WPI

Increased Sugar Conversion in Hops-Free Beer

A Major Qualifying Project
submitted to the Faculty of
WORCESTER POLYTECHNIC INSTITUTE
in partial fulfilment of the requirements for the
degree of Bachelor of Science
in the field of Chemical Engineering

Sponsored By:
Purgatory Beer Company
670 Linwood Ave Building C
Whitinsville, MA 01588

Report Prepared By:
Maggie Gaffney
Madison Govaert
Erinn Jambor
Emily Osterloh

Submitted on 28 April 2021

Advised By:
Professor Stephen J. Kmiotek, PhD

Abstract

This report serves to summarize the processes and data gathered regarding the formulation of a hops-free beer with increased sugar conversion and a desirable flavor profile. The ultimate goal of this project was to develop a hops-free beer utilizing juniper berries and raspberries as flavoring agents that had a greater sugar conversion than the test batch and had a desirable flavor. This report details the different brew methods used and their impact on taste. It also details the use of a GC-MS to identify compounds present in the beers and to correlate the compounds present with the tastes of the different beers. Additionally, information about the use of HPLC data to determine sugar conversion is also within the contents of this report. An analysis of possible error is provided, along with the conclusions generated by the team and the recommendations given for students attempting to further this study in future years.

Acknowledgements

The team would like to thank the following individuals and groups for their contribution to this project:

- Professor Stephen Kmiotek for always fielding our questions, concerns, and random thoughts, as well as aiding in creative solutions for how to address MQP during a global pandemic.
- Purgatory Brewing Co., for their part in sponsoring this MQP and its precursors.
- Chepachet Hardware store, for their brewing advice and for their emergency supply of glass carboys.
- Deanna Lauro, Prachi Patel, Sebastian Stypulkowski, Colleen West, Guillaume Poisson, Nicholas Fleury, Jakob Field, and Hunter Kortz for their work on the previous MQPs that inspired this one.
- Professor Geoffrey Tompsett, Feng Cheng, and Ziyang Zhang for the enormous amount of help they gave in running the laboratory machines.
- Alia Brown for allowing the team to brew in her apartment during B-Term.
- David Gaffney Jr. and Kathleen, Shane, and Brian Jones for their help brewing over winter break and the spring semester.
- Amy Gaffney for her help procuring brewing equipment, sanitizing brewing equipment, transporting the brew kit before winter break, and helping run tasting panels when the team was under lockdown in C-Term.

Table of Contents

Table of Figures

Introduction

In conjunction with Purgatory Beer Company of Northbridge, MA., several of our fellow WPI undergraduate students last year sought to improve upon and specialize techniques in microbrewing that would individualize Purgatory among the competitors in the world of craft beer. This project looks to continue the work of the *Development and Optimization of a "Hop Free" Beer* (Lauro et al) and the *Brewing Process Optimization: Mash Efficiency* (Field et al) projects. Ultimately, the goal of this project is to develop a beer without using hops as preservatives that could be marketable as such by Purgatory, as well as analyze which brewing method yielded the highest conversion rates of sugars such as fructose, maltose, and sucrose. Using Lauro et al.'s findings, the alternative to hops was chosen to be juniper berries, with raspberries added for flavoring. The brewing methods were chosen from Field et al.'s work.

Methods

Sanitization Methods

For all brews, all brew equipment was sanitized with a solution of water and StarSan sanitizing agent. For brews seven and eight, spring water was used for sanitization. This change was made in an attempt to remove an unpleasant taste from the beer that was thought to be the result of the chlorides in tap water.

Brew Methods

Over the course of this project, eight different brew methods were used. The amount of grain used in each brew, in addition to the amount of juniper berries, was held constant.

Brew one was the control batch. The recipe in Appendix A was followed, with five pounds of fresh raspberries added during primary fermentation.

Brew two utilized Vorlauf sparging, wherein during the sparge stage of brewing, the beer was sparged an additional time. The recipe in Appendix A was otherwise followed, with five pounds of fresh raspberries added during primary fermentation.

Brew three involved preheating the brew bag by placing it in the brew kettle while the water was room temperature and then allowing the brew bag to remain in the kettle until the sparge stage. The recipe in Appendix A was otherwise followed, with five pounds of fresh raspberries added during primary fermentation.

Brew four involved increasing the mash time during brewing by ten minutes. The recipe in Appendix A was otherwise followed with five pounds of fresh raspberries added during primary fermentation.

Brew five involved continuously mixing the wort while it brewed. The recipe in Appendix A was otherwise followed, with five pounds of fresh raspberries added during primary fermentation.

Brew six was a second control batch, wherein the recipe in Appendix A was followed. However, only three pounds of fresh raspberries were added during primary fermentation. This change was performed as an attempt to reduce the bitterness of the beer.

Brew seven involved a mash time increased by ten minutes. The recipe in Appendix A was otherwise followed. In this brew, only three pounds of raspberries were added during primary fermentation. Frozen, thawed raspberries were also used over fresh because the thawed raspberries would have an increased amount of sugar, which was desired in order to reduce the

bitterness of the beer. The raspberries for this brew were also washed in spring water to avoid the bitter taste previously attributed to the chlorides in tap water.

Brew eight involved a mash time increased by twenty minutes. The recipe in Appendix A was otherwise followed. In this brew, three pounds of thawed raspberries were added during primary fermentation. The raspberries for this brew were also washed in spring water for the same reason that the raspberries in brew seven were.

Increased mash time was used in brews seven and eight because brew four, the original brew in which increased mash time was used, had the most satisfactory data, as seen in the results section.

Extraction Methods

For these methods, we followed the procedures of Bosco et al. In order to begin the process of extracting the wort samples for HPLC and GC-MS, each sample was mixed with dichloromethane (DCM) in a 1:2 ratio. Most often, this meant 6 mL of wort were placed with 12 mL of DCM in a centrifuge vial. Adding salt to each vial may also help in the separation process of each sample. Each vial was gently shaken for 10 minutes, then placed in a centrifuge at room temperature for 10 minutes, running at 2000 rpm. After centrifuging, the samples are separated and stable enough to store for however long until the next step.

Next, it is necessary to have hypodermic needles, syringes, and syringe filters, as well as 1.5 mL autosampler vials for the HPLC and spring water for dilution. The needles are sterile, 0.5 inches diameter, and individually-packaged as 25 needles per pack. The Luer-Slip syringes are non-sterile, disposable, and hold up to 3 mL of liquid. The barrel of the syringe is made of polypropylene, and the plunger is made of polyethylene. These are packaged at 100 syringes per pack. Syringe filters come in packs of 100, made of polytetrafluoroethylene (PTFE), non-sterile, 13 mm in diameter and have pore sizes of 0.22 μm. The autosampler vials are made of clear glass and caps with pre-inserted PTFE/silicone. Vials are 12x32 mm in size with a writing patch on the side to make sure samples are correctly identifiable. Both the organic-phase and aqueous phase, containing the DCM and wort, respectively, should be extracted from each sample with a filtered needle and syringe. The DCM may be placed directly into an autosampler vial, while the wort sample should be diluted with a 1:4 ratio of wort to water (i.e., diluting 2 mL of wort with 8 mL of spring water) before placing in a vial. Finally, the samples are ready to run HPLC and GC-MS on. It is recommended to try these methods on a sample of generic beer first to ensure extraction runs without causing error to the wort samples.

GC-MS Methods

Gas Chromatography Mass Spectroscopy (GC-MS) was used to determine what compounds were present in the different brewing methods. Because of COVID-19 restrictions we were unable to analyze the samples ourselves and each wort sample was given to several PhD students to be analyzed. Their method of analyzing the samples consisted of using a GC-2010 Plus gas chromatograph, a QP2010 SE mass spectrometer, and an AOC-20i auto injector. The injection temperature performed was 290℃ with an ion source at 260℃. From each wort sample, a smaller 4μL sample was extracted and injected into the GC-MS system in a split mode 25:1. A constant flow rate of 3 mL/min was set for the helium carrier gas. The programmed temperature started at 30℃ for 4 minutes and was then heated up to 290℃ at a rate of 3℃/min, the temperature was then held at 290℃ for 5 min. The ionization energy that the mass spectrometer was operated at was a m/z 35-500 scan range. From this GC-MS procedure, tables of the retention time and area% were formed for each wort sample and shared with our group to be analyzed.

Sugar Calibration Curve Generation Methods

In order to identify the concentrations of sugars in each brew, calibration curves were created following a similar procedure as that of the Brewing Process Optimization group (Field et al, 2020). The team utilized samples of known concentrations of fructose, maltose, and sucrose from Sigma Aldrich. A standard all-malt wort will contain approximately 12% monosaccharides (such as fructose), 5% sucrose, and 47% maltose (Kapral, n.d.). Therefore, a range of concentrations for each sugar was prepared encompassing a wide amount of percentages in the possible case of variance. For example, we set a range of sucrose samples differing in percentage from 1% to 20% to cover a broad range, with the expected percentage still being considered.

Each sample was prepared through dilution of liquid samples of fructose and sucrose and through the combination of water and solid samples of maltose in varying ratios. Calculations were performed to determine the volumes of each compound necessary to reach proper concentrations. Sample calculations can be found in Appendix C.

After each sample was prepared, 1 mL of each sample was added through disposable and non-sterile Luer-Slip syringes and to a 1.5 mL autosampler vial. Each vial was labeled depending on the sugar content and concentration (e.g., 7% Fructose) and all vials were grouped together for analysis using High Performance Liquid Chromatography (HPLC).

HPLC Methods

Both the centrifugation and the filtration methods utilized during the extraction of the samples were helpful in ridding the wort of potential impurities for HPLC data collection. It also preserved the quality of the wort, as it was less likely to expire after such procedures were taken.

Tasting Methods

For each beer, a sample of individuals ranging from age twenty one to eighty eight sampled the beer. Both men and women sampled each brew, as did both individuals who regularly drink beer and also individuals who would not typically taste beer.

Oral feedback from participants was noted. Since brews one, two, three, four, and five were all tasted at once, the feedback from those brews influenced the decision to brew with fewer raspberries in brew six. After brew six was tasted, the results from that taste test impacted the brew methods from brews seven and eight.

Results

GC-MS Results

The beer tasting panel gave a qualitative view of the effects of the different brewing methods on the taste of the beer. To gather a more quantitative look into the taste of each beer, GC-MS analysis was completed. Just prior to the addition of the yeast during the brew, wort samples were taken from the beer and tested using the GC-MS, data on the area percentage and retention time of the compounds were given in the form of an excel file. A graph of the area percent vs the retention time was created for each wort sample. A list of compounds was created for each wort sample and from these lists, compounds were compared and researched for their impact on the flavor profile of the beer. The compounds and their area percentages were analyzed for each brew to help determine which brewing method produced the highest quality beer.

Due to time constraints, GC-MS results were only analyzed for beers one through five. The detailed GC-MS data can be found in Appendix B.

Brew one:

Brew one, the control brew, yielded two wort samples that were gathered and tested. The chromatograph was analyzed for compounds with an area percentage above one, research was completed on these compounds to determine if they had a significant impact on the flavor profile.

Tetradecane and tridecane had significant peaks of 4.77 and 5.3 respectively, these compounds most likely produce a bad taste and smell (National Center for Biotechnology Information, 2021A). Additionally, the compound dodecane, 4,6-dimethyl also has a negative effect on the taste/smell and is present in this sample. The area percentage of dodecane, 4,6-dimethyl is only 1.17, however this could be significant enough to negatively affect the beer (National Center for Biotechnology Information, 2021B).

In this sample, Phenol, 2,4-bis(1,1-dimethylethyl)- was produced. According to research, a beer consisting of phenols could have either positive or negative results to the beer. Some phenolic flavors are welcome in beer while others should be avoided. The area percentage of this compound was 2.95 which is a significant peak. This phenol occurs naturally in allspice, which could have a positive effect on the flavor of the beer (The good scents company - Aromatic/Hydrocarbon/Inorganic Ingredients catalog information, n.d.A).

After analyzing the GC-MS data for brew one, it was determined that more negative compounds were present than positive compounds. This was consistent with the taste test considering the results concluded the beer did not taste good.

Brew two:
Brew two utilized the Vorlauf method during the sparge stage of brewing and 1 wort sample was gathered and tested. The chromatograph was analyzed for compounds with an area percentage above one, these compounds were then analyzed to determine their effect on the flavor profile.

Similarly to method 1, dodecane, 4,6-dimethyl- was also present in brew two. However, three additional peaks of this compound were also present. Having almost four times as much dodecance, 4,6-dimethyl in this sample compared to the previous, could have a significant negative impact on the flavor profile (National Center for Biotechnology Information, 2021B).

Phenol, 2,4-bis(1,1-dimethylethyl)- was also present in beer two, similarly to beer one, however 1-hexadecanesulfonyl chloride was also detected. Initially the phenol being present is considered a positive, but with the detection of a chloride, a negative result could occur. Phenols and chlorides could combine to create chlorophenols, which have an off-putting taste, similar to band aids. The chloride could be a result from chlorine present in the water used in any part of the brewing process (Hines, 2017).

After analyzing the GC-MS data for brew two, the high amount of dodecance, 4,6-dimethyl and the combinations of the phenol and chloride, could have been responsible for the negative taste of the beer, which was concurrent with the tasting panel results.

Brew Three:
Brew three involved preheating the mash bag during the boiling stage of brewing and two samples were taken. Both samples were analyzed for compounds that could have a significant effect on the flavor profile.

Both of the previous brews contained Dodecane, 4,6-dimethyl-, similarly to brewing method 2, Dodecane, 4,6-dimethyl- had 4 four peaks causing there to be around 4 times as much as brewing method 1 and about the same as brewing method 2. This amount of Dodecane, 4,6-dimethyl- would have similar impacts on the flavor profile as brew two (National Center for Biotechnology Information, 2021B).

Additionally, this brewing method produced Pentadecane and Sulfurous acid with a peak of 4.49 and 1.12 respectively. Both of these compounds have a positive impact on the flavor profile (Bickham, 2013). Pentadecane has an alkane and waxy taste and is often found in peppers, papaya, and lemons. Sulfurous acid has a sweet and/or sour flavor (Pentadecane, n.d.).

Similarly, to brew one and two, Phenol, 2,4-bis(1,1-dimethylethyl)- was also detected with a peak of 3.95, which is larger than both the previous peaks. This compound will have a more significant impact on the flavor profile than the previous two brews (Hines, 2017).

Brew three contained three compounds that had a positive impact on the flavor profile and a significant amount of one compound that negatively affected the flavor. This was concurrent with the qualitative data that described beer three as better tasting than the others.

Brew Four:
Brew four utilized a mash time increased by ten minutes during the brewing process and two wort samples were gathered. Both samples were analyzed for compounds that could have a significant effect on the flavor profile.

A chlorophenyl was detected in this brew. This has a negative effect on the flavor profile as chlorine should be avoided. Similarly, to brew two, this compound could add an off putting taste. The chlorine could be a result from chlorine present in the water used in any part of the brewing process (Hines, 2017).

Similarly to brew one, tetradecane and tridecane were detected in the beer. Tetradecane produced two peaks of 3.46 and 1.42, while tridecane produced a peak of 2.17. As previously stated, both of these compounds are similar to tridecane 1-iodo which has a negative effect on the flavor profile therefore these compounds are most likely to produce a bad taste and smell (National Center for Biotechnology Information, 2021A).

With the compounds, chlorophenyl, tetradecane, and tridecane all having negative impacts on the flavor profile of the beer, it was no surprise that the tasting panel matched these results.

Brew Five:
Brew five utilized continuous mixing during the brew stage and two wort samples were gathered. Once again, both samples were analyzed for compounds that could have a significant effect on the flavor profile.

Similar to previous brews dodecane, 4,6-dimethyl was present, however, the amount of dodecane, 4,6-dimethyl was similar to brew two and three and had the same impact on the flavor of the beer as it had in those two brews (National Center for Biotechnology Information, 2021B).

Tetradecane and tridecane were detected with peaks of 4.51 and 4.07 respectively. The presence of these compounds was similar to brew one and had a similar impact on the taste (National Center for Biotechnology Information, 2021A).

Additionally, propanoic acid was detected with a peak of 3.82. Propanoic acid has a flavor that is acidic and tastes similar to dairy. The presence of propanoic acid had a negative effect on the flavor of brew five (The good scents company - Aromatic/Hydrocarbon/Inorganic Ingredients catalog information, n.d.B).

Overall, the presence of the compounds dodecane, 4,6-dimethyl, tetradecane, tridecane, and propanoic acid all had a negative effect on the flavor profile. These results are concurrent with the tasting panel, wherein brew five was considered the most bitter and unpleasant of the first five brews.

HPLC Results

In the resulting data, each sugar concentration had a corresponding peak area and retention time. These values were recorded in conjunction with the known concentrations to graph the calibration curves. For all three sugars, curves were plotted with the peak area being the dependent variable (y-axis) and the concentration being the independent variable (x-axis). At this point, the wort samples were analyzed. Each wort sample contained several substances represented as peaks on a chromatogram (and these chromatograms can be viewed in Appendix E. However, the sugars our group examined were determined based on their retention times, as each sugar is present in the HPLC apparatus for a unique amount of time. For example, fructose was found to have a retention time of around 11 minutes, so we selected the peak whose retention time was closest to that value. From there, the peak area for that sugar was listed and was then interpolated along the calibration curves to find the concentration of that sugar in the wort. The calibration curve for each sugar can be seen in Appendix D.

Interpolation occurred by forcing a best-fit line through the data points on the curve. While we did examine a control sample of water, there was a non-zero value for peak area, and generally our group would have anticipated no area along with a zero sugar concentration. Therefore, the line was generated through a (0,0) point and the equation was formatted in the way below:

$$Peak\ Area\ = A * (Sugar\ Concentration)$$

Where A is a constant, and Peak Area would be our y-value since it depends on the Sugar Concentration in the wort sample, our x-value. From plugging in the Peak Area values provided in the results, as well as using the best-fit line, sugar concentration was properly determined. The

initial best-fit lines contained an additional constant added to the end of the equation - this was neglected, as it did not fit in our format and would yield a negative concentration.

The values are shown in the figure below:

Sample/Product (Wort #, Sample #)	Fructose Concentration (%)	Maltose Concentration (%)	Sucrose Concentration (%)
W1S1	3.87E-05	0.000802	0.000575
W1S2	5.09E-06	0.000741	0.000432
W2S1	3.56E-06	0.001916	0.001275
W3S1	1.43E-06	0.000762	0.000618
W3S2	8.75E-05	0.000512	0.000521
W4S1	0.000171	0.009051	0.001965
W4S2	6.08E-05	0.003921	0.000868
W5S1	0.000119	0.003991	0.002123
W5S2	5.41E-06	0.001669	0.000724

Figure 1: HPLC Data

Our group thus determined that Wort four, Sample one contained the highest sugar concentration overall. The fructose and maltose concentration percentages surpassed other values for this sample, and while the sucrose percentage was not the largest, it was certainly close to the maximum value in Wort five, Sample one. Wort four was yielded from the parameter corresponding to an increased mash time of ten minutes - this informed our future brew batches and Brews 7 and 8 were performed optimizing this parameter in hopes of continuing the trend of increased sugar content, as well as a better-tasting beer.

HPLC data were only analyzed for brews one through five.

Qualitative Results

All eight beers were a pink color as shown in Appendix F. The beers were also all very carbonated. In beers one through six, small particulates could be seen in some of the bottles due to small chunks of raspberry falling through the filter.

Beers one through seven were all described as being bitter, though beers three and seven were described as less bitter than the others. Beer eight was described as tart but still a beverage that could be enjoyed by those who enjoy raspberry flavor. Figure 1 depicts the overall tasting consensus for each beer.

Brew	Brew Method	Amount and Type of Raspberries	General Taste Descriptor Given By Tasting Panel
1	Control	5lbs, Fresh	Bitter, unpleasant
2	Vorlauf	5lbs, Fresh	Bitter, unpleasant
3	Preheat	5lbs, Fresh	Less bitter, unpleasant
4	Increased Mash Time (10 minutes)	5lbs, Fresh	Bitter, unpleasant
5	Continuous Mixing	5lbs, Fresh	Very bitter, unpleasant
6	Control	3lbs, Fresh	Bitter, unpleasant
7	Increased Mash Time (10 minutes)	3lbs, Frozen	Less bitter, unpleasant
8	Increased Mash Time (20 minutes)	3lbs, Frozen	Tart, pleasant

Figure 2: Tasting Panel Data

Our team thus determined that a mash time increased by twenty minutes utilizing three pounds of frozen and thawed raspberries washed in spring water yielded the best taste results.

Limitations

Brewing Error

Some errors that could have contributed to the results were present during brewing. In between some of the brews, the brew kettle was washed with tap water and left to air dry. The tap water contained chlorides which may have contributed to the bitter taste, this would have been compounded had the kettle not been completely dry prior to the next brew. The raspberries were also washed in tap water in beers one through six, which would have had a similar bitter affect due to the chlorides.

In brew eight, the brew bag was approximately half a pound short of the two row wheat, which had the potential to make the beer weak, though brew eight ended up being the best tasting of the beers.

Analytical Error

The team acknowledges that there were areas where error may have been introduced into our data analysis. The percentages of the sugars were much smaller and out of the range expected for each sugar analyzed during this process. We imagine the miniscule numbers may directly correspond with the small wort concentrations extracted for HPLC analysis. The wort samples were intended to only be 1 mL, but even then, this was not always the case. The extraction process was incredibly challenging at times - the wort was not easily suctioned into the syringe/filter combination, and it would often take an hour just to reach a quantity of 0.5 mL. The 1:4 ratio was maintained in all cases, but the quantity of wort involved was much smaller such that the percentages may have been smaller than expected as well.

The lengthiness of extraction led to our inability to continue the process for Brews 6 through 8, because several days of work did not allow for even the slightest bit of the liquid to travel through to our syringe. Given the time constraints and circumstances, and knowing our group already had enough data to form a cogent analysis from our first five brews, we elected not to perform extraction for the remaining brews, meaning we can only draw valid conclusions from quantitative results for brews one through five.

It may have also been likely that the centrifuge issues we ran into affected our results. After the first couple of runs for samples with the centrifuge, the equipment unfortunately broke down. Several days passed before we ended up having to switch to newer machinery for the remainder of samples. While we still used the same parameters for time and speed, the differences in age of the machines may have affected how well the samples were separated and perhaps could have affected how much sugar was actually present in the wort. This means maybe there would have been a higher sugar concentration had the two liquid phases been more completely separated.

There also could have been computational errors in calculating concentrations with the calibration curves. Indeed there were challenges in finding these values, as negative concentrations arose initially without utilizing a (0,0) value for the control (water) or with the additional constant being added onto the best-fit line. It took some extra work and manipulation to obtain concentrations which were not below zero. The best-fit lines were not always quite exact to the points either, so there is plenty of room for error here. The values may have been more exact if the equation for the line was more precise, but a best-fit line that demonstrated linear behavior was found to be the most appropriate for this scenario given that it yielded a positive concentration and was often similar in shape to those yielded by the data points. Nonetheless, these are approximate numbers and thus this leads to lower precision and accuracy.

On the brewing side of it, it could make sense that an increase in mash time positively impacted the sugar concentration. A longer mash time is said to create a more fermentable wort, especially for brews run at lower temperatures, because enzymes are active for a long period of time and can produce more maltose, increasing fermentability (which may explain why the highest concentration of maltose is present for the wort with a higher mash time) (Colby, 2014)(Troester, 2008). However, it should also be noted that the increase in fermentability, and hence sugar concentration, is not as substantial with an increase in mash time, and that may explain why the values of the percentages across all samples are all relatively close to one another, however small these percentages are (Colby, 2014). It may explain also why Brew 4 had an okay taste - though still not preferable according to the tasting panel.

Still, the numbers come across as abnormally small, and we have reason to believe perhaps fermentation did not occur completely during our brewing process with these first five batches - a “stuck fermentation.” A stuck fermentation entails a fermentation that stops at a gravity significantly higher than the targeted gravity - the roots of such an issue in our case could have been a lack of nutrients to promote growth (which is often a problem if high proportions of sugars are added), insufficient oxygen dissolved in the wort, pitching the yeast into the wort at too high of a temperature (which could have drastically reduced viability of the yeast), not enough yeast being pitched (meaning a very long lag time before fermentation is noticed or occurs - though there is more chance of spoilage that way or lower quality performance of yeast), or even flocculent yeast (where fermentation is sluggish from flocculation of yeasts, even if pitched in the right amount) (Foster, 2014).

With regards to the GC-MS data, error could have occurred on the operator end of the process. It is possible that the sample was not entirely pure due to contaminants present during the injection process required to place the sample in the GC-MS machine. Analytical error could have also

been present with regards to the GC-MS data, since the naked eye was used to compare the data generated by this project to the data for known compounds.

In any case, a stuck fermentation could have led to lower sugar concentrations. Because we did not have a hydrometer for quite some time, we did not take specific gravity readings during any of the brews. Therefore, we were not able to quantitatively conclude that the fermentation stopped at a higher gravity - and even if we wanted to, we would have needed to do a forced fermentation where a sample was pitched with an excess of yeast and the final gravity was checked against the gravity of the wort. This would have been redundant and impractical for our brewing process, and the idea of stuck fermentation was a possibility our team was not aware of when we brewed. Regardless, the concept may substantiate why our sugar concentrations ended up so low for the first five batches, and why the taste of our first seven brews were not satisfactory to begin with.

Conclusions and Recommendations

Several conclusions were drawn at the completion of this project. Firstly, beer eight was the most pleasant beer to consume. Thus, three pounds of frozen and thawed raspberries added during primary fermentation and the use of spring water at all points where the recipe calls for water contributed heavily to a better flavor profile.

In addition to the better flavor associated with beer eight, it was concluded that increased mash time led to more efficient fermentation. This was evidenced by the increased sugar conversion in beer four, denoted by the HPLC data earlier in the report.

Next, chloride generation in beer was concluded to be responsible for the bitter, unpleasant taste present in beers one through six. Since the chlorides were most likely the result of using tap water during sanitizing the equipment and washing the raspberries, spring water was substituted during these steps during brews seven and eight. Since the bitterness decreased in those two brews, this conclusion was deemed accurate by the team.

Finally, the most important conclusion made by the team is that, while this project was unable to reach the desired goal set at the beginning of the project, it is possible to make juniper-raspberry flavored beer with an increased sugar conversion and without the presence of hops.

As far as recommendations for future projects, the team noted several significant pieces of advice. First, spring water should be used for all parts of the brewing, sanitizing, fermentation and bottling processes whenever water is called for. Second, it would be beneficial to have a trained tasting panel with a better palette, an opportunity not afforded to this team due to the COVID-19 pandemic. Third, it is very important to ensure that the beer is stored in a cool, dark place with relatively consistent temperature for the best fermentation practices. Fourth, if access to a hydrometer is not inhibited, specific gravity readings at every stage during the brewing process would allow for a group to know if the beer was experiencing a stuck fermentation. Next, it is important to ensure maximum separation between the DCM and the wort during the extraction phase of the laboratory work. The separation is important to prevent impurities in the wort from being analyzed with the sample. Additionally, it is important to fill the glass amber bottles only three quarters of the way with beer because foam-over or even exploding bottles become risks when the bottles are overfilled. Finally, it is recommended to use frozen raspberries that have been thawed in order to ensure consistency in the ripeness of the berry and also to take advantage of the increased sugar conversion provided by the thawing process.

References

Bosco, J,, Pickett, J., Schaeffer, Z., & Smith, S.. (2019) *Investigation of the Effects of Oxygen and Other Considerations on the Shelf-Life of Craft Beer*. : Worcester Polytechnic Institute

Colby, C. (2014, March 5). How long should you mash? Retrieved March 27, 2021, from https://beerandwinejournal.com/mash-how-long/

De Keukeleire, Denis. (2000). Fundamentals of beer and hop chemistry. *Química Nova*, *23*(1), 108-112. https://doi.org/10.1590/S0100-40422000000100019

Field, J., Poisson, G., Fleury, N., & Kortz, H. (2020). *Brewing Process Optimization.* : Worcester Polytechnic Institute.

Foster, T. (2017, December 01). Preventing (& fixing) STUCK FERMENTATIONS. Retrieved March 27, 2021, from https://byo.com/article/stuckfermentationstechniques/#:~:text=The%20only%20real%20way%20to,the%wort%20in%20the%20fermenter

Hines, W. (2017, November 22). These are the chemical compounds that make beer taste so good. Retrieved February 26, 2021, from https://vinepair.com/articles/chemical-compounds-beer/

Kapral, D. (n.d.). The oxford companion to beer definition of wort. Retrieved March 27, 2021, from https://beerandbrewing.com/dictionary/nfffzoYQNF/

Lauro, D., Patel, P., West, C., & Stypulkowski, S. (2020). *Development and Optimization of a "Hop Free" Beer.* : Worcester Polytechnic Institute.

National Center for Biotechnology Information (2021A). PubChem Compound Summary for CID 545617, 1-Iodotridecane. Retrieved March 29, 2021 from https://pubchem.ncbi.nlm.nih.gov/compound/1-Iodotridecane.

National Center for Biotechnology Information (2021B). PubChem Compound Summary for CID 545627, 4,6-Dimethyldodecane. Retrieved March 29, 2021 from https://pubchem.ncbi.nlm.nih.gov/compound/4_6-Dimethyldodecane.

Palmer, J. J. (2006). *How to brew: Everything you need to know to brew beer right the first time*. Boulder, CO: Brewers Publications.

Pentadecane (n.d.) FoodB. Retrieved February 22, 2021 from https://foodb.ca/compounds/FDB011800#:~:text=Pentadecane%20is%20a%20hydrocaron%20lipid,an%20alkane%20and%20waxy%20taste.

The good scents company - Aromatic/Hydrocarbon/Inorganic Ingredients catalog information. (n.d.A). Retrieved February 28, 2021, from http://www.thegoodscentscompany.com/data/rw1001661.html.

The good scents company - Aromatic/Hydrocarbon/Inorganic Ingredients catalog information. (n.d.B). Retrieved February 26, 2021, from http://www.thegoodscentscompany.com/data/rw1163331.html

13-Docosenamide (CAS 112-84-5). (n.d.). Retrieved February 28, 2021, from https://www.caymanchem.com/product/90377/13-

Troester, K. (2008). Effects of mash parameters on fermentability and efficiency in single infusion mashing. Retrieved March 27, 2021, from https://braukaiser.com/wiki/index.php/Effects_of_mash_parameters_on_fermentability_and_efficiency_in_single_infusion_mashing

Wefing, P., Conradi, F., Trilling, M., Neubauer, P., & Schneider, J. (2020). Approach for modelling the extract formation in a continuous conducted “β-amylase rest” as part of the production of beer mash with targeted sugar content. *Biochemical Engineering Journal, 164*, 107765. doi:10.1016/j.bej.2020.107765

What gives beer its bitterness & flavour? (2016, January 11). Retrieved February 26, 2021, from https://www.compoundchem.com/2014/07/10/beerchemicals/

Appendices

Appendix A: Beer Recipe

This recipe was adapted from the recipe given in the Hops-Free beer report from the 2019-2020 academic year (Lauro et al).

These are instructions for a five gallon batch of juniper-raspberry flavored beer.

Equipment:

- Brew Pot (~5 gallons)
- Thermometer
- Brew Bag
- Large Ladle
- Siphon (with tubing)
- 5 gallon food grade fermentation bucket with lid
- Stove
- Star-San sanitizing agent
- 5 gallon glass carboy
- Aerator
- Food scale
- 2 cases of amber glass bottles (24 bottles per case)
- Capping tool
- Bottle caps

Ingredients:

- 6 lbs 2-Row
- 2 lbs White Wheat
- 0.75 lbs Flaked White Wheat
- 0.5 lbs Caramel
- 1 oz Juniper Berries (crushed)
- Raspberries (in desired amount)
- Safale US 05 Dry Ale Yeast
- Priming Sugar
- Spring Water

Sanitization Steps:

1. Mix the appropriate amount of star san with spring water,
2. Agitate mixture slightly, until bubbles are visible.

3. Fully submerge all equipment in mixture.
4. Let submerged equipment sit in the mixture for three minutes.
5. Rinse equipment with spring water until there is no visible sanitizer or bubbles.

Brew Day Steps:

1. Prepare brew bag with all varieties of grain (2-Row, white wheat, flaked white wheat, caramel).
2. Add four gallons of spring water to the brew pot. Heat to 158°F.
3. Add brew bag and steep at 149°F for 60 minutes.
4. Mash out for 10 minutes at 168°F.
5. Transfer wort to fermentation bucket.
6. Sparge the brew bag using 3.75 gallons of spring water.
7. Heat the sparge water to 175°F.
8. Combine the wort and sparge water in the brew kettle. Boil for 60 minutes. Add Juniper berries at 15 minutes.
9. Turn off heat and allow the wort to cool to 80°F.
10. Transfer wort through filter into fermentation bucket.
11. Pitch yeast.
12. Close bucket. Store wort in a cool, dry place.

Primary Fermentation Steps:

1. Allow beer to ferment in fermentation bucket for 7-10 days.
2. Add pureed raspberries to glass carboy using the siphon.
3. Transfer beer to the carboy and agitate.
4. Put stopper and aerator into neck of carboy.
5. Allow 2 weeks for secondary fermentation.

Bottling Steps:

1. Prepare priming sugar solution.
2. Add priming sugar to carboy. Agitate.
3. Fill bottles ~¾ of the way up with beer. Cap bottles.
4. Store bottles in a cool, dark place for 2 weeks.

Appendix B: GC-MS Data

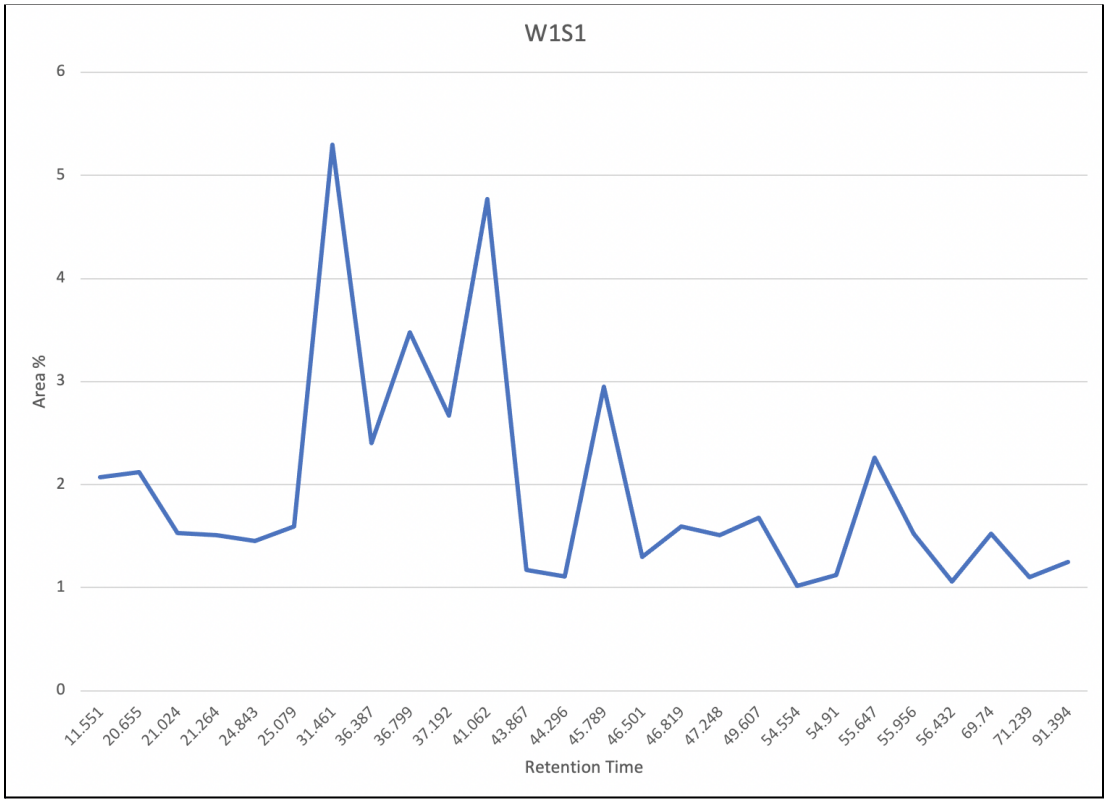

Figure 3: Brew One Wort Sample One GC-MS Data

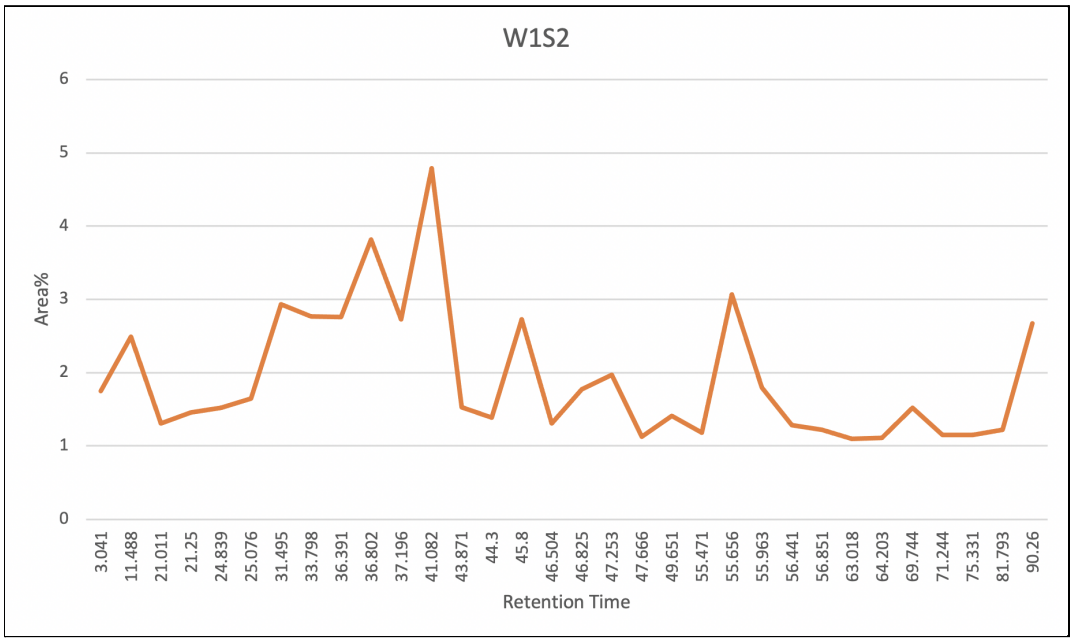

Figure 4: Brew One Wort Sample Two

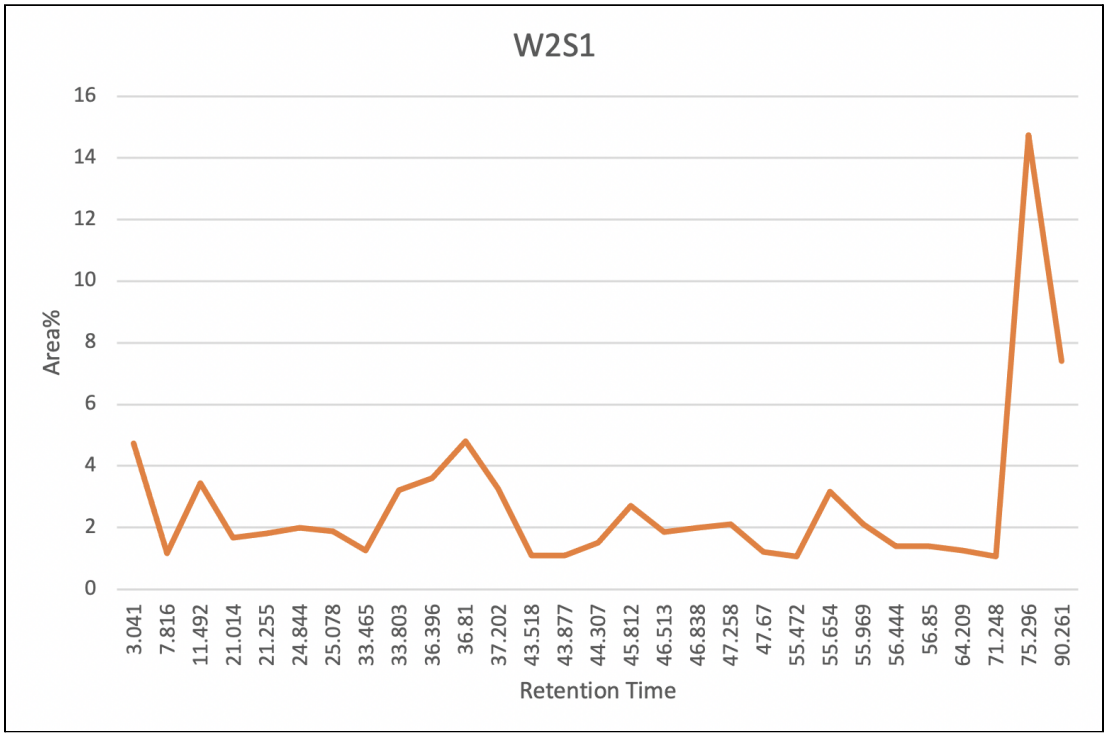

Figure 5: Brew Two Wort Sample One

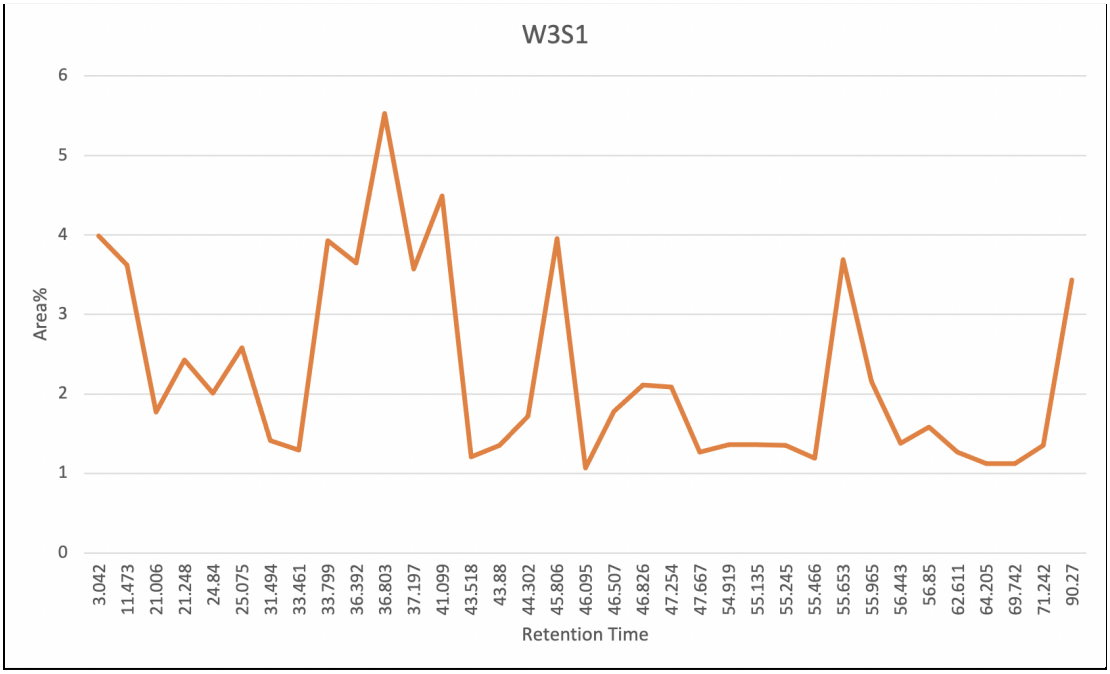

Figure 6: Brew Three Wort Sample One

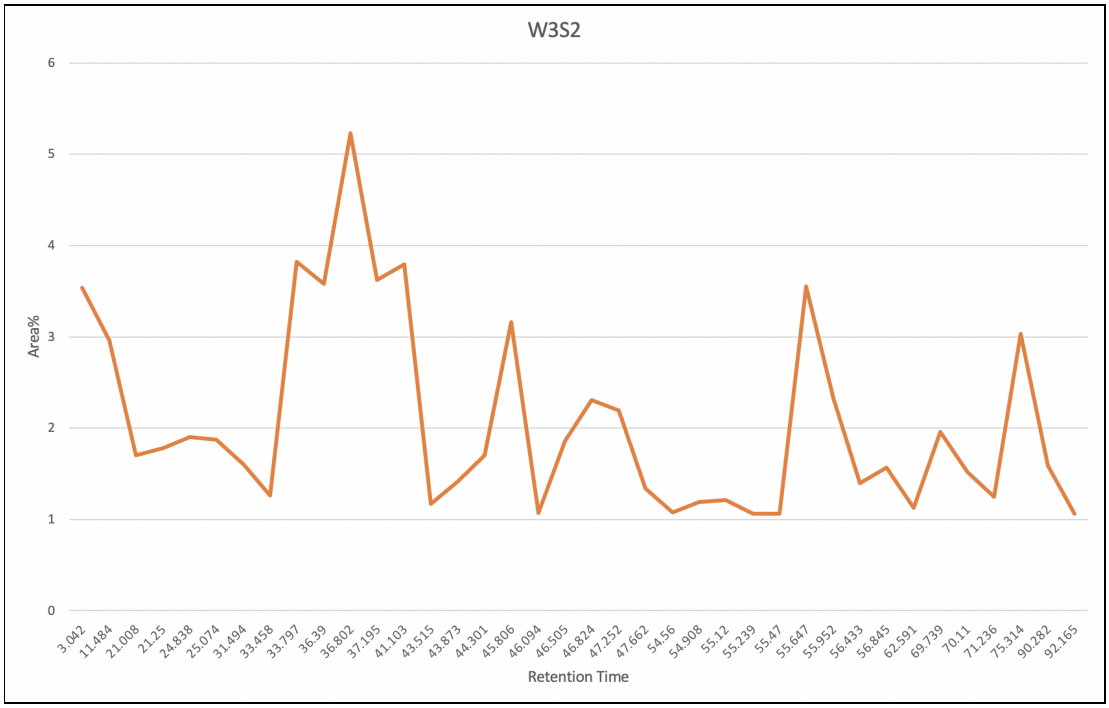

Figure 7: Brew Three Wort Sample Two

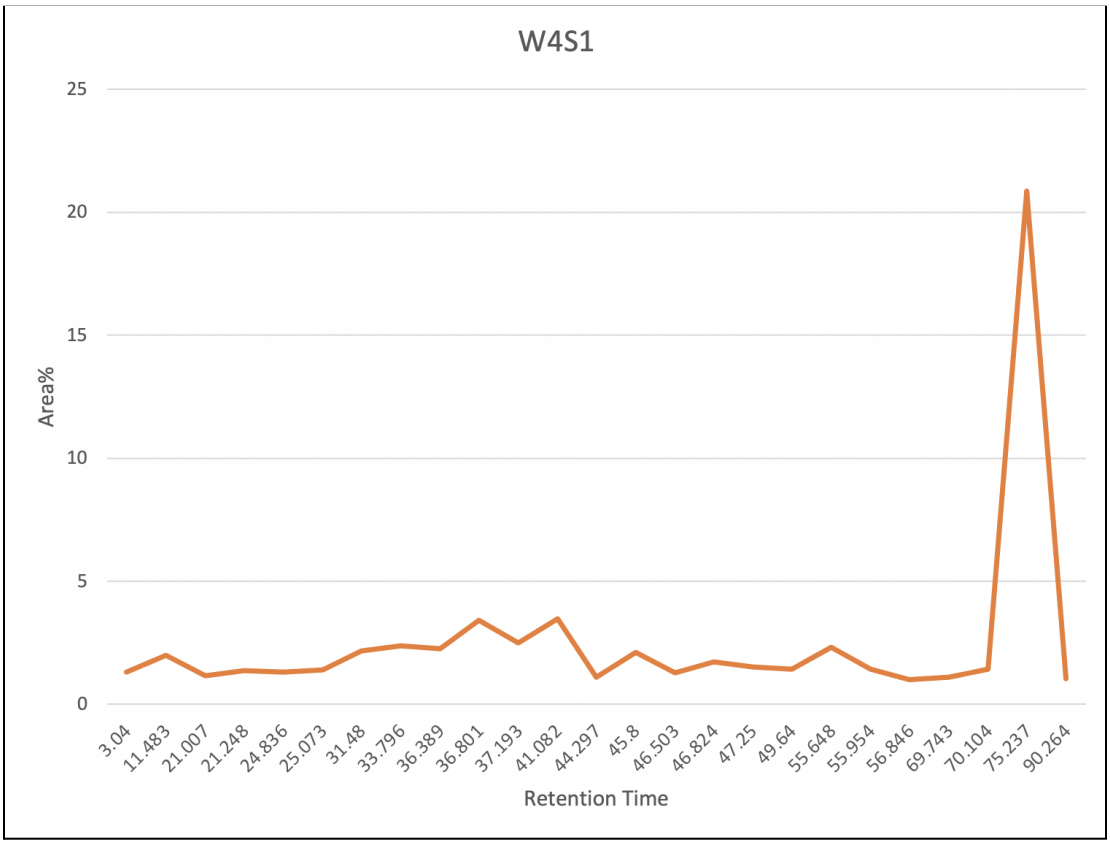

Figure 8: Brew Four Wort Sample One

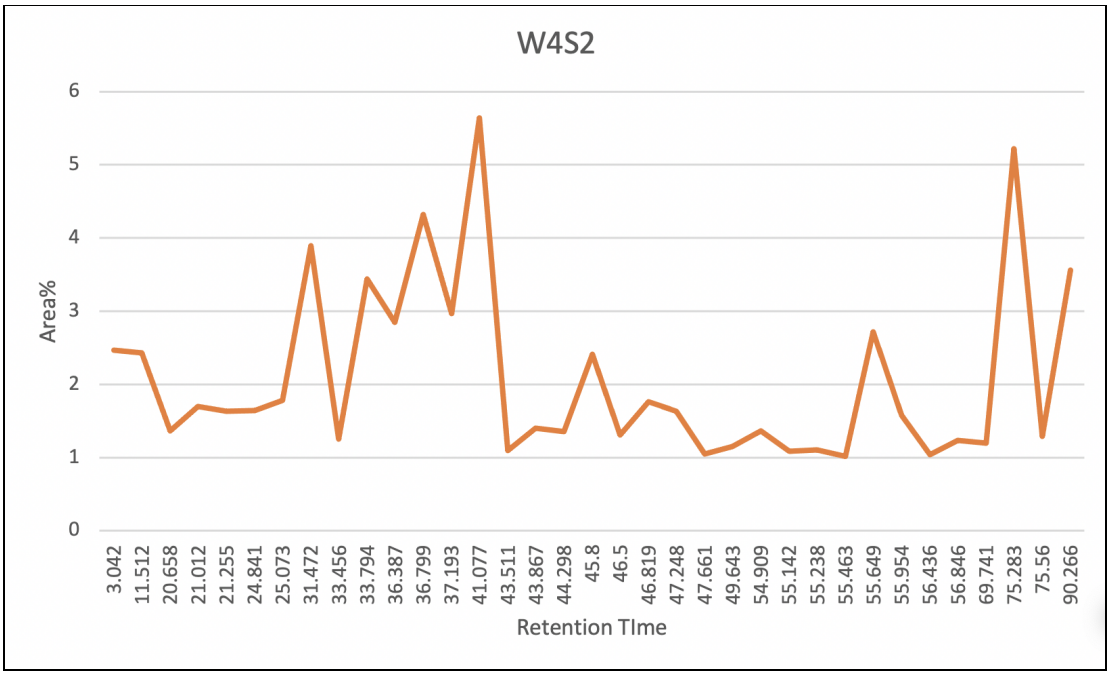

Figure 9: Brew Four Wort Sample Two

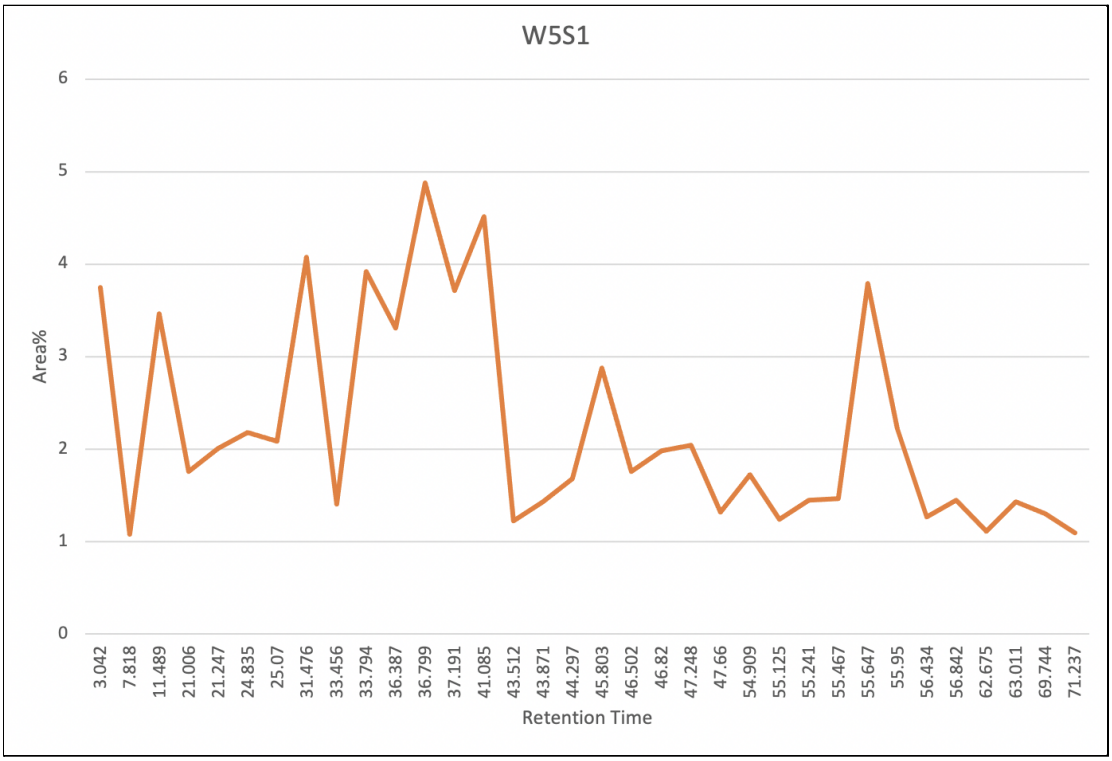

Figure 10: Brew Five Wort Sample One

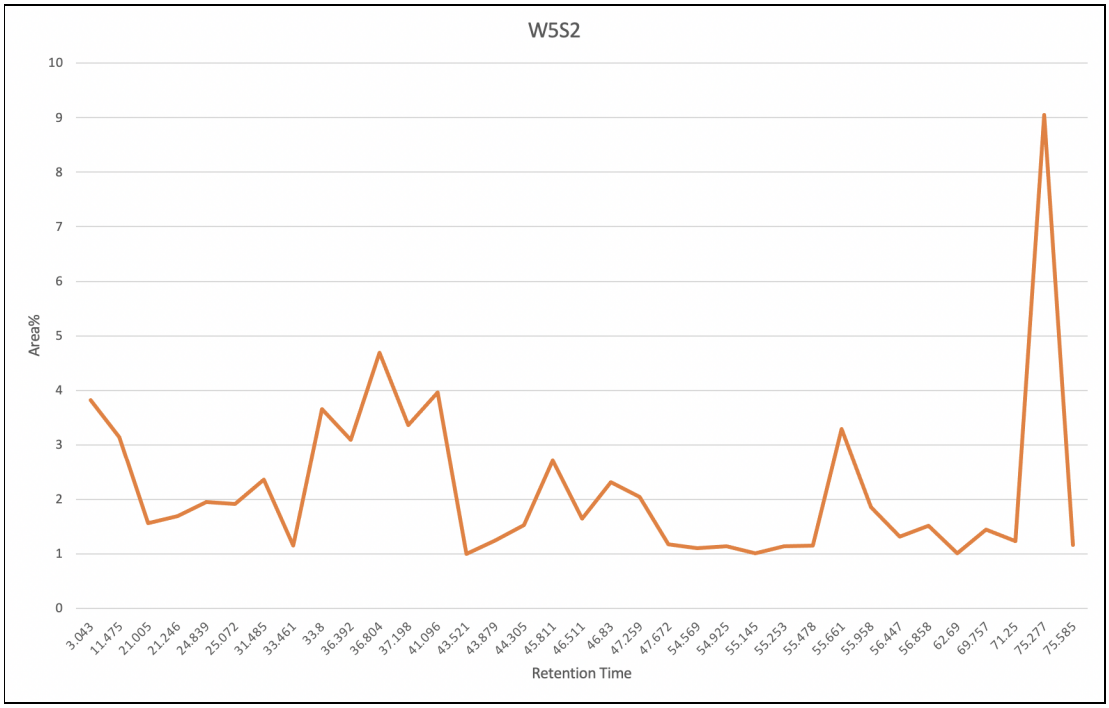

Figure 11: Brew Five Wort Sample Two

Appendix C: Sugar Calibration Curve Sample Calculations

Sample Calculations for Maltose (Solid Form of Sugar):

$For\ 20\%\ Maltose\text{:}\ \frac{20\ g\ maltose}{100\ g\ solution} \times \frac{1\ mL/1.54\ g}{100\ g\ solution} \rightarrow \frac{12{,}99\ mL\ maltose}{12.99\ mL\ maltose + 80\ mL\ H_2O} = \frac{12{,}99}{92.99} = 0.1397\ (13.97\ mL\ \%$

$M_1V_1 = M_2V_2$ where M_1 is the found percent of sugar in the solution, V_1 is the corresponding volume of the sugar solution, M_2 is the concentration we want to achieve in the total sugar solution, and V_2 is the volume of the total sugar solution that should be used.

$(0.1397)(100\ mL) = (0.100)V_2 \rightarrow V_2 = 139.7\ mL,\ so\ add\ 39.7\ mL\ H_2O$

Due to the large scale of solution needed for the above calculation, we repeated it with a smaller solution size of 5 mL, so the value of M_1 changes.

$(0.699)(5\ mL) = (0.100)V_2 \rightarrow V_2 = 34.93\ mL,\ so\ add\ 29.93\ mL\ H_2O$

Sample Calculations for Sucrose (Liquid Form of Sugar):

$\frac{1\ g}{1.59\ g/mL} = 0.63\ mL$

$\frac{0.63\ mL}{x} = \frac{1\ mL}{100\ mL} \rightarrow x = 63\ mL$

Knowing that the weight is 0.25 g, 15.75 mL H_2O/g sucrose should be used.

Appendix D: Sugar Calibration Curves

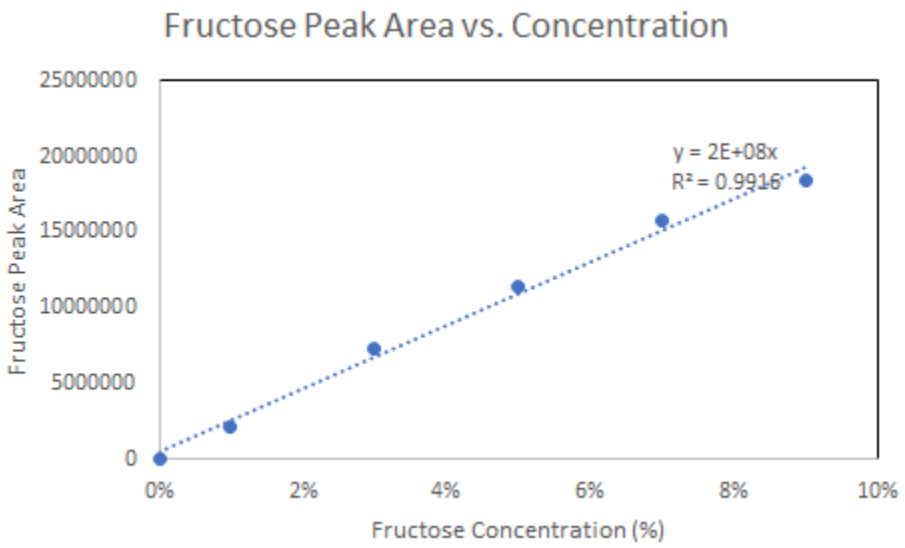

Figure 12: Fructose Calibration Curve

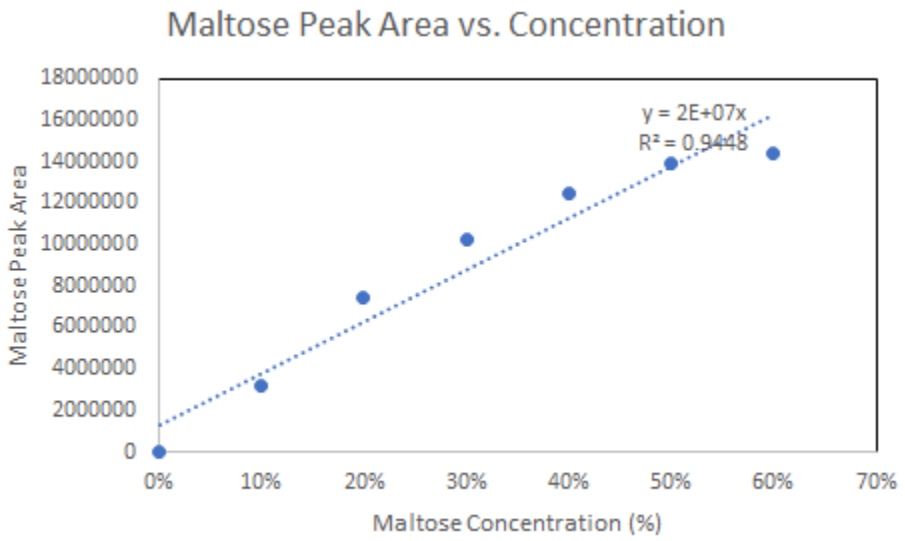

Figure 13: Maltose Calibration Curve

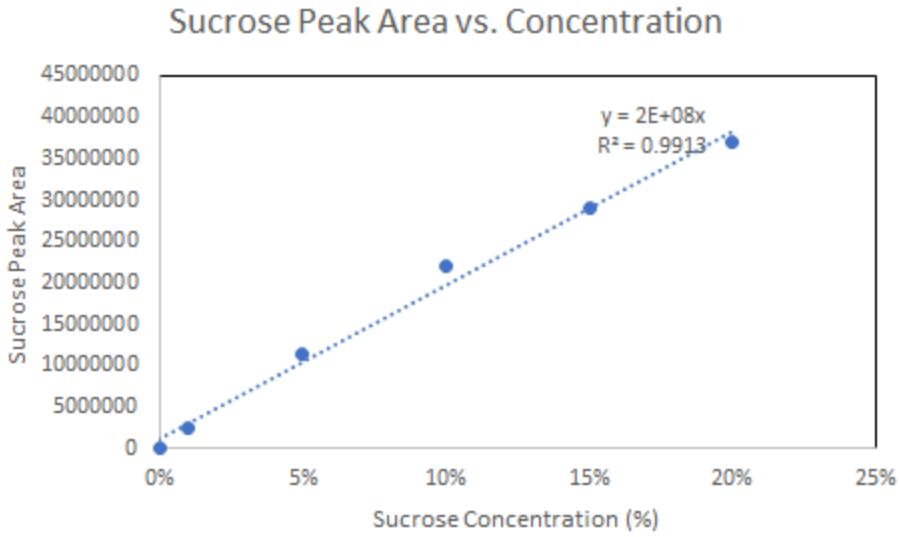

Figure 14: Sucrose Calibration Curve

Appendix E: HPLC Data

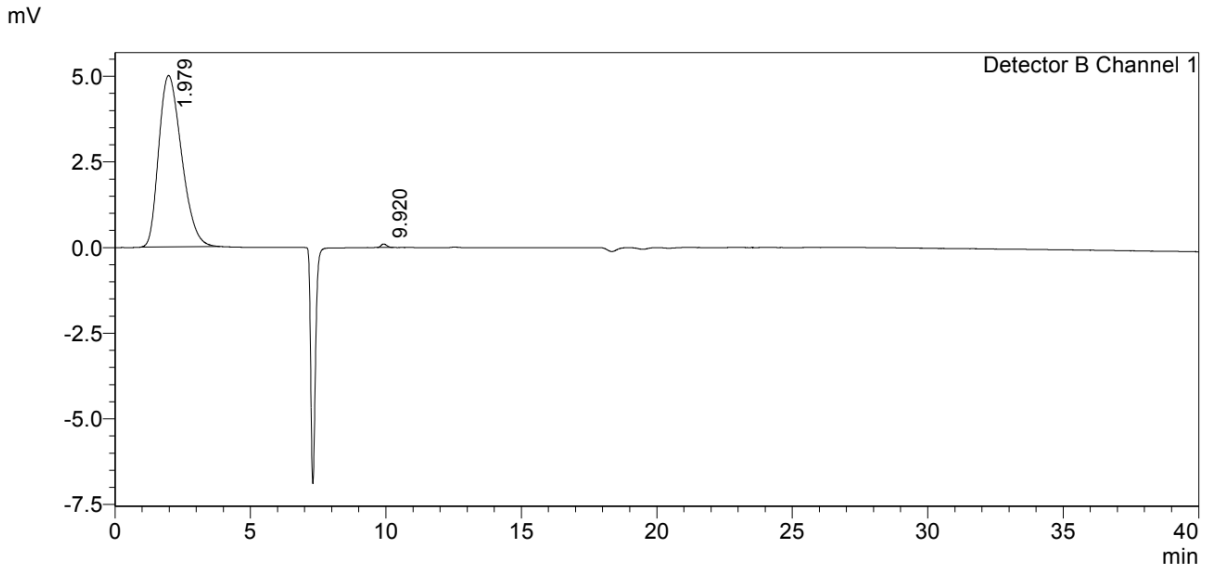

Figure 15: Control Sample Chromatogram

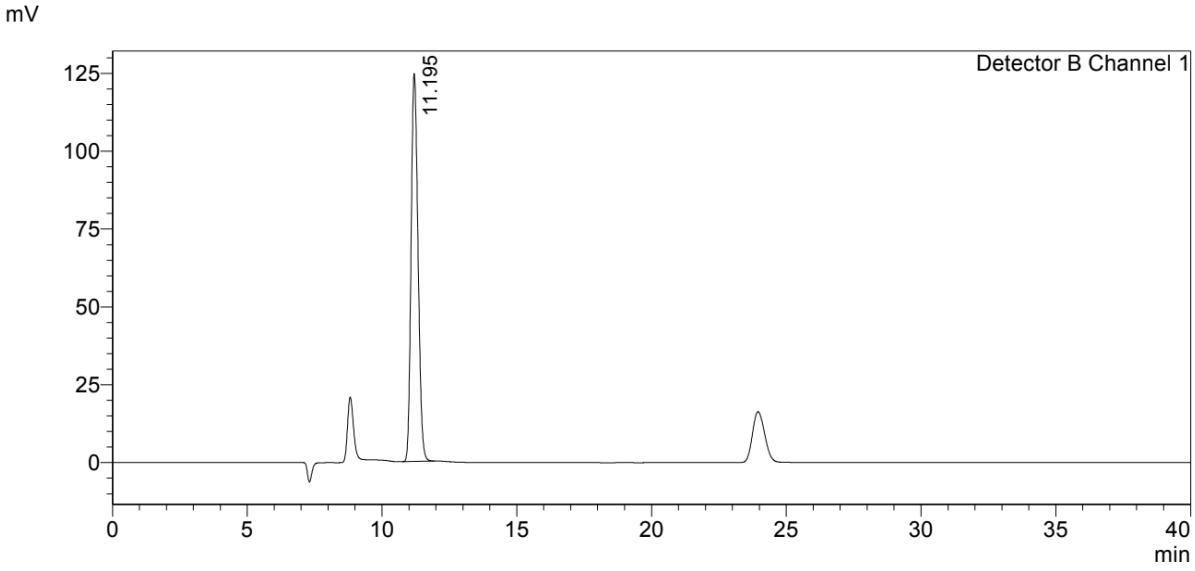

Figure 16: 1% Fructose Sample Chromatogram

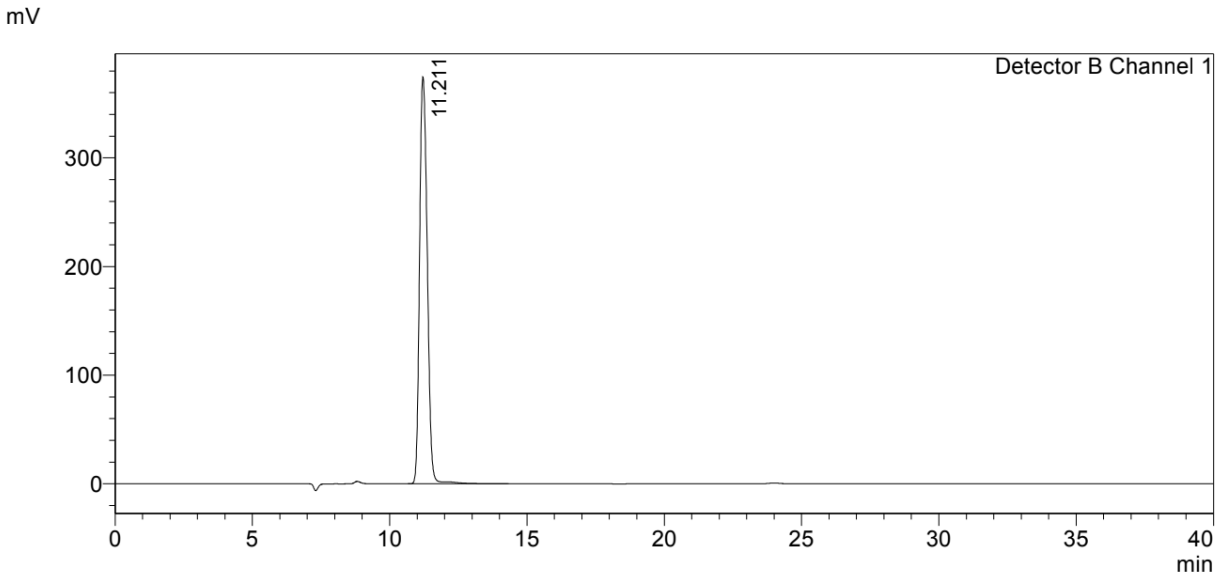

Figure 17: 3% Fructose Sample Chromatogram

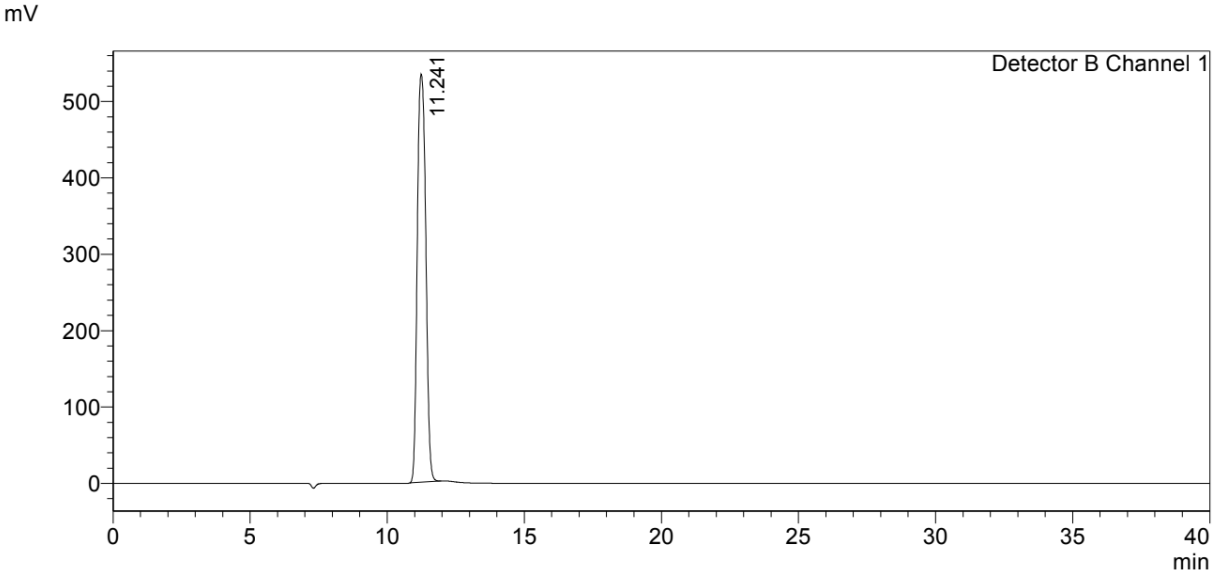

Figure 18: 5% Fructose Sample Chromatogram

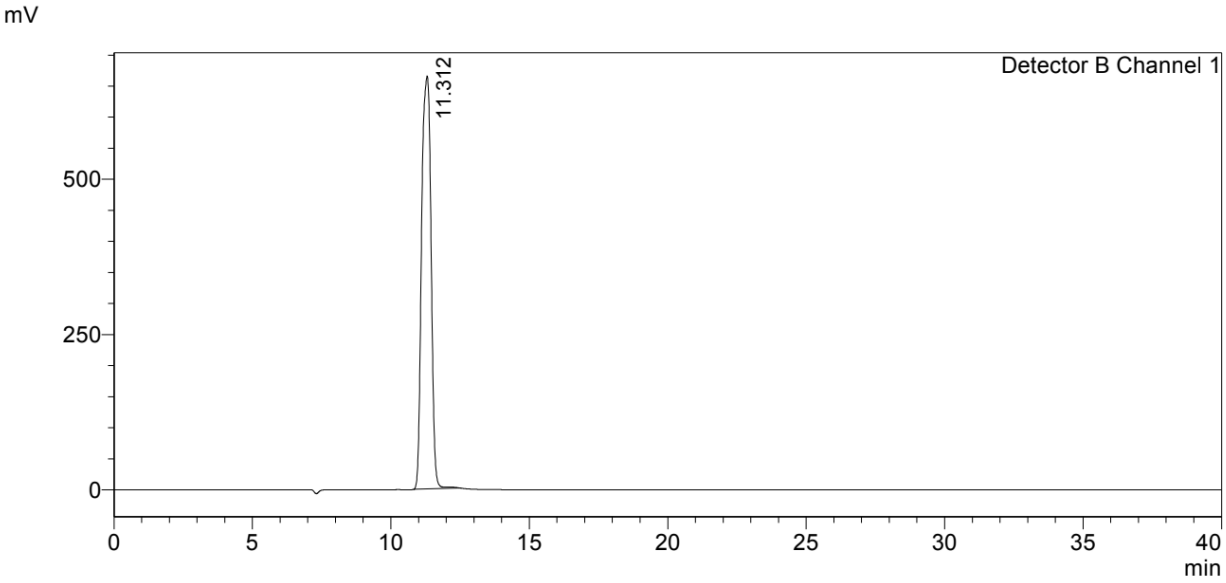

Figure 19: 7% Fructose Sample Chromatogram

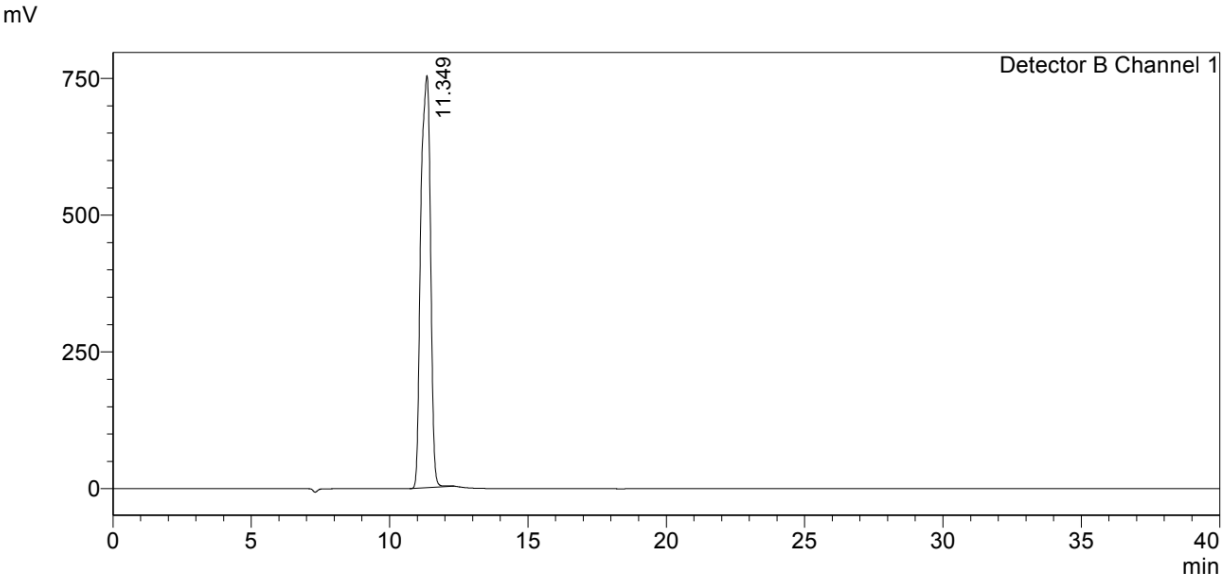

Figure 20: 9% Fructose Sample Chromatogram

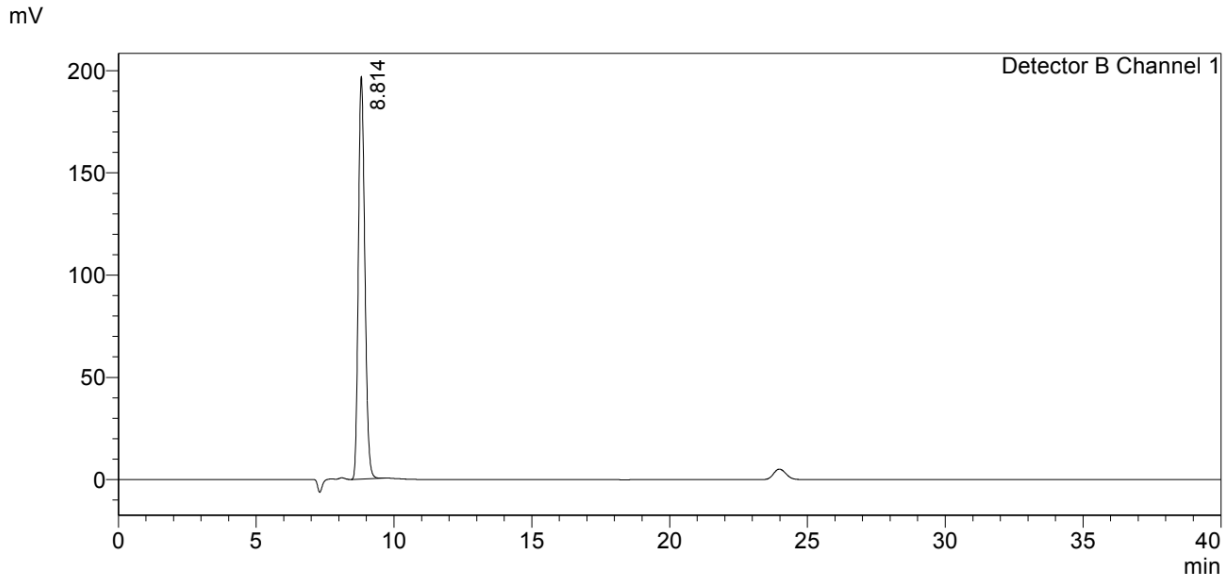

Figure 21: 10% Maltose Sample Chromatogram

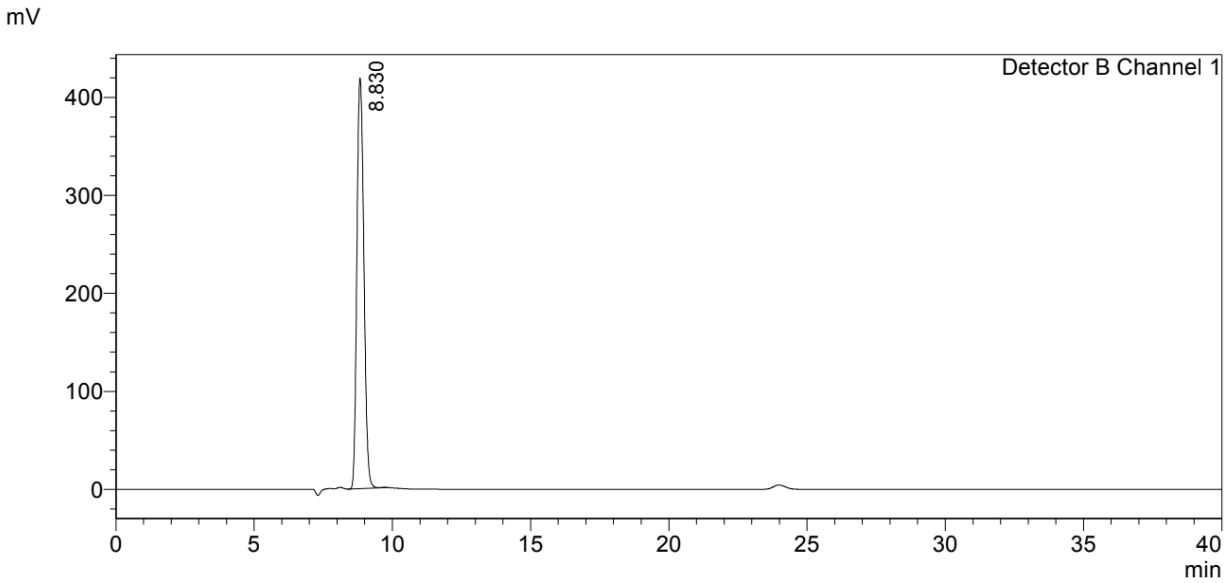

Figure 22: 20% Maltose Sample Chromatogram

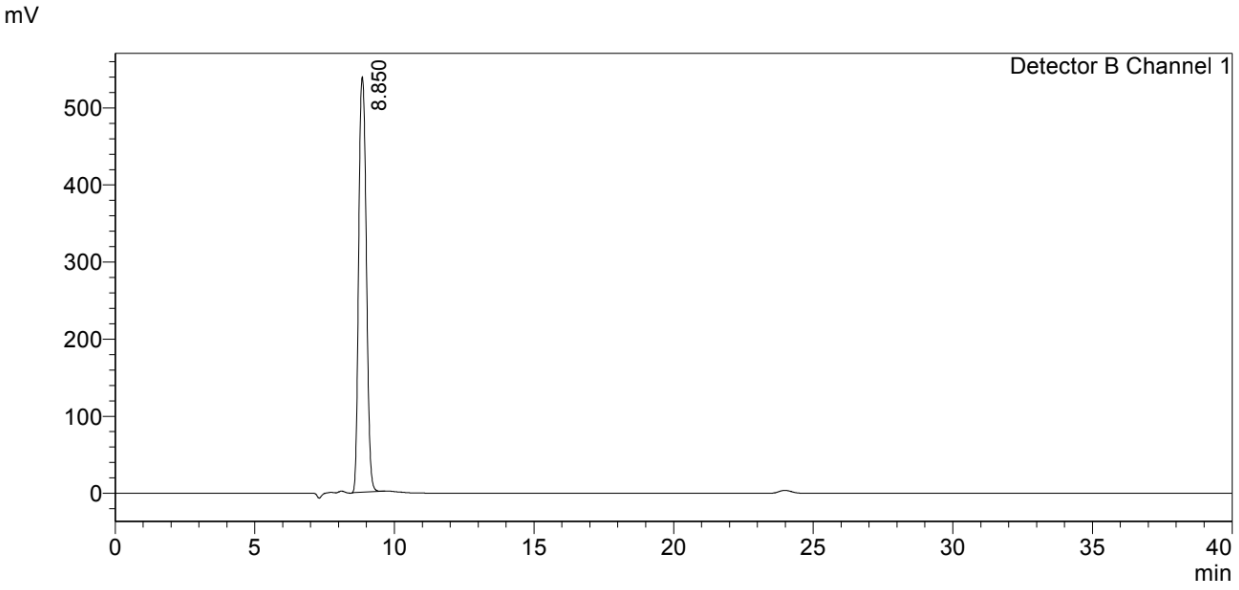

Figure 23: 30% Maltose Sample Chromatogram

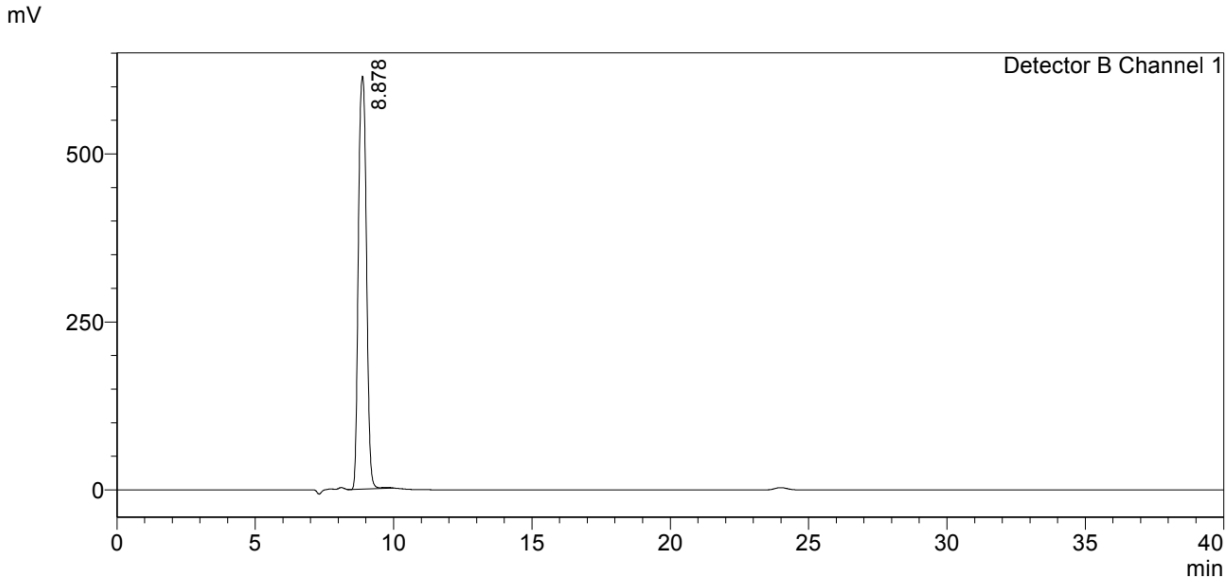

Figure 24: 40% Maltose Sample Chromatogram

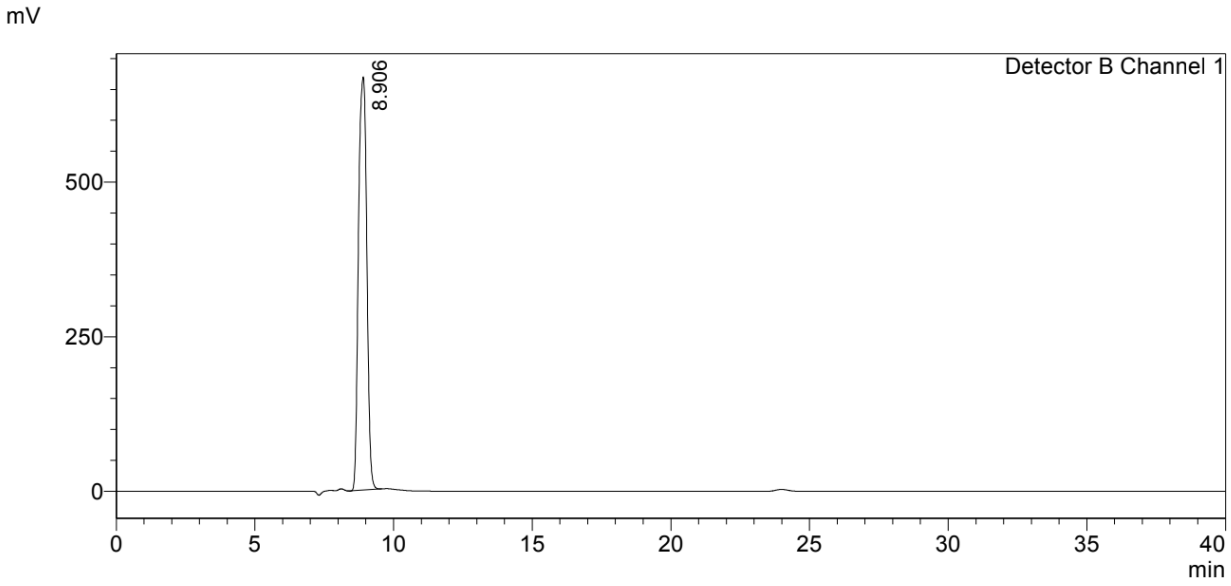

Figure 25: 50% Maltose Sample Chromatogram

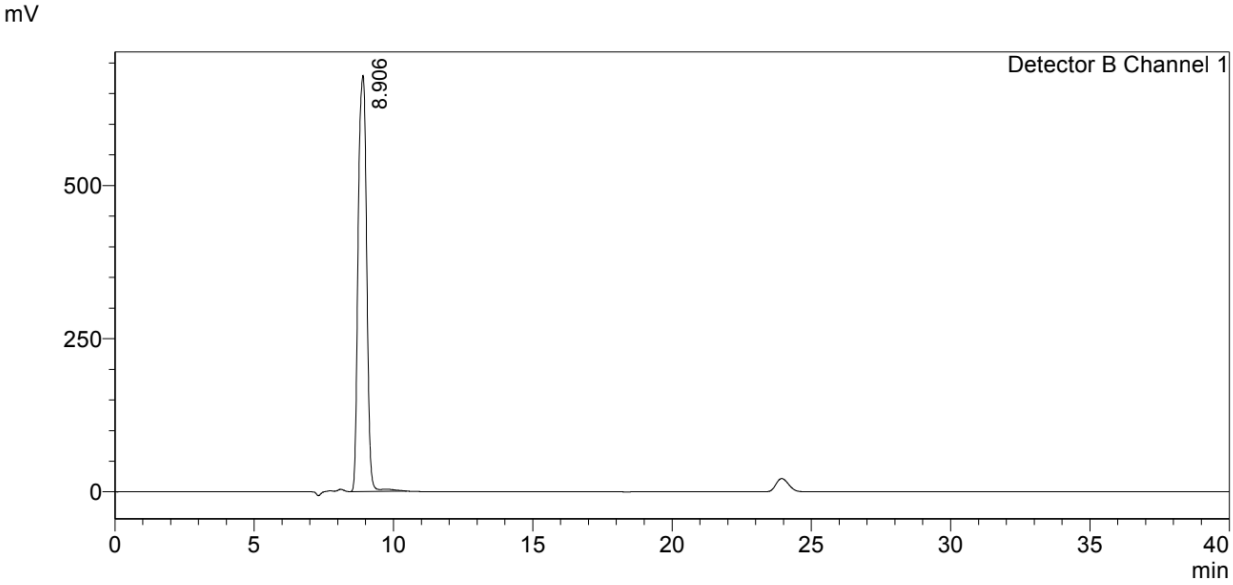

Figure 26: 60% Maltose Sample Chromatogram

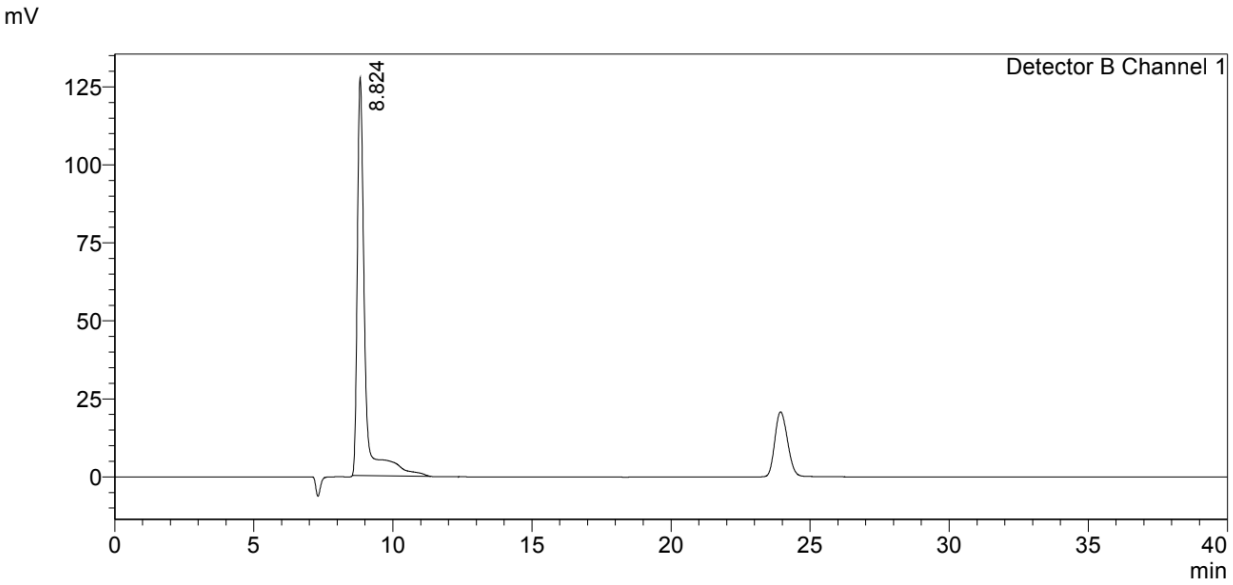

Figure 27: 1% Sucrose Sample Chromatogram

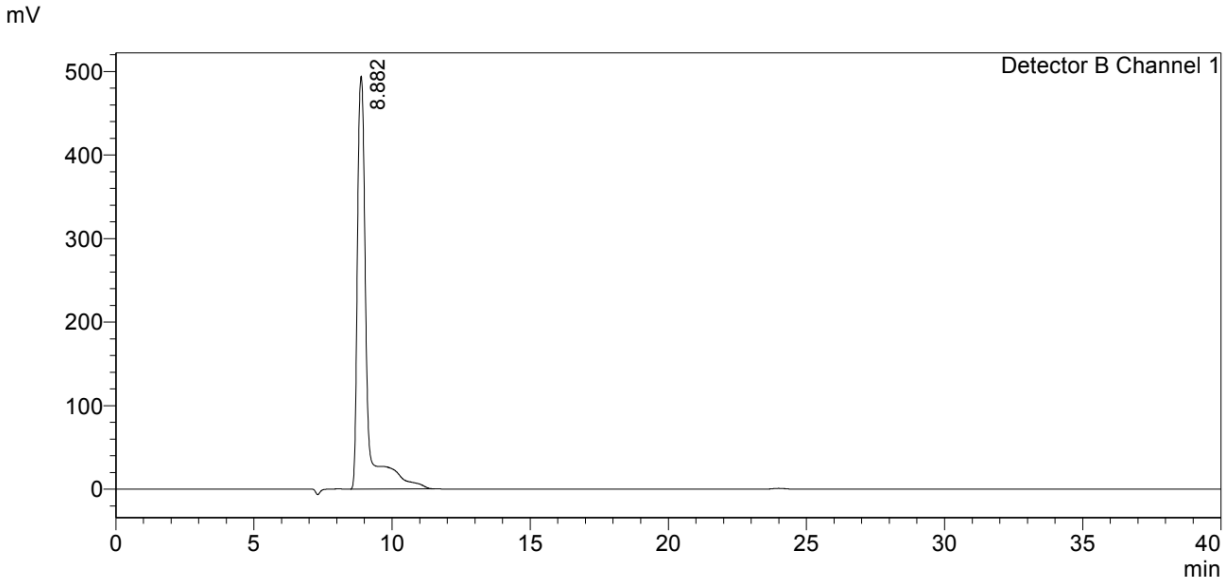

Figure 28: 5% Sucrose Sample Calculations

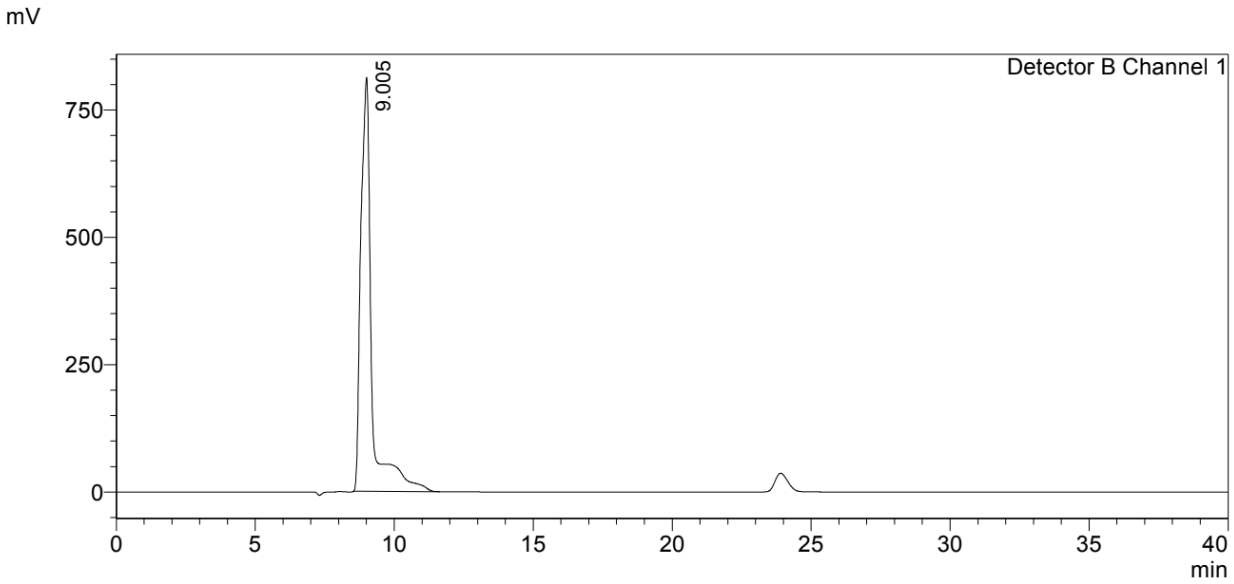

Figure 29; 10% Sucrose Sample Calculations

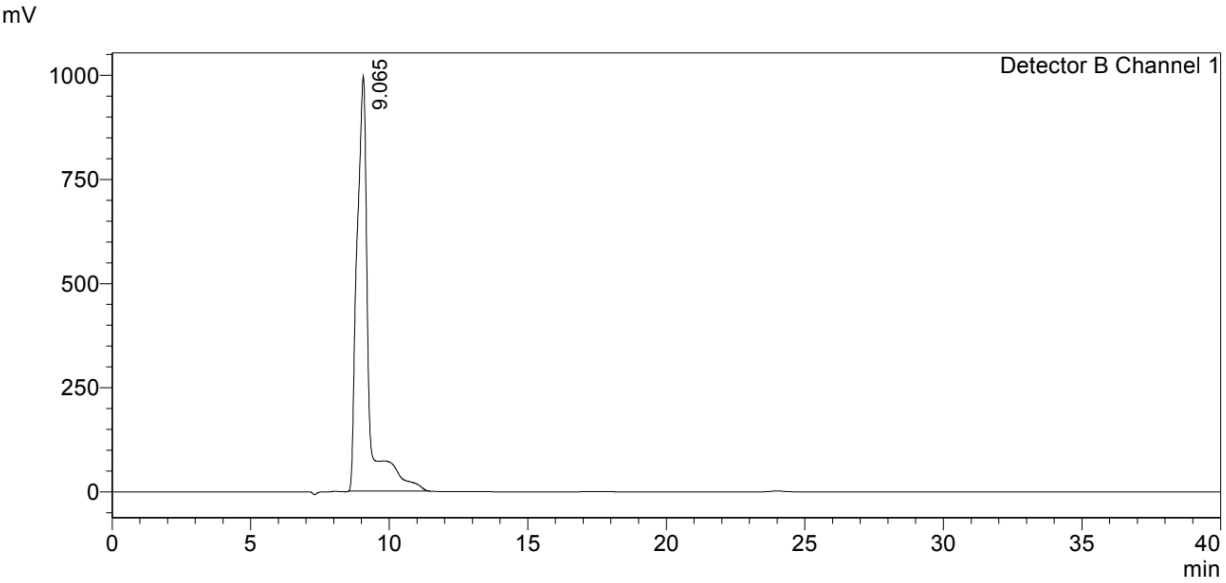

Figure 30: 15% Sucrose Sample Calculations

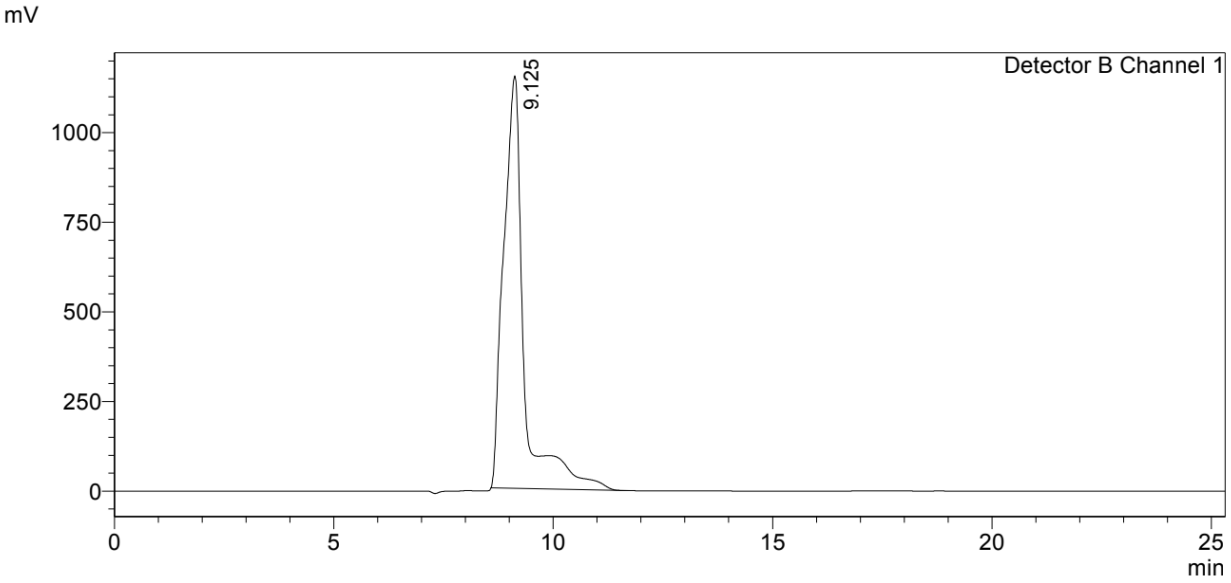

Figure 31: 20% Sucrose Sample Calculations

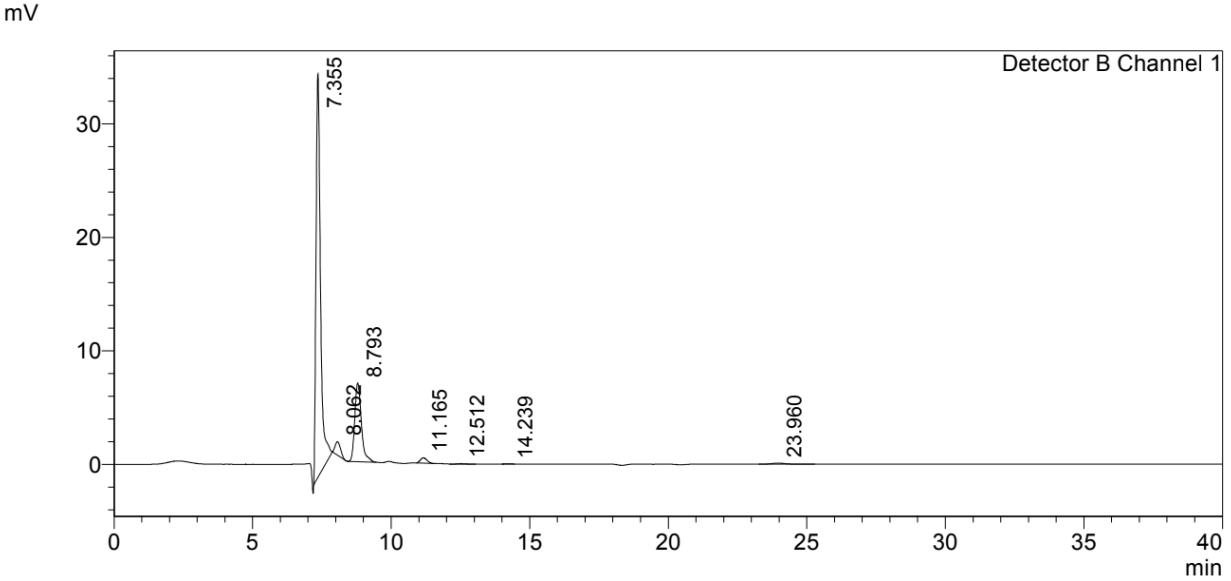

Figure 32: Wort 1, Sample 1 Chromatogram

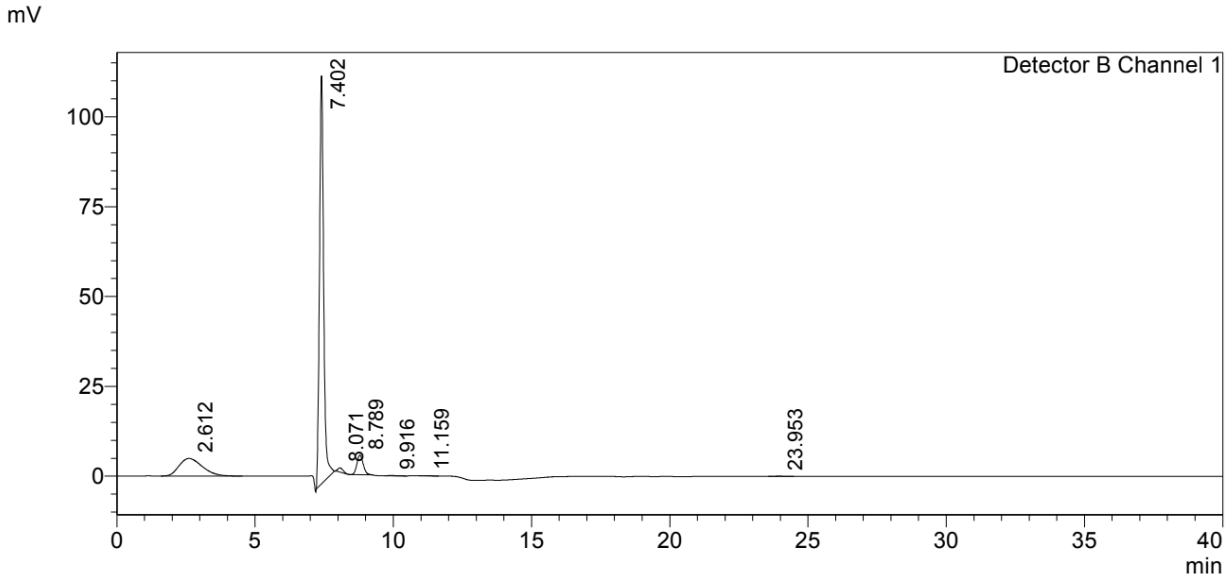

Figure 33: Wort 1, Sample 2 Chromatogram

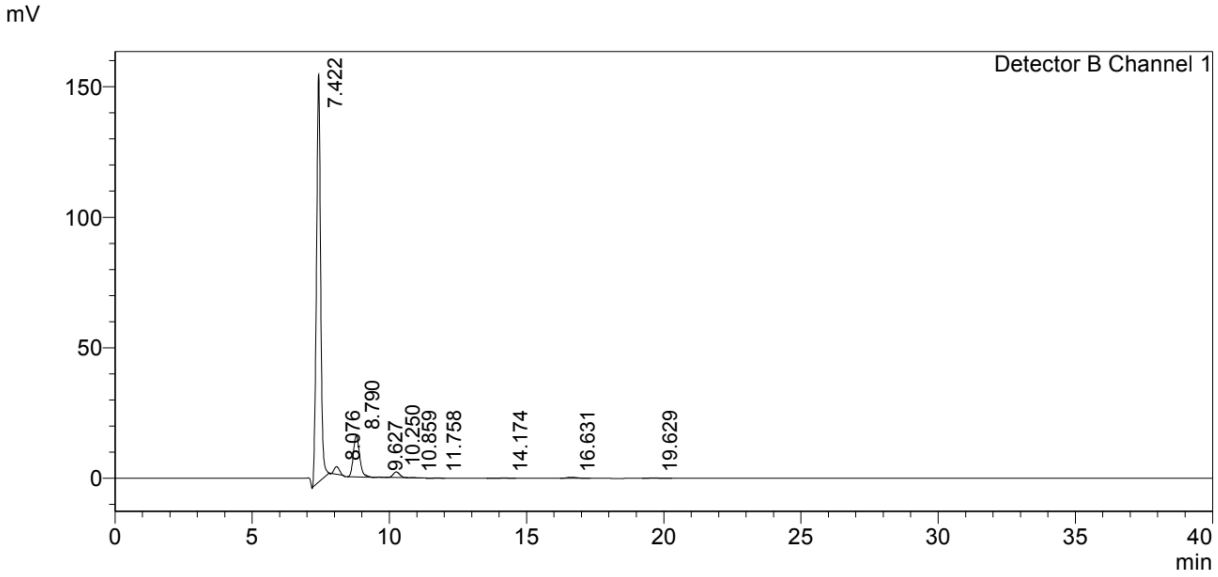

Figure 34: Wort 2, Sample 1 Chromatogram

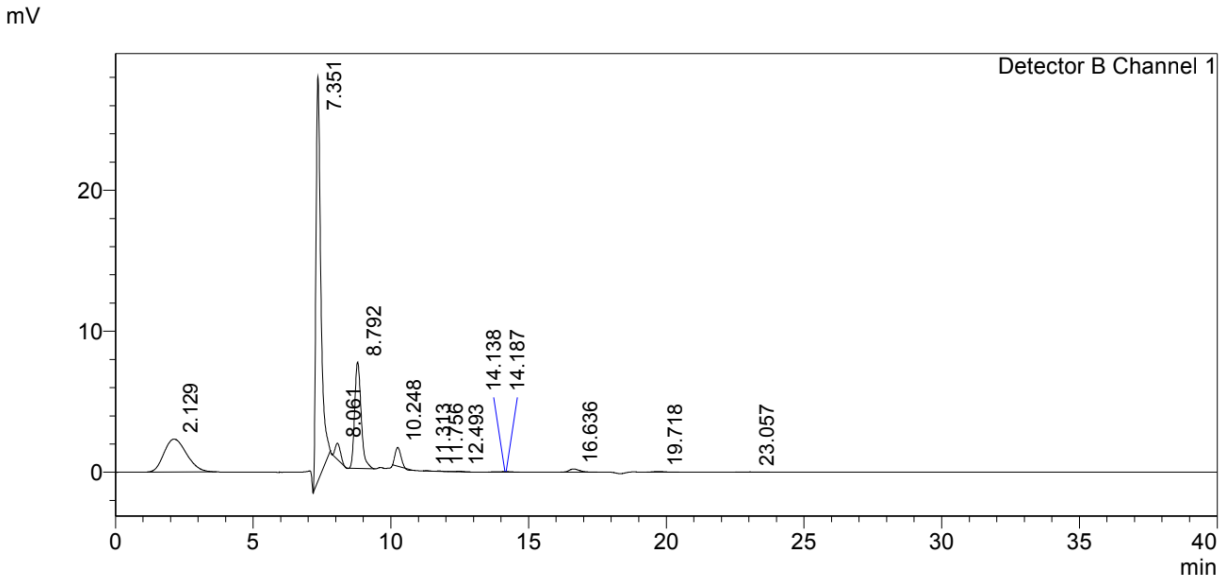

Figure 35: Wort 3, Sample 1 Chromatogram

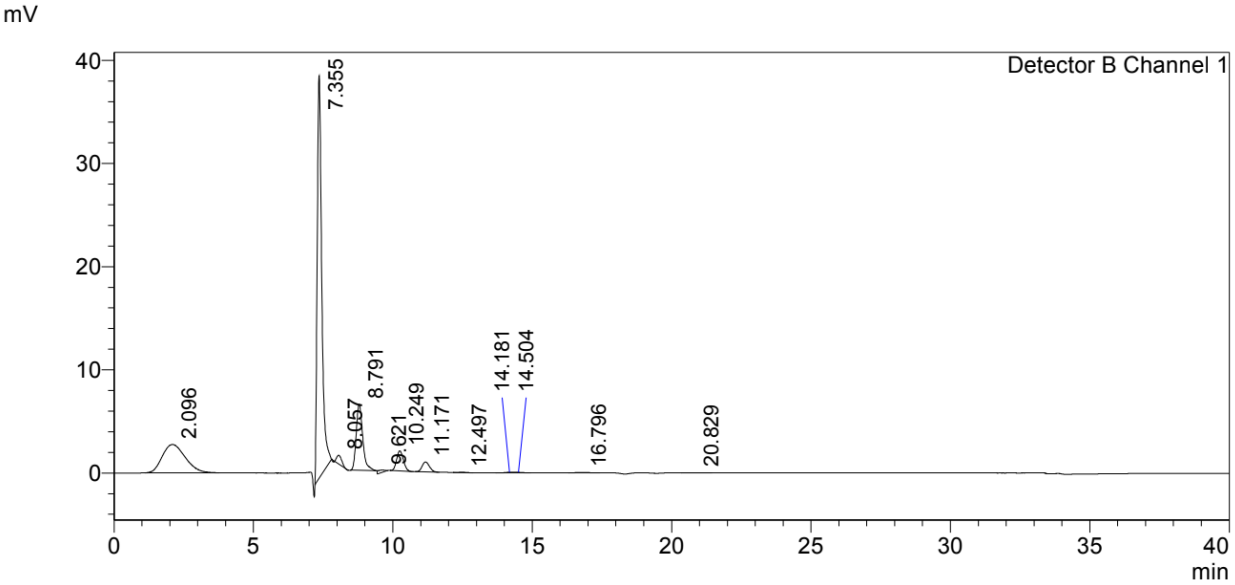

Figure 36: Wort 3, Sample 2 Chromatogram

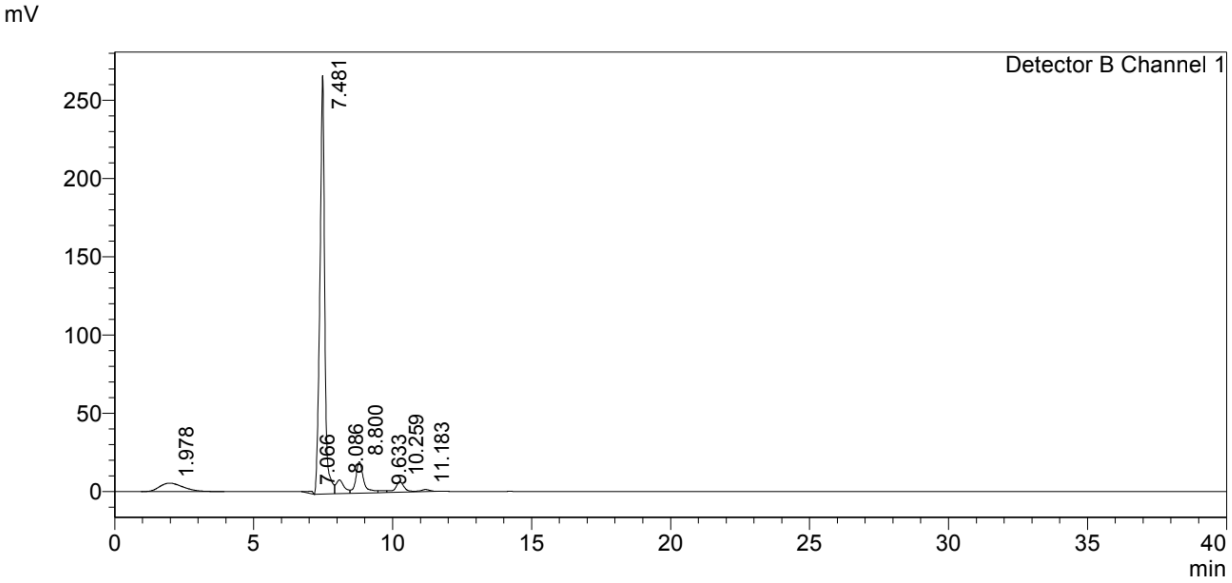

Figure 37: Wort 4, Sample 1 Chromatogram

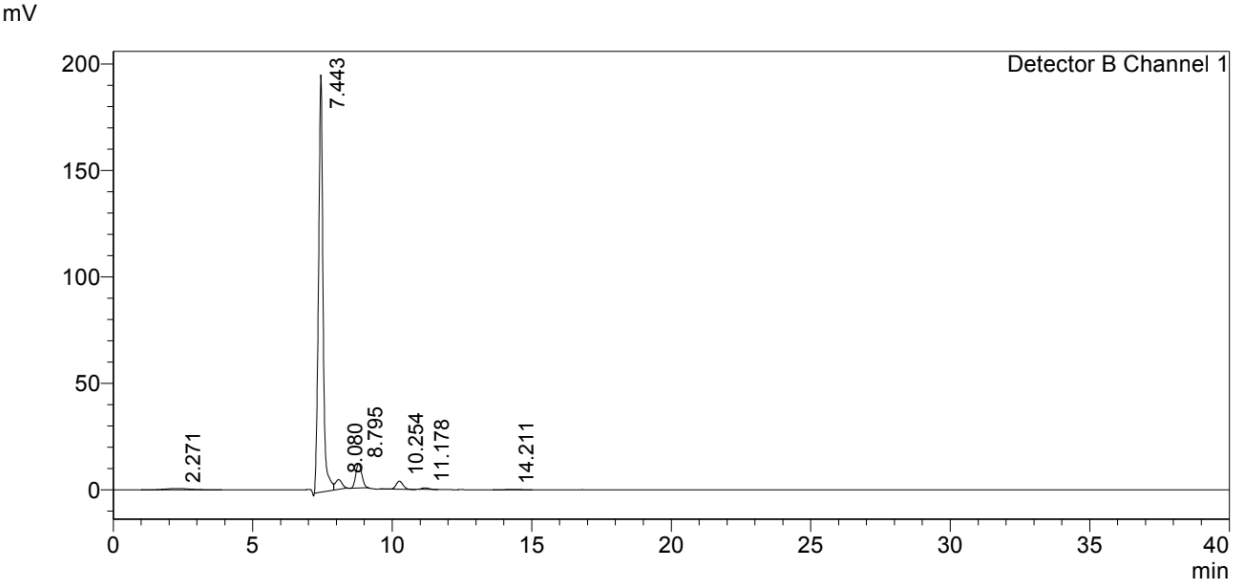

Figure 38: Wort 4, Sample 2 Chromatogram

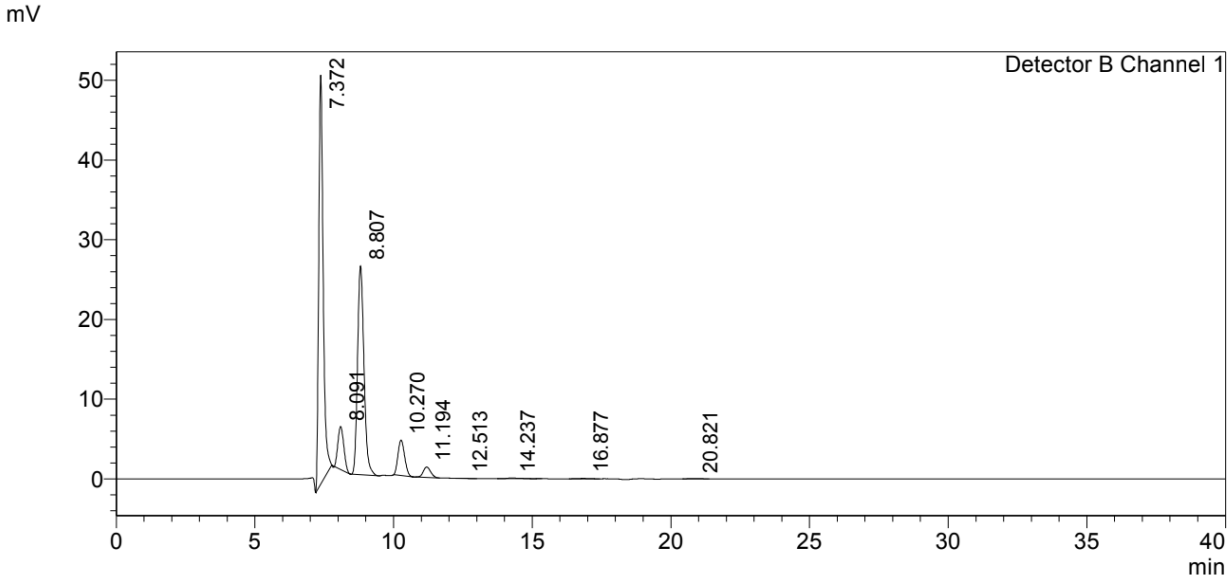

Figure 39: Wort 5, Sample 1 Chromatogram

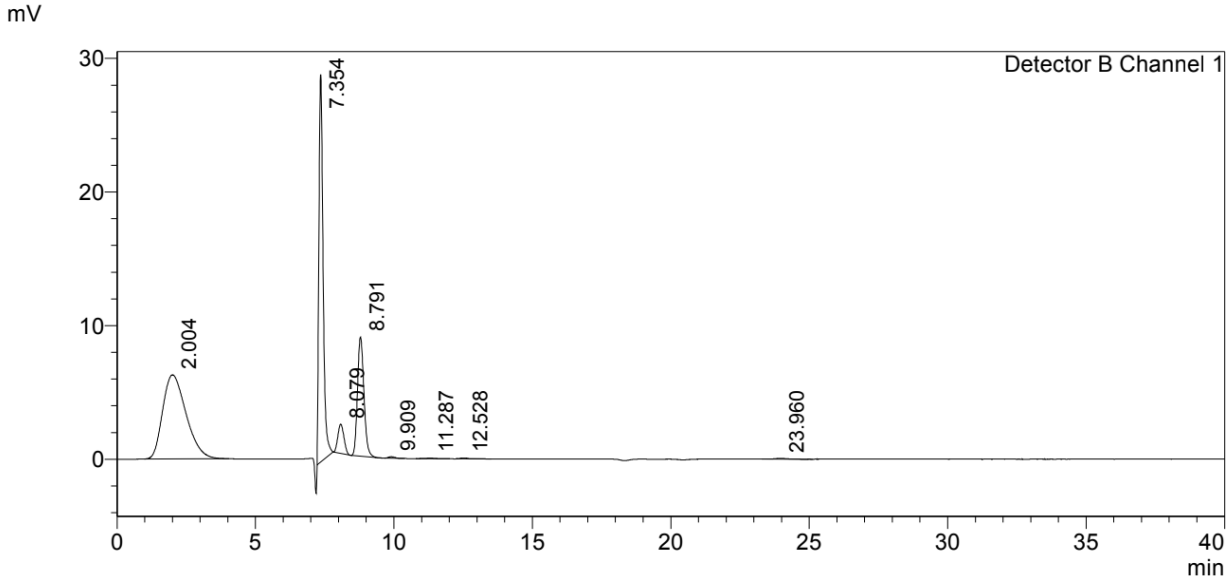

Figure 40: Wort 5, Sample 2 Chromatogram

Appendix F: Photographs of the Brewing Process

Figure 41: Photos taken of Brew 1 in fermentation bucket

Figure 42: Photos taken of Brew 2 in fermentation bucket

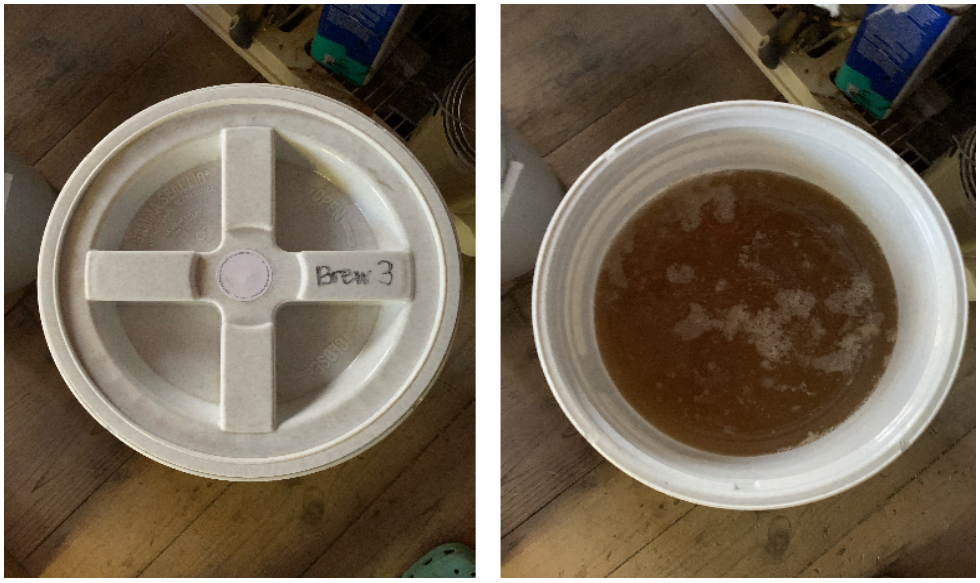

Figure 43: Photos taken of Brew 3 in fermentation bucket

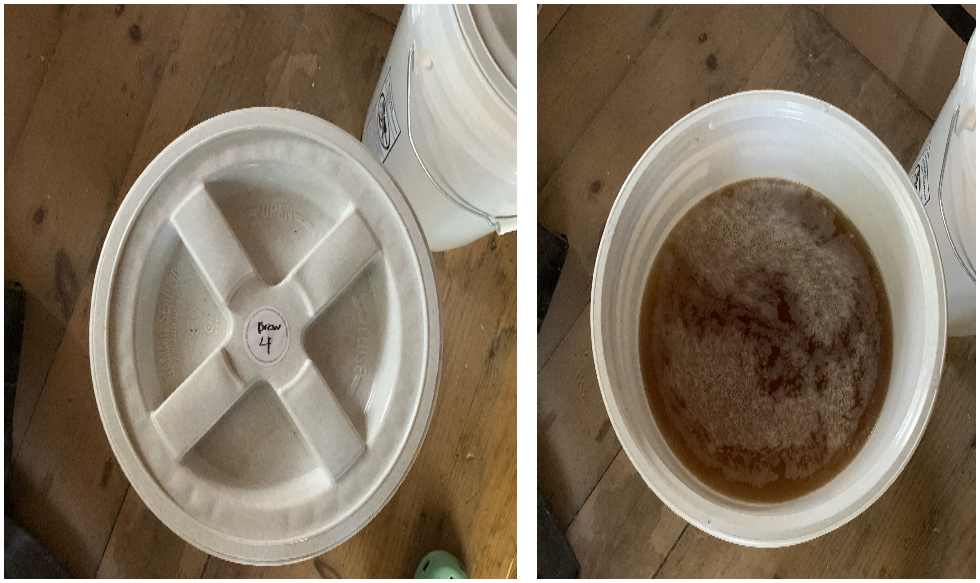

Figure 44: Photos taken of Brew 4 in fermentation bucket

Figure 45: Photo taken of Brew 5 in fermentation bucket

Figure 46: The process of siphoning beer from the primary fermentation bucket to a carboy for secondary fermentation with raspberries

Figure 47: Photos of each brew in carboys for secondary fermentation

Figure 48: All brews are pictured bottled and 2 weeks away from consumption