-titration RGB color values for the refrigerated samples.

Refrigerated Samples											
Date	Days	Mean All	Mean Red	Mean Green	Mean Blue	Normalized	Normalized	Normalized	Average	Average	Average
						Red	Green	Blue	Normalized	Normalized	Normalized
1252019	11	73.21	94.78	101.97	22.90	1.29	1.39	0.31	1.24	1.41	0.35
	11	72.32	89.44	102.75	24.79	1.24	1.42	0.34			
	11	73.28	88.93	104.08	26.83	1.21	1.42	0.37			
	11	80.36	98.07	112.12	30.88	1.22	1.40	0.38			
2012019	18	67.90	89.59	99.13	14.98	1.32	1.46	0.22	1.27	1.46	0.39
	18	67.94	87.07	100.46	16.28	1.28	1.48	0.24			
	18	68.51	86.12	99.89	19.52	1.26	1.46	0.28			
	18	68.61	86.12	100.33	68.59	1.26	1.46	1.00			
	18	68.51	86.12	100.40	19.02	1.26	1.47	0.28			
	18	69.13	86.20	99.81	21.39	1.25	1.44	0.31			
2082019	25	73.27	115.68	100.18	3.96	1.58	1.37	0.05	1.53	1.40	0.07
	25	73.15	111.92	101.88	5.65	1.53	1.39	0.08			
	25	72.53	110.39	102.20	5.00	1.52	1.41	0.07			
	25	73.54	111.30	103.57	5.75	1.51	1.41	0.08			
	25	73.50	111.79	104.55	4.17	1.52	1.42	0.06			
	25	74.15	112.39	105.25	4.81	1.52	1.42	0.06			
2152019	32	68.79	94.71	99.73	11.92	1.38	1.45	0.17	1.33	1.46	0.21
	32	68.48	91.35	99.49	14.60	1.33	1.45	0.21			
	32	67.46	89.88	99.45	13.06	1.33	1.47	0.19			
	32	69.73	91.95	101.53	15.73	1.32	1.46	0.23			
	32	69.22	90.57	100.46	16.63	1.31	1.45	0.24			
2222019	39	73.17	107.33	102.52	9.67	1.47	1.40	0.13	1.45	1.39	0.16
	39	73.62	104.84	102.15	13.85	1.42	1.39	0.19			
3122019	46	77.16	117.20	106.63	7.65	1.52	1.38	0.10	1.52	1.39	0.10
	46	76.93	116.34	107.40	7.05	1.51	1.40	0.09			
3222019	53	75.98	112.95	100.65	14.33	1.49	1.32	0.19	1.50	1.33	0.17
	53	75.11	113.48	100.91	10.94	1.51	1.34	0.15			



**Figure 11.** Selected area of the beer under identical light conditions for color analysis. Both the beer color before (TOP) and after (BOTTOM) the indigo carmine dye is added is shown. This beer sample was aged for 67-days (collected on 03/22/2019) under refrigerated conditions.



**Figure 12.** Full pictures of the beer within the sample vials for the post-titration color analysis. All samples are from the refrigerated group and are aged 11 days (LEFT), 32 days (CENTER), and 67 days (RIGHT).

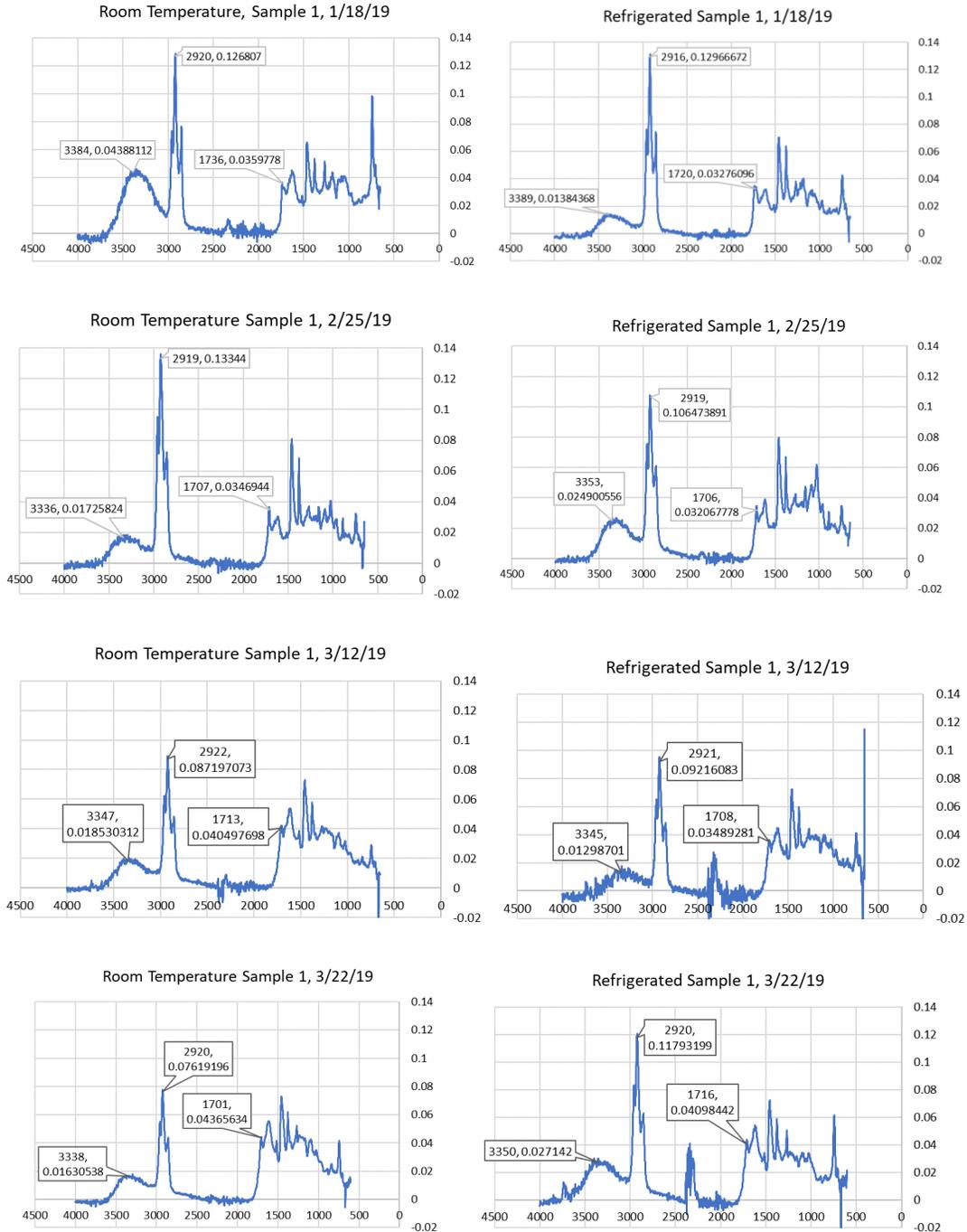
## Appendix F: Estimated Dissolved Oxygen Values

**Table 14.** Compilation of average normalized red color values correlated with the estimated dissolved oxygen concentration.

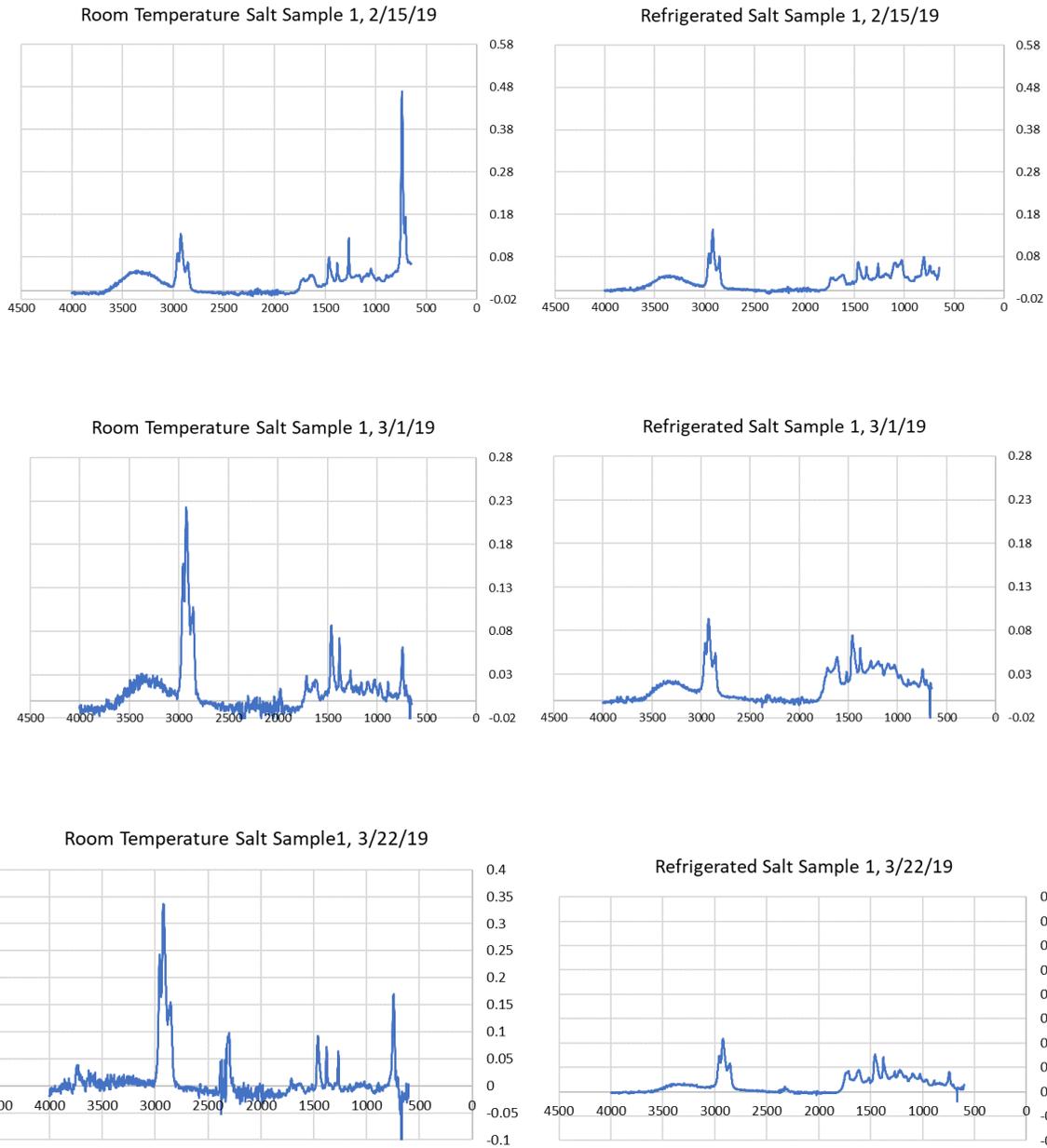
Days	ROOM TEMPERATURE		REFRIGERATED	
	Average Normalized Red	Estimated Dissolved Oxygen Concentration [ppb]	Average Normalized Red	Estimated Dissolved Oxygen Concentration [ppb]
11	1.36	11.5	1.24	11.8
18	1.55	9.00	1.27	11.5
25	1.74	7.32	1.53	9.28
32	1.69	7.74	1.33	10.9
39	1.62	8.34	1.45	9.90
57	1.67	7.90	1.52	9.38
67	1.66	8.00	1.50	9.50

## Appendix G: Normalized IR data

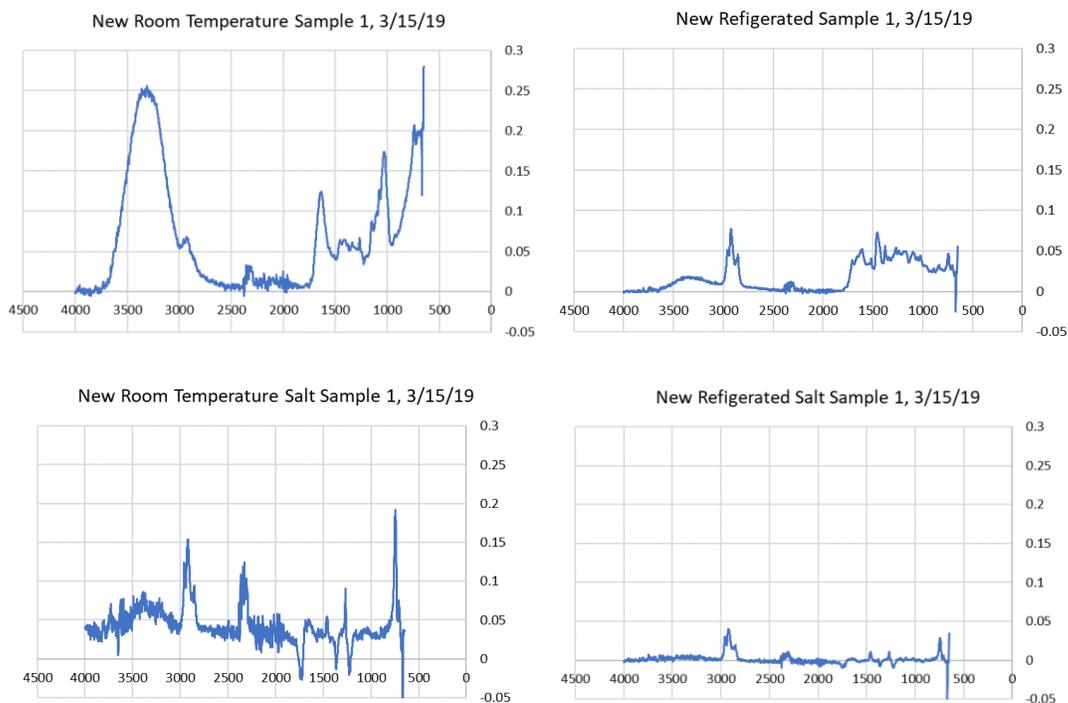
The following appendix contains the IR spectra from selected samples on selected testing dates. The spectra have been normalized for analysis.



**Figure 13:** IR Spectra from selected samples of beer on selected dates, all separated using solely DCM. Peaks of interest are labeled, along with the name of each sample and the date tested.



**Figure 14:** IR Spectra from selected samples of beer on selected dates, all separated using crushed calcium chloride and DCM. Names of each sample and dates tested are labeled. Note: axis ranges change between differently dated samples.



**Figure 15:** Selected IR Spectra from the second batch of Two-Car Garage DIPA. Names of each sample and dates teste are labeled. Spectra including “salt” in name (the bottom two spectra) denote samples separated using crushed calcium chloride along with DCM. All other samples separated with solely DCM.

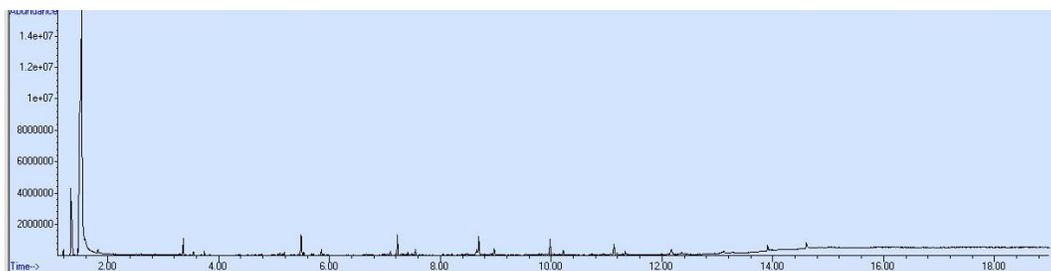
## Appendix H: Haze in Beer



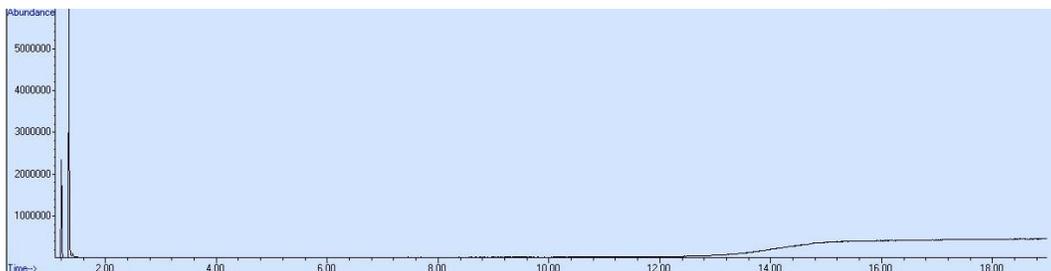
**Figure 16.** Side-by-side comparison of the refrigerated (LEFT) and room temperature (RIGHT) for the beer aged for 67 days. There is an observed difference in the haziness between the two samples.

## Appendix I: GC/MS data

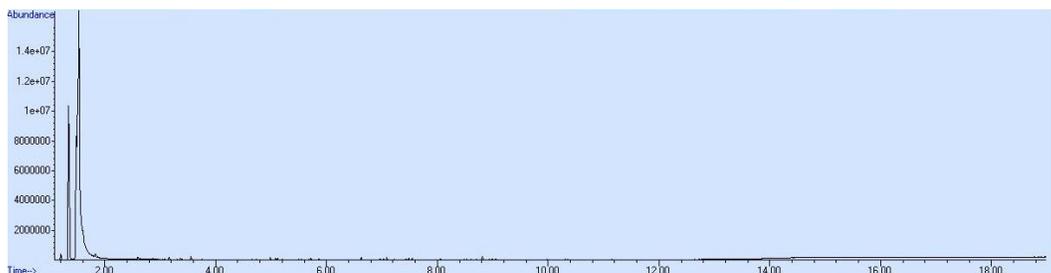
This section shows selected GC/MS spectra. This data was not usable for analysis. As shown below, the spectra remained relatively constant throughout the entire testing period, and only showed compounds that were due to the oils on the hops. Another challenge with the data from GC/MS was that if the samples were not run immediately, the solvent would evaporate. This led to samples needing to be rehydrated with dichloromethane, resulting in diluted samples and unusable data.



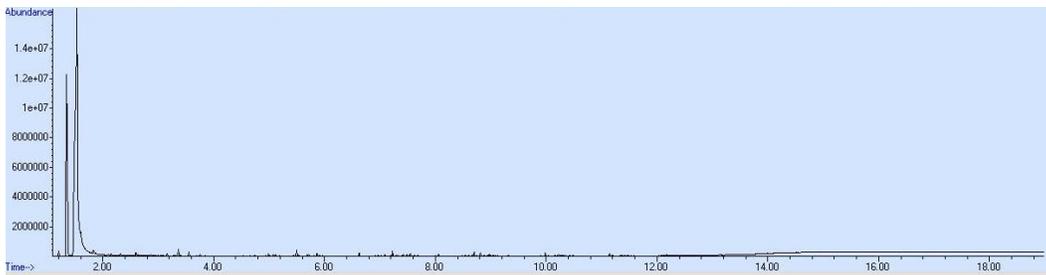
**Figure 17:** Spectrum from the refrigerated sample on January 18, 2019



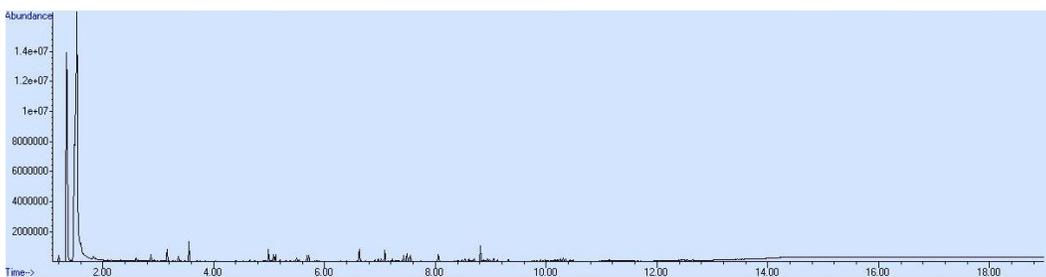
**Figure 18:** Spectrum of room temperature sample from January 18, 2019



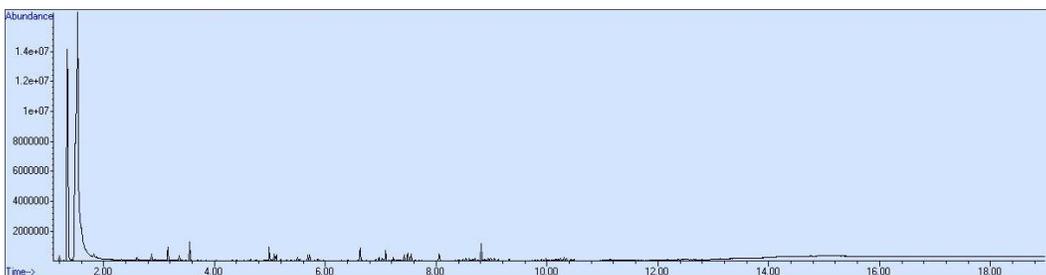
**Figure 19:** Spectrum of the refrigerated sample from March 22, 2019



**Figure 20:** Spectrum of the room temperature sample from March 22, 2019



**Figure 21:** Spectrum of the salted refrigerated sample from March 22, 2019



**Figure 22:** Spectrum of the salted room temperature sample from March 22, 2019