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Wachusett Filtration Project

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Abstract

This project examined and analyzed beer sample from Wachusett Brewing Company for haze properties and flavor compounds. The goal of experimentation was to evaluate the effect of centrifugation on the flavor of Wachusett beer. Reduction of haze was conducted with centrifugation of beer. The effects on flavor compounds were subsequently analyzed using SEC and GCMS methods. It was found that centrifugation significantly reduces haze without reducing the relative abundance of flavor-contributing compounds.
Acknowledgments

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Professor Sabyasachi Sarkar

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Additionally, we would like to thank following people for the valuable tools and information they provided. Without the help of these individuals, this project would not have been possible.

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Paula Moravek
Professor Andrew Butler
Tom Partington
Fred Hutson
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1 Introduction

In the United States there is a preference for bright, clear beers. The clear, almost transparent appearance of beers such as pale lagers have led to the widespread popularity of bright beers. In a 2015 study by online beer rating website Beer Advocate, out of the top 20 best-selling beers in the United States, 95% of the beers were pale lagers [Braskier, 2015]. A similar study conducted by the Grocery Headquarters showed that 80% of the beers sold were also pale lagers [Grocery Headquarters].

As suggested by their name, pale lager type beers are characterized by their light color. One technique used in the creation of clear or translucent beer is filtration, which removes sediment—typically yeast and protein particles—created during the brewing process. However, the effect of filtration on the beer’s flavor is not completely understood. There is an ongoing debate regarding the differences in flavor between unfiltered and filtered beers. In an article from the Guardian, proponents of unfiltered beer claim a superiority of flavor that is lost through filtering, as well as being vegan-friendly by not using animal-derived fining agents [Naylor, 2014]. In contrast, opponents have claimed in the All About Beer Magazine that the differences are negligible and arise primarily from personal bias. They go on to state that filtering beer removes residual organic matter suspended in the beverage, resulting in a stable product with an extended shelf life [Lyke, 2012].

Recently, Wachusett Brewing Company has received feedback from some of its customers via social media regarding an inconsistency in the quality of their unfiltered beers. Some of the beers exhibited an unexpectedly high level of cloudiness that was visually discouraging to customers. Wachusett Brewing Company currently utilizes diatomaceous earth as a media for their filtered beers and is in the process of installing a centrifuge for additional separation. The scope of this project is an investigation of methods to improve the clarity of Wachusett Brewing Company’s unfiltered beers without affecting their flavor, by means of studying the nature and properties of the particulates causing the turbidity issue. During this investigation, the team conducted a series of experiments using size exclusion chromatography, gas chromatography-mass spectrometry, and centrifugal separation to characterize the particulates. In addition, a time separation experiment was run in order to observe the natural settling process of the beer.
2 Background

This chapter outlines information relevant to the brewing process, beer composition, haze formation and the experiments used to determine and analyze the nature and properties of haze-forming particulates for Wachusett Brewing Company.

2.1 Brewing Process

Most beers follow the same basic brewing process. First a grain such as barley is malted, meaning that the barley is allowed to soak in water, germinate, and then dry. After the drying process is completed the barley is referred to as malt. The malt is then mixed with hot water in order to release the fermentable sugars. At this point in the process the mixture is known as wort. The wort is boiled and seasoning is added in the form of hops and other additives to give the beer its own individual taste. The hot wort is then cooled and moved to a fermenting tank where yeast is added. The yeast consumes the fermentable sugars in the wort producing ethyl alcohol and carbon dioxide. From this point the beer is carbonized and sent into post processing or to packaging where it is ready to be brought to market [Palmer, 2006, p. 1]. A graphic of the basic brewing process is provided in Figure 1.

The brewing process for beer can be universally summarized into these basic steps. The difference in beer flavor stems from the methods implemented by the brewer to achieve these steps.

2.2 Flavor Components

This study will focus on the components of beer that contribute to flavor or haze formation. The basic ingredients of a beer are the yeasts, malt, and hops. During the brewing process, yeasts allow for the fermentation that produces the alcohol while the malt and hops produce proteins and polyphenols respectively. These ingredients are the precursors to the flavor and haze forming particles.

Beers come in a variety of flavors that can be traced to several sources. In general however, the primary source of beer flavor comes from esters, polyphenols, and sugars which correlate to the fruity, bitter, and sweet flavors of beer. Esters and polyphenols are produced by the yeast during
the fermentation process. Sugars are usually derived from the raw ingredients used to produce beer. [Meilgaard, 1982]

The flavor active esters in beer are organic compounds derived from alcohols and acids. Common esters in beer are ethyl acetate, isoamyl acetate, phenylethyl acetate, and isobutyl acetate. Alongside ethanol, esters are a product of the fermentation process of yeast through a biochemical pathway. Esters give beer a fruity flavor likened to bananas, pears, or other light fruits. Generally, the amount of esters in a beer is at or around the threshold value [Verstrepen et al, 2003]. The threshold value is defined as the amount of substance added or subtracted that can be detected by an individual with an accuracy of 50% [Aron and Shellhammer, 2010]. Even though they occur in relatively low
concentrations in comparison to the other compounds found in beer that contribute to flavor, their effects on flavor are easily detected and thus have the potential to greatly influence the flavor profile of an individual beer.

Polyphenols are another group of organic compounds that are important to the flavor of beer. Usually coming from the hops as well as from the malt, polyphenols such as tannins and flavonols give beer astringency and bitterness. They are released from their source during the fermentation process. Catechin and epicatechin are both flavonols found commonly in plants that have a demonstrated ability to increase the bitterness of a beer when added to the beverage. In a study, a panel of tasters sampled beer with 0, 100, and 200 mg/L of added polyphenol extract derived from spent-hop material. The panelists assessed that the beers with the added polyphenol content were substantially more bitter than the control batch [Steiner et al., 2010].

Sweet flavors in beer either come directly from sweeteners or latent sugars within the other beer ingredients such as malts. Sugars can be added to beer in its raw form or via fruits or syrups and honey. In the case of the ingredient malts, the carbohydrates of the grains are broken down into simpler sugars. There are many different varieties of grain that can be used to produce malt and as such there are many variations on the flavor that can be produced, ranging from caramel to chocolate flavors [Aron and Shellhammer, 2010].

2.3 Haze

Haze in beer is the suspended sediment that results in reduced clarity of the beverage. Since most consumers prefer their beer to be bright, reduced haze in most beer is preferred. Haze formation is the result of colloidal instability in the beer. The instability is usually produced by large-sized particles produced during the brewing process. However, relatively small particles that would not by themselves contribute significantly to haze formation can coalesce into larger particles that affect and produce haze.

The particulates that cause beer haze are mostly comprised of proteins and polyphenols stemming from hops and malt. These result in the formation of particles that contribute to beer turbidity. Analysis has shown that the majority of haze particles are proteins (constituting about 40-75% of
haze), however polyphenols (approximately 17% of haze), when combined with proteins, can have a strong presence in the composition of haze [Ward, p. 2].

Barley can be a cause of water-soluble proteinaceous polypeptides that can be found within beer, although only one third of the overall protein content appears in the finished product. In addition to proteins, protein breakdown products are also important to the formation of beer haze, and are more prevalent in beer than the proteins themselves. These breakdown products differ from the proteins in that they are water soluble and do not precipitate when boiled. Hops and malt introduce polyphenolic compounds to the beer, which bind with proteins to form particulates large enough to form sediment and contribute significantly to turbidity levels. The ratio of polyphenols and proteins is a strong indicator of the amount of haze that can be formed, as the amount of haze tends to be greatest when the number of binding sites of polyphenols and proteins is nearing equality. Oxidation of the polyphenols can also cause them to condense into polymerised products that are active precursors in haze production. In addition, other reactive species can also be oxidized producing molecules that cause haze to form themselves or aid another particles in doing so. Since oxygen has the potential to be introduced to the beer in many stages of the brewing process, care must be taken to reduce this source of haze formation. [Steiner et al., 2010, p 360-361]

Two primary variations of beer haze exist. The first type, chill haze, is formed as protein-polyphenol compounds begin to polymerize. Upon decreasing temperature, the compounds precipitate and become visible as chill haze [Ward, p. 3]. The formation of chill haze is a reversible process, as the compounds dissolve when the temperature of the beer is increased. Over time however, chill haze can give rise to the second type of beer haze, called permanent haze. As its name suggests, permanent haze is irreversible, and forms when protein-polyphenol compounds further polymerize. Covalent bonds form between polypeptides and polyphenols, resulting in compounds that are unaffected by temperature change [Steiner et al., 2010, p. 360].

2.4 Size Exclusion Chromatography (SEC)

Size exclusion chromatography (SEC) is a method of separating molecules based on their size by passing a mixture through a column of porous packing media. Smaller molecules will diffuse into the pores
of the packing media while larger molecules will travel in the column’s void volume [Mori et al., 1999, p. 11]. As such, the exiting flow is separated in order of decreasing molecular weight. At the exit of the column is a detector, which will output detection peaks vs time graphs. For example, if the detector measures conductivity, one can expect an output of conductivity vs time. Additionally, standards can be passed through the column in order to determine the time that a certain sized molecule should make it through the column. This is useful for determining the relative sizes of molecules during testing. A visual representation of the SEC process is shown in Figure 2.

In order to achieve proper separation several factors must be considered. The first factor to take into consideration is the media fractionation range. This range dictates the size of the pores and thus the size of the molecules that will be able to diffuse into the porous sites. It is important to understand the desired degree of separation when making this choice. The smaller the fractionation range, the higher the resolution that can be achieved.

The second factor one must take into consideration is the column dimensions and packing. Typically, a longer column will result in increased resolution, and a larger column diameter will allow for more mixture to be passed into the column. Additionally, one must consider the degree of packing in the column. If the column is packed too tightly then the pores of the gel media will be crushed.

Figure 2: Size Exclusion Chromatography Outline [Yonemoto, 2009]
and not allow for the proper fractionation of the mixture. This will in turn diminish the resolution. Conversely, an under-packed column increases the void volume. The increased void volume will result in broader, less resolved peaks. In an under packed column we can also expect to see dead volume at the top of the column, which allows the sample to diffuse prior to entering the column bed. This results in broadening of peaks and less resolution. [BIORAD, 2016]

Lastly, it is important to consider the flow rate of the sample being passed through the column. If a sample is passed too slowly then there is more time for the molecules to diffuse. This results in less resolved, broader peaks. Conversely, a flow rate that is too high will decrease the amount of time for molecules to diffuse. This will result in overlapping of detection peaks [BIORAD, 2016].

2.5 Gas Chromatography Mass Spectrometry

Gas chromatography mass spectrometry (GCMS) is a technique that allows for the identification of compounds in a volatile or semi volatile mixture [Evans Analytical Group, 2016]. Gas chromatography (GC) is used to separate the mixture into their individual components based on molecule size. This is done by injecting a set volume into the GC column where the mixture is volatilized. A carrier gas, usually an inert gas such as helium, carries the volatilized sample through the column [Douglas, 2010]. The column is lined with a stationary phase which, much like SEC, causes the individual components to partition themselves via increasing molecular weight [Douglas, 2010]. The now separated gas flows into the mass spectrometer (MS) which is used in the identification of the individual components in the gas stream. First, the separated gaseous sample passes through an electron beam where the molecules are ionized. The ionized molecules are then accelerated by an ion accelerator and move into the quadrupole mass filter. Ions that pass through the quadrupole have differing trajectories based on their mass/charge ratio. The ions are then detected and a mass spectra is produced. The mass spectrum can then be checked versus a library of known mass spectrum and assigned a degree of probability to be a particular molecule.

A generic diagram of the GCMS is shown in Figure 3
2.6 Centrifugal Separation

Centrifugal separation is a process that uses centrifugal force to achieve settling of particles in a solution, by way of a centrifuge. As the centrifugal force of the machine is used, higher density particles can be expected to settle to the bottom of the solution, with less dense particles forming layers on top of the more dense sediment. For a particle of mass \( m \) in a centrifuge of radius \( R \) rotating at a speed of \( \omega \) rpm, the centrifugal force \( F \) on the particle is expressed as \( F = 0.01097\omega^2R \). This, in conjunction with the force of gravity, achieves rapid settling of the particles in solution. [Lenntech]

Since various centrifuges can have very different design or operation parameters, a standard, dimensionless quantity is utilized to compare the forces applied by different centrifuges. This dimensionless quantity is traditionally called relative centrifugal force (RCF) and is measured in multiples of Earth’s standard gravity, colloquially known as “g’s.” The equation for calculating RCF is shown in Equation 1. This equation can also be represented in terms of RPM and the radius of the centrifuge as shown in Equation 2.

\[
RCF = \frac{\omega^2 r}{g} \quad (1)
\]

\[
RCF = 1.118 * r * \frac{RPM^2}{1000^2} \quad (2)
\]

For the purposes of this project, centrifugal separation was used to separate particulate matter for further analysis. Figure 4 shows the centrifuge used during experimentation for this project.
The speed at which the centrifuge operated was related to the maximum power of the centrifuge such that 10% power corresponded to 90 rpm, 20% power corresponded to 3675 rpm, 30% power corresponded to 6050 rpm, 40% power corresponded to 8250 rpm and 50% of the max power corresponded to 10000 rpm.

2.7 Time Dependent Separation

Time dependent separation is the process by which objects, such as haze and other particulate matter, separate into various layers over time. When a keg of beer is first delivered to the customer the beer inside is homogenized. As the keg sits at the restaurant or bar the haze and other particulate matter will separate and settle to the bottom of the keg causing excessively hazy pours and thus inconsistencies between each pour. The reason that the particulate matter separates is due to Archimedes’ Principle, which states that “the upward buoyant force that is exerted on a body immersed in a fluid is equal to the weight of the fluid that the body displaces.”[Boundless, 2016] In other words, the particulate matter will separate into regions of similar densities as the weight of the particulate comes to equilibrium with the buoyant force acting upward on it.
3 Methods

The goal of this project is to investigate methods of improving the clarity of the unfiltered beer products of Wachusett Brewing Company. Inconsistencies have recently been reported from customers in the clarity of some of the company’s unfiltered beers. Wachusett Brewing Company currently uses diatomaceous earth as a media in its filters. In order to preserve the vegan nature and simplicity of their product, they are hesitant to use additional fining agents to improve their clarity. The reason for this is that the majority of these fining agents are derived from animal components or artificial chemicals.

For this project, a series of experiments were run to investigate methods to improve the clarity of the beer. The experiments are an observation of natural particulate matter settling in a column over time, centrifugation followed by IR spectrography and measure of visual light transmittance, and size selective chromatography. From these experiments the team hopes to learn the conditions of the particles that are settling to cause turbidity, the effectiveness of mechanical separation via centrifuge as well as characteristics of the separated sediment.

3.1 Determination of Beer Components

Determination of beer components is vital for analyzing the impact of centrifugation on flavor components of beer. Compounds which contribute to flavor have already been categorized and comparing compounds present in the unfiltered beer sample provided by Wachusett Brewing Company to a database is an effective method of analyzing flavor components in their unfiltered beers. Relative abundance of compounds were measured and compared across three trials, an uncentrifuged sample, a 3675 rpm centrifuged sample, and a 10000 rpm centrifuged sample.

3.1.1 Size exclusion separation of beer components

The size exclusion chromatograph in this experiment did not incorporate a peristaltic pump, but instead beer sample was forced through the separation medium gravitationally. Additionally, the separated eluate was collected for analysis later by means of GCMS. Thus the only components of
this SEC were a separation column, a separation media, and test tubes for collecting samples. The full experimental setup can be seen in Figure 5a.

A slurry was prepared of 30 mL of 0.1 M 7.0 pH tris-HCl buffer and 1.25 g of G-75 sephadex. This slurry was mixed thoroughly and then poured into the size-exclusion column. The column was continuously filled with additional tris-HCl buffer so that the sephadex would not dry out, as is shown in Figure 5b.

If the sephadex does dry out, the uniformity of the media would be ruined, which could potentially create inconsistencies in elution time.

After ten minutes, the sephadex was settled and compacted within the column enough to create a medium for separation. The tris-HCl buffer was not poured into the column for some time until the level of tris-HCl was even with the top of the separation medium as can be seen in Figure 6b. This was to avoid any dilution of the beer sample when it was poured into the column.

The beer sample was then poured into the column along a glass stirring rod as to not disturb the surface of the sephadex. As the level of the beer sample reached the top of the sephadex, more tris-HCl buffer was added to the column to avoid drying the medium. The buffer was added as late as possible to avoid dilution of the beer sample.
The beer sample was observed as it passed through the medium and eluate was collected at the bottom of the separation column in thirty 1.0 mL test tubes from the time the beer sample was added to the top of the column until well past the time the last of the beer sample had eluted. The first ten test tubes were discarded as those test tubes contained only tris-HCl buffer and would not be useful for data collection. The remaining twenty samples were prepared for GCMS analysis by filling Thomson PTFE standard 0.45 $\mu$m filter vials. This whole process was repeated for each of the three beer samples.

3.1.2 Analysis of separated beer components via GCMS

The Thomson filter vials were loaded into the auto-sampler to be analyzed by the GCMS. An appropriate method was written for the voltage of the mass filter to analyze the full range of masses of components of the sample. The GCMS was run for sixty-three samples comprised of three sets of twenty-one samples each. Figure 7 shows the GCMS used for this experiment. For each set, twenty samples were separated eluate from the SEC and the last sample was a ‘control’ sample not run through the SEC, with each sample taking twenty minutes to analyze. The GCMS analysis program created a graphical output of abundance versus time. This graphical output was
then examined thoroughly to identify flavor-contributing components of the beer sample.

The Agilent analysis software used a National Institute of Standards and Technology (NIST) database of chemical compounds to determine identities of compounds in the beer sample. Each peak on the graph was examined for potential flavor contributing compounds that had a quality above 65%. Once flavor contributing compounds are found in one sample the same components can be expected to elute in other samples around the same time frame. This allows for the user to quickly navigate to the appropriate location on the abundance-time graph and then compare samples to one another.

3.2 Centrifugation and IR Spectrography

In addition to characterization of the the haze in its beer, Wachusett Brewing Company was also interested in clarity enhancement techniques that would not affect the quality or integrity of their products. Traditionally, fining agents consist of egg whites, blood, milk, isinglass, and other animal products. In addition, modern agents PVPP, activated carbon, and copper sulfate are also used. Wachusett Brewing Company prefers not to use such additives to keep their products as vegan and organic as possible. One method of improving clarity mechanically without the need of additives is through centrifugation.

The goal of the centrifuge experiments was to investigate what operating parameters can increase separation and thus improve the clarity of beer samples placed through it. The clarity of the beer
was measured by the percent of light transferred through beer. Ten samples were produced for this experiment. Test tubes 45 mL in volume were filled with a sample of beer and run through the centrifuge, physically separating particles in the beer samples by forcing them to settle. A series of five samples were run under constant power and increasing duration while another five samples were run under constant duration and increasing power, for a total of ten samples. The constant power samples were run with the centrifuge at 50% of its maximum power (corresponding to approximately 10000 rpm) with durations of six, twelve, eighteen, twenty-four, and thirty minutes. The constant duration samples were run for ten minutes at 10%, 20%, 30%, 40%, and 50% power (corresponding to approximately 90, 3675, 6050, 8250, and 10000 rpm, respectively).

After being centrifuged, the separated sediment that settled to the bottoms of the test tubes was collected. In order for the samples to be analyzed, they were first dried into pellets. A control sample of uncentrifuged beer was also dried to produce a pellet. Analysis of the ten pellet samples previously produced was done using a Fourier transform infrared spectroscopy (FTIR) machine. FTIR was used to measure the percent transmittance of IR light through a film made from a crushed pellet. The analysis produced graphs of percent transmittance vs wavelength of light. The percent transmittance at a given wavelength was compared between the samples. An increase in the percent transmittance can be related to an increase in the clarity of the beer since more light is able to pass through.

3.3 Visual Spectrum Analysis of Centrifuged Beer

To further examine the effectiveness of centrifugation in enhancing beer clarity, an analysis of visual light transmittance through centrifuged beer samples was also conducted. The same ten samples of beer used during the FTIR analysis were used in this experiment. Five samples were time-dependently centrifuged over six-minute intervals ranging from six to thirty minutes at a constant 50% centrifuge power and another five samples were power-dependently centrifuged at a constant time of ten minutes over 10% power intervals ranging from 10 to 50% of the maximum centrifuge power.

To measure the transmittance of visual light through a beer sample, a Vernier Software and Technology light sensor was used, and data was recorded using LoggerPro. The light sensor was highly sensitive to outside light sources, which would skew the data recorded. To mitigate this issue,
an apparatus was constructed using a styrofoam box in order to block unwanted light. Three holes were cut in the box, two in perpendicular faces to accommodate the light sensor at different angles and one for the desired light source. This desired source was a desk lamp with a three-Watt LED light bulb, which was kept on at all times to achieve consistent readings. A layer of masking tape and a sheet of white notebook paper were placed over the light source hole to reduce the intensity of the light source. Figure 8 shows the experimental setup of the complete apparatus.

![Figure 8: Representation of the apparatus used in the visual spectrum analysis](image)

During data collection, the lid of the apparatus was removed to minimize reflection within the box and other than the light source, the room was kept dark.

Two tests were conducted on each sample, one for which the light sensor was at a 90 degree angle to the light source and a second for which the sensor was kept at a 180 degree angle to the source. The two light sensor holes in the apparatus were used for this purpose. Holes in the apparatus that were not being used were covered during data collection. For each test, a 1.5 mL cuvette of a given sample was affixed to the base of the box using masking tape. The light sensor was placed flush against the cuvette at one of the two angles and held in place with a piece of masking tape at the top of the cuvette, so as not to affect transmittance readings. The rectangular cuvettes that were utilized had different textures on perpendicular faces, with two parallel faces being slightly more opaque than the other two. For consistency, the light sensor was always flush to one of the two less opaque faces.
After the light sensor and cuvette were in place and any unused openings in the apparatus were covered, data was recorded. Three or four sets of data were recorded for each test to make up for outliers and any undesired light that happened to be registered by the sensor. The data sets were then averaged to determine a single lux reading for the test. The process was repeated for all ten samples at both 90 and 180 degree angles, in addition to two similar control tests using water, and all data was recorded using Microsoft Excel.

3.4 Time Dependent Separation

One liter of beer sample was transferred to a one liter graduated cylinder sealed with Parafilm. The graduated cylinder filled with hazy beer used in this study is shown in Figure 9.

![1000ml Column with Hazy Beer](image)

Figure 9: 1000ml Column with Hazy Beer

The graduated cylinder was left to rest over the course of several days. Every twelve hours, a picture with a white background was taken of the graduated cylinder to measure the progression of visual clarity as the beer haze settles.
4 Results

4.1 Gas Chromatography Mass Spectrometry

In order to determine if the flavor of the beer would be impacted by centrifugation it was important to quantify any change in flavor contributing molecules in the beer across a sample of varying centrifugation rates. As prescribed in the GCMS methods, flavor contributing molecules were quantified for a control, 20% power and 50 % power samples. As expected, the elution of these compounds were around the same time frame. This is evident by noting the time at which peaks appear in the abundance-time graphs shown in figures 10, 11 and 12.

![Figure 10: Control GCMS Abundance-Time Data](image)
Figure 11: 20% Power GCMS Abundance-Time Data

Figure 12: 50% Power GCMS Abundance-Time Data
The raw data from these runs are shown in Appendix C. The flavor contributing compounds and their flavor contributions found across the three samples are shown in Table 1. Ideally, the abundance of these flavor compounds would show minimal fluctuation across control, 20%, and 50% powers. However, in comparing the control abundances to the other trials there was an observed increase in abundance of these compounds. The comparison of control to the 20% and 50% powers are shown in Tables 2 and 3.

Since GCMS abundance measurements are relative, the positive change in abundance for flavor contributing compounds indicates that significantly more haze particulate was removed than flavor compounds. If the volume of a sample remains constant, a decrease in abundance of one compound (haze creating particles) would result in a relative increase in other compounds in the volume (flavor contributors).

This data shows that centrifugation at the powers tested, does not have a significant impact on beer flavor.

<table>
<thead>
<tr>
<th>Name from GCMS</th>
<th>IUPAC Name</th>
<th>FEMA</th>
<th>Flavor Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maltol</td>
<td>3-hydroxy-2-methylpyran-4-one</td>
<td>2656</td>
<td>Chocolate</td>
</tr>
<tr>
<td>Phenylethyl alcohol</td>
<td>2-Phenylethanol</td>
<td>2858</td>
<td>Honey, Rose</td>
</tr>
<tr>
<td>1,2-benzenediol</td>
<td>Benzene-1,2-diol</td>
<td>2532</td>
<td>Wood, Smoke</td>
</tr>
<tr>
<td>1H-Indol-3-ethanol</td>
<td>2-(1H-Indol-3-yl)ethanol</td>
<td>N/A</td>
<td>Almonds[Hui, 2007]</td>
</tr>
<tr>
<td>Pentanoic acid, propyl ester</td>
<td>ethyl pentanoate</td>
<td>2462</td>
<td>Apples</td>
</tr>
<tr>
<td>Butanoic acid, 3-methyl-, propyl ester</td>
<td>propyl 3-methylbutanoate</td>
<td>2899</td>
<td>Strawberry</td>
</tr>
<tr>
<td>2(3H)-Furanone, dihydro-5-propyl-</td>
<td>5-propyloxolan-2-one</td>
<td>2539</td>
<td>Caramel</td>
</tr>
<tr>
<td>benezethanol, 4-hydroxy</td>
<td>4-(hydroxymethyl)phenol</td>
<td>3987</td>
<td>Nuts</td>
</tr>
</tbody>
</table>

Table 1: Flavor Attributes of Compounds Identified by GCMS. Flavor notes from the Flavor & Extract Manufacturers Association [FEMA]
<table>
<thead>
<tr>
<th>Common Name</th>
<th>IUPAC Name</th>
<th>Percent Change in Abundance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maltol</td>
<td>3-hydroxy-2-methylpyran-4-one</td>
<td>+19%</td>
</tr>
<tr>
<td>Phenethyl alcohol</td>
<td>2-Phenylethanol</td>
<td>0 %</td>
</tr>
<tr>
<td>Catechol</td>
<td>Benzene-1,2-diol</td>
<td>+4%</td>
</tr>
<tr>
<td>Tryptohol</td>
<td>2-(1H-Indol-3-yl)ethanol</td>
<td>+17%</td>
</tr>
<tr>
<td>Ethyl valerate</td>
<td>ethyl pentanoate</td>
<td>0%</td>
</tr>
<tr>
<td>Propyl isovalerate</td>
<td>propyl 3-methylbutanoate</td>
<td>+8%</td>
</tr>
<tr>
<td>gamma-Heptalactone</td>
<td>5-propyloxolan-2-one</td>
<td>0%</td>
</tr>
<tr>
<td>4-Hydroxybenzyl alcohol</td>
<td>4-(hydroxymethyl)phenol</td>
<td>+7%</td>
</tr>
</tbody>
</table>

Table 2: Change in abundance of flavor-contributing compounds between a non-centrifuged and 20% power centrifuged sample

<table>
<thead>
<tr>
<th>Common Name</th>
<th>IUPAC Name</th>
<th>Percent Change in Abundance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maltol</td>
<td>3-Hydroxy-2-methyl-4H-pyran-4-one</td>
<td>+19%</td>
</tr>
<tr>
<td>Phenethyl alcohol</td>
<td>2-Phenylethanol</td>
<td>0%</td>
</tr>
<tr>
<td>Catechol</td>
<td>Benzene-1,2-diol</td>
<td>+19%</td>
</tr>
<tr>
<td>Tryptohol</td>
<td>2-(1H-Indol-3-yl)ethanol</td>
<td>+11%</td>
</tr>
<tr>
<td>Ethyl valerate</td>
<td>ethyl pentanoate</td>
<td>0%</td>
</tr>
<tr>
<td>Propyl isovalerate</td>
<td>propyl 3-methylbutanoate</td>
<td>+13%</td>
</tr>
<tr>
<td>gamma-Heptalactone</td>
<td>5-propyloxolan-2-one</td>
<td>+28%</td>
</tr>
<tr>
<td>4-Hydroxybenzyl alcohol</td>
<td>4-(hydroxymethyl)phenol</td>
<td>+20%</td>
</tr>
</tbody>
</table>

Table 3: Change in abundance of flavor-contributing compounds between a non-centrifuged and 50% power centrifuged sample
4.2 Centrifugation

From the centrifuge experiments, it was found that centrifugation can be used to improve the clarity of beer samples by reducing the suspended particles through separation. Optical observations demonstrated a progression of improving beer clarity as the centrifuge was operated with increasing power. Before being centrifuged the test tubes of beer were opaque similar to the appearance of apple cider. After being centrifuged the samples became translucent, but cloudy with suspended particles visible, and with higher or longer centrifugation, they finally became clear with most of the sediment deposited at the bottom of the test tubes. A comparison of uncentrifuged samples with two centrifuged samples is shown in Figure 13.

![Figure 13](image)

(a) No centrifugation  (b) Low centrifugation  (c) High centrifugation

Increased clarity in the beer samples was confirmed via additional analysis of the IR and visual spectra.

4.2.1 IR Spectroscopy

Analysis of the IR transmittance through the separated sediment showed that, as expected, the samples possessed a transmittance pattern very similar to water. IR spectroscopy experiments initially began with direct analysis of the liquid samples of the beer. However, due to the aqueous
nature of the samples, they were nearly indistinguishable from pure water.

![Figure 14: Aqueous Centrifugation at 10000 RPM and 30 minutes](image)

In order to obtain measurable results, the liquid beer was evaporated to produce pellets of sediment which were then analyzed by the spectrometer. After the adjustment to the experiment, the samples demonstrated differences in the peaks along the wavenumbers of approximately 1700 to 1200 cm\(^{-1}\) that generally increased with increasing centrifuge power. As the centrifuge was increased from 10% to 50% of the centrifuge’s maximum power, the average transmittance of IR light through these peaks increased from 72% to 90%.

It can be seen from Figures 16 & 17 that at the wavenumbers 1750 and 1500 cm\(^{-1}\) that there is a positive slope on the plots of power vs percent of light transmitted through the beer. As the RPM of the centrifuge increases, the clarity of the beer increases. It should be noted that the relationship between time and power with respect to beer clarity is only a general trend. In both the power dependent as well as the time dependent trials, there were values that fit the expected trend when compared to one or more of its neighboring trials. An explanation for this variation can be found in the operation of the FTIR machine. In order for the machine to analyze solid samples, they must first be crushed into a fine powder and compacted by an arm over the analyzing aperature. Fluctuations in the thickness of this compacted layer can affect the results of the analysis.
Figure 15: IR Control Test, Water

Figure 16: Percent Transmittance at 1750 cm\(^{-1}\)

\[ y = 0.18x + 74.6 \]
Figure 17: Percent Transmittance at 1500 cm$^{-1}$
### 4.2.2 Visual Spectrum

In addition to the IR spectrography, analysis of the visual spectrum was also conducted with a light sensor. These experiments were conducted to corroborate the results of the IR spectrography as well as to test liquid samples.

The tests using visible light confirmed the result of the IR tests. In most of the cases, the lux of the light passing through the beer samples increased as they were either centrifuged longer or at a greater speed.

![Figure 18: Measured Lux Values of Power Dependent Centrifugation at 180°](image)

Another significant finding was that the placement of the light probe at different angles relative to the light source affected the measurable differences between samples. When the light probe was perpendicular to the light source the greatest differences in the measured lux values were 3 and 10 lux for the power and time dependent centrifuge experiments respectively. In comparison, when run with the light probe in line with the light source, the respective lux differences measured through the samples were 33 and 17 lux.
Finally, it was found from the test using visible light that centrifuge separation does not cause an increase in chill haze. The average difference between chilled and room temperature samples was 14 lux with the smallest difference being 0.65 lux, the greatest being 27 lux and the mode lux difference approximately 16 lux.

4.3 Time Dependent Separation

From the time dependent separation experiment, it was found that the particulate matter that makes up permanent haze settled to the bottom of a 1000ml graduated cylinder in approximately 9 days. As described in the methods section, pictures with time stamps were taken of the column in approximately twelve hour increments. These photos were then examined and assigned an estimated haze level. Examples of the haze level estimates are shown in figures 20a and 20b.

From this and the time stamp on each image it was possible to graphically relate the haze position
to an elapsed time. The data could then be fit with an appropriate trend line. In this case, a linear trend line best fit our data. The rate of change of the haze level is then represented as the slope of the trend line which is shown in figure 21 as -0.17cm/h. All data used in creating figure 21 is attached in Appendix B.
Figure 21: Change of Haze Position Over Time

\[ y = -0.1715x + 36.483 \]
5 Analysis

5.1 Determination of Haze Particulate Sizes

Calculations involving the settling rate of particles can be used to determine the size range of haze particulate. These calculations involve the balance of drag forces and buoyant forces. Drag force is given by:

\[ F_d = \frac{1}{2} C_D A \rho_f v^2 \]  

(3)

Where \( C_D \) is the drag coefficient, \( A \) is the cross-sectional area of the sediment particle, \( \rho_f \) is the density of the fluid, and \( v \) is the settling velocity.

Buoyant force is given by:

\[ F_b = \frac{\pi d^3}{6} (\rho_p - \rho_f) g \]  

(4)

Where \( d \) is the diameter of the settling particle, \( g \) is acceleration due to gravity, and \( \rho_p \) is the density of the settling particle.

We can set these two forces equal to each other and solve for the diameter of the particle:

\[ d = \frac{3}{4} C_D \frac{v^2}{g} \frac{\rho_f}{\rho_p - \rho_f} \]  

(5)

From §4.3, Figure 21, it can be seen that the settling rate is 0.1715 cm/h. From the literature, density of yeast, and thus density of it’s broken down components, is 1.05 g/cm³. Because these particles are small, the particles can be approximated as spheres, and therefore the \( C_D \) of the particles is 0.50. Solving for \( d \), yields a value of 17.36 Å.

5.2 Centrifuge Scale-up

The Gt-method is a simple, qualitative method in which a centrifuge process can be scaled up from laboratory scale to production scale. A value of the RCF multiplied by time is called a Gt-value. This is shown in Equation 6. Equating two Gt values will result in the same degree of centrifugation.
\[ Gt = RCF \times t = t \times 1.118 \times r \times \frac{RPM^2}{1000^2} \] (6)

RPM, radius (mm), and time (min) values are all measurable on the lab sized centrifuge and as such a Gt value can be made for any of our centrifuge runs. In order to scale up the lab sized centrifuge it is important to realize the parameters that the brewery can control. The radius \((r_c)\) of the brewery’s centrifuge is fixed and thus this parameter cannot be manipulated. The residence time \((t_c)\) of the beer in their continuous centrifuge is also a parameter that is fixed based on their required production rate. This leaves the RPM of the brewery’s centrifuge as the control parameter. A new equation can then be written in terms of the RPM of the brewery’s centrifuge shown in equation 7.

\[ RPM_c = \sqrt{\frac{r_l \times RPM_L^2 \times t_L}{r_c \times t_c}} \] (7)

In equation 7 all the lab sized centrifuge values ie: \(RPM_L, t_L,\) and \(r_L\) are known or measurable at the time of testing. These values can be taken from our data or manipulated on another centrifuge to achieve any desired visual clarity. Since the radius of the production centrifuge is fixed and the residence time of beer is set by the flow rate of beer, all variables to solve for the \(RPM_c\) are known. This essentially means that any visual clarity that can be produced on a lab sized centrifuge can be reproduced on a production scale centrifuge providing the \(RPM_c\) calculated from equation 7 is mechanically achievable by the production centrifuge. A sample calculation using the 50% power figures is shown in Appendix D

### 5.3 Calculation of Permittivity of the Separation Medium

A useful parameter for generalizing the dynamics of a separation column is the permittivity of the exclusion medium. From the product sheet on GE’s website, Sephadex G-75 behaves as spheres obeying Darcy’s Law. Therefore, the diffusion rate of particulate through the medium can be modeled by:

\[ \Phi = -\frac{\kappa}{\eta} \frac{dP}{dz} \] (8)
Where $\Phi$ is the flow rate through the column, $\eta$ is the viscosity of the fluid, $\frac{dP}{dz}$ is the instantaneous change in pressure along the vertical axis of the column,\(^1\) and $\kappa$ is the permittivity of the separation medium. Because the column has a fixed length, the differential can be exchanged for a difference:

$$\Phi = -\frac{\kappa \Delta P}{\eta \Delta z} \tag{9}$$

Where $\Delta P$ is the change in pressure between the top of the column and the bottom of the column and $\Delta z$ is the height of the separation medium. Since the SEC was gravitationally driven, the pressure difference within the column is a result of the weight of the fluid:

$$\Delta P = \rho_f \times (V_q + V_{\text{void}}) \times g \times \frac{1}{A_{\text{col}}} \tag{10}$$

Where $\rho_f$ is the density of the fluid, $A_{\text{col}}$ is the cross-sectional area of the separation column, $g$ is the acceleration due to gravity, $V_q$ is the queued volume\(^2\), and $V_{\text{void}}$ is the void volume:

$$V_{\text{void}} = V_{\text{total}} - V_w \tag{11}$$

Where $V_{\text{total}}$ is the total volume of the separation medium in the column and $V_w$ is the volume of the wet sephadex, given by:

$$V_w = V_d \times \frac{V_{pw}}{V_{pd}} \tag{12}$$

Where $V_d$ is the dry volume of the sephadex, $V_{pw}$ is the volume of a single wet sephadex bead, and $V_{pd}$ is the volume of a single dry sephadex bead.\(^3\) From here, it is possible to calculate $\kappa$.

Solving for permittivity with equations 9, 10, 11, 12:

$$\kappa = \frac{-\Phi \times \frac{\Delta z \times \eta \times A_{\text{col}}}{\rho_f \times (V_q + V_{\text{total}} - V_d \times \frac{V_{pw}}{V_{pd}}) \times g}}{13}$$

\(^1\)The generalization of Darcy’s Law calls for a gradient over all coordinates of pressure; however, by restricting large scale flow to a single axis of motion, this gradient reduces to a one-dimensional derivative.

\(^2\)The queued volume is the portion of liquid which has not yet entered the separation medium and is resting above this medium within the column.

\(^3\)This $\frac{V_{pw}}{V_{pd}}$ term is equivalent to the swelling factor of the sephadex.
From the appropriate literature and experimentally determined values, the value of $\kappa$ for this SEC with this separation medium is:

$$\kappa = 0.00225 \pm 0.00013$$
6 Conclusion

The goal of this project was to assist Wachusett Brewing Company by determining the viability of methods to maintain visual consistency among their unfiltered beers without impacting their flavor. Based on centrifugation experiments in conjunction with IR and visual spectrum analysis, it can be shown that centrifugal separation is an effective method for reducing beer turbidity and haze. In addition, centrifugation does not increase the presence of chill haze in low temperature samples of beer. Further GCMS analysis showed that flavor compounds are still present in centrifuged beer, thus the quality of flavor is not impacted by centrifugation. We recommend that Wachusett Brewing Company utilizes centrifugal separation to reduce haze and increase the consistency between batches of unfiltered beer.
## Appendix A  Centrifugation Data

<table>
<thead>
<tr>
<th>Power</th>
<th>Avg Normalized Transmittance $^4$</th>
<th>Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.674</td>
<td>0.089</td>
</tr>
<tr>
<td>10%</td>
<td>0.841</td>
<td>0.121</td>
</tr>
<tr>
<td>20%</td>
<td>0.852</td>
<td>0.126</td>
</tr>
<tr>
<td>30%</td>
<td>0.851</td>
<td>0.094</td>
</tr>
<tr>
<td>40%</td>
<td>0.869</td>
<td>0.049</td>
</tr>
<tr>
<td>50%</td>
<td>0.853</td>
<td>0.135</td>
</tr>
</tbody>
</table>

Table 4: Light Transmittance Values of Centrifuged Beer Taken at 90°

<table>
<thead>
<tr>
<th>Power</th>
<th>Avg Normalized Transmittance $^5$</th>
<th>Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.626</td>
<td>0.034</td>
</tr>
<tr>
<td>10%</td>
<td>0.800</td>
<td>0.117</td>
</tr>
<tr>
<td>20%</td>
<td>0.884</td>
<td>0.043</td>
</tr>
<tr>
<td>30%</td>
<td>0.875</td>
<td>0.089</td>
</tr>
<tr>
<td>40%</td>
<td>0.975</td>
<td>0.058</td>
</tr>
<tr>
<td>50%</td>
<td>1.010</td>
<td>0.050</td>
</tr>
</tbody>
</table>

Table 5: Light Transmittance Values of Centrifuged Beer Taken at 180°

$^4$Normalized to water

$^5$Normalized to water
# Appendix B  Time Dependent Separation

## B.1 Data

<table>
<thead>
<tr>
<th>Sample</th>
<th>Time Recorded</th>
<th>Minutes Elapsed</th>
<th>Level of Haze (ml)</th>
<th>Level of Haze (cm)$^6$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>9:26 PM</td>
<td>0</td>
<td>1050</td>
<td>37.24</td>
</tr>
<tr>
<td>2</td>
<td>9:44 AM</td>
<td>738</td>
<td>975</td>
<td>34.67</td>
</tr>
<tr>
<td>3</td>
<td>9:08 PM</td>
<td>1414</td>
<td>900</td>
<td>32.00</td>
</tr>
<tr>
<td>4</td>
<td>9:07 AM</td>
<td>2133</td>
<td>830</td>
<td>29.51</td>
</tr>
<tr>
<td>5</td>
<td>10:16 PM</td>
<td>2922</td>
<td>790</td>
<td>28.09</td>
</tr>
<tr>
<td>6</td>
<td>9:37 AM</td>
<td>3603</td>
<td>725</td>
<td>25.78</td>
</tr>
<tr>
<td>7</td>
<td>9:12 PM</td>
<td>4288</td>
<td>680</td>
<td>24.18</td>
</tr>
<tr>
<td>8</td>
<td>10:25 AM</td>
<td>5081</td>
<td>615</td>
<td>21.87</td>
</tr>
<tr>
<td>9</td>
<td>10:49 PM</td>
<td>5825</td>
<td>575</td>
<td>20.45</td>
</tr>
<tr>
<td>10</td>
<td>9:48 AM</td>
<td>6484</td>
<td>500</td>
<td>12.78</td>
</tr>
<tr>
<td>11</td>
<td>9:20 PM</td>
<td>7166</td>
<td>450</td>
<td>16.00</td>
</tr>
<tr>
<td>12</td>
<td>9:42 AM</td>
<td>7908</td>
<td>400</td>
<td>14.22</td>
</tr>
<tr>
<td>13</td>
<td>10:24 AM</td>
<td>9390</td>
<td>275</td>
<td>9.78</td>
</tr>
<tr>
<td>14</td>
<td>9:41 AM</td>
<td>10787</td>
<td>150</td>
<td>5.33</td>
</tr>
<tr>
<td>15</td>
<td>8:50 AM</td>
<td>12176</td>
<td>50</td>
<td>1.78</td>
</tr>
</tbody>
</table>

Table 6: Time Stamp and Total Time Elapsed Between Photographs

---

$^6$These height measurements were made using a Starrett No.C305R 6in. Scale. Conversions done on the basis of 1in to 2.54cm.
B.2 Time Dependent Separation Images Marked for Clarity

Figure 22
Figure 23

(a) 2-18-2016 9:08pm  
Haze Height 
~900ml

(b) 2-19-2016 9:07am  
Haze Height 
~830ml
Figure 24

(a) 2-19-2016
10:16pm

(b) 2-20-2016 9:37am

Figure 24
Figure 25
Figure 26

(a) 2-21-2016 10:49pm
(b) 2-22-2016 9:48am

Haze Height
~575ml

Haze Height
~500ml
Figure 27

(b) 2-23-2016 9:42am
Figure 28
Appendix C  GCMS Data

C.1 Control

This section displays NIST library detection graphs for the GCMS control runs

Figure 30: Maltol Mass Spectrometry
Figure 31: Phenylethyl Alcohol Mass Spectrometry

Figure 32: 1,2-benzenediol Mass Spectrometry
Figure 33: $1H$-Indol-3-ethanol Mass Spectrometry

Figure 34: Pentanoic Acid. propyl ester Mass Spectrometry
Figure 35: Butanoic Acid. 3-methyl-, propyl ester Mass Spectrometry

Figure 36: 2(3H)-Furanone,dihydro-5-propyl- Mass Spectrometry
Figure 37: Benzeneethanol, 4-hydroxy Mass Spectrometry
C.2 20% Power

This section displays NIST library detection graphs for the GCMS 20% power runs.

Figure 38: Maltol Mass Spectrometry
Figure 39: Phenylethyl Alcohol Mass Spectrometry

Figure 40: 1,2-benzenediol Mass Spectrometry
Figure 41: 1H-Indol-3-ethanol Mass Spectrometry

Figure 42: Pentanoic Acid. propyl ester Mass Spectrometry
Figure 43: Butanoic Acid. 3-methyl-, propyl ester Mass Spectrometry

Figure 44: Benzeneethanol, 4-hydroxy Mass Spectrometry
C.3  50% Power

This section displays NIST library detection graphs for the GCMS 50% power runs.

Figure 45: Maltol Mass Spectrometry
Figure 46: Phenylethyl Alcohol Mass Spectrometry

Figure 47: 1,2-benzenediol Mass Spectrometry
Figure 48: 1H-Indol-3-ethanol Mass Spectrometry

Figure 49: 2(3H)-Furanone,dihydro-5-propyl- Mass Spectrometry
Figure 50: Benzeneethanol, 4-hydroxy Mass Spectrometry
Appendix D  Sample Calculations for Centrifuge Scale-up

Given:
\( t_L = 10 \text{ min} \)
\( \text{RPM}_L = 10,000 \pm 500 \)
\( r_L = 65.5 \text{ mm} \pm 2.5 \)

Find \( \text{RPM}_c \):
To do this we require the radius \((r_c)\) of the process centrifuge and the residence time \((t_c)\) of the beer in the process centrifuge. To find \( t_c \) we first assume a production of 15,000 bbl/year [Kalispell, 2016]. This equates to 465,000 gal/year of beer. Assume that only half of this production needs to be centrifuged. Assume a 40 hour work week and 50 week year. Assume that pump uptime of 65%. Converting this to effective gallons/hr and gallons/min:

\[
\frac{465,000 \text{ gal}}{\text{year}} \times \frac{1 \text{ year}}{50 \text{ week}} \times \frac{1 \text{ week}}{40 \text{ hour}} \times \frac{1}{2} \times \frac{1}{65\%} = \frac{178.85 \text{ gal}}{\text{hour runtime}} = \frac{2.98 \text{ gal}}{\text{minute runtime}}
\]  

Calculating residence time \((t_c)\) assuming process volume of 3 gallons:

\[
t_c = \frac{\text{Process Volume}}{\text{Process Flowrate}} = \frac{3 \text{ gal}}{\frac{2.98 \text{ gal}}{\text{min}}} = 1 \text{ minute}
\]

Calculate \( r_c \) from assumption of 3 gallon process volume:

\[
3 \text{ gal} = 0.01136 \text{ m}^3
\]

\[
V_s = \frac{4}{3} \times \pi \times r_c^3
\]

\[
0.01136\text{m}^3 = \frac{4}{3} \times \pi \times r_c^3
\]

\[
r_c = \sqrt[3]{0.01136\text{m}^3 \times \frac{3}{4} \times \frac{1}{\pi}} = 0.14 \text{ m} = 140 \text{ mm}
\]
Combine into $RPM_c$ equation 7:

$$RPM_c = \sqrt{\frac{65.6 \text{ mm} \times 10,000 \cdot RPM_L^2 \times 10 \text{ min}}{140 \text{ mm} \times 1 \text{ min}}} = 21,600 \pm 860 \text{ RPM} \quad (20)$$

To achieve the same visual clarity as our centrifuge at 10,000 RPM and 10 min runtime one would have to run the process centrifuge at 21,600 ± 860 RPM.
References


