Project Number: MQP SJK-AANT

Improvement of Hard Cider Production

A Major Qualifying Project
Submitted to the Faculty of
Worcester Polytechnic Institute
In partial fulfillment of the requirements for the
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Sponsored by:
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Abstract

The goal of our project was to enhance the quality and efficiency of Ricker Hill's hard cider production. This was accomplished by identifying the species of bacterial infections, developing cider and juice flavor compounds, determining yeast assimilable nitrogen (YAN) levels in juice samples, and optimizing Ricker Hill's cross-flow filter. As a result of our work, *Acetobacter* was identified as the primary infecting bacteria. We also concluded through YAN testing that all samples were nutrient deficient and produced sulfur byproducts during fermentation. The presence of sulfur compounds was confirmed in the GC-MS analysis. Finally, we found the optimal pressure and flowrate for Ricker Hill's crossflow filter.

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Introduction

Ricker Hill Orchards, located in Turner, Maine has been growing a variety of apples on their 1,000 acre farm since 1803. Included on their farm are a variety of other fruits, such as pears, cranberries, and pumpkins. Ricker Hill is best known for their McIntosh apples, which they sell up and down the east coast. In August 2014 Ricker Hill started their hard cider brewery with the help of Mr. Justin Lagassey. The entire process takes place in their new brewing facility located on the farm. Their flagship product is Mainiac Gold, which is made primarily from Golden Delicious apples. This product is 5% alcohol by volume and is packaged in an aluminum can. It can be found in stores throughout Maine and will be available in various stores in New England.

I. Ricker Hills' Brewery Operation

Fresh cider for fermentation is obtained from a nearby juice press. The cider is first filtered using a crossflow filter, shown in Figure 1, and then pumped into fermentation tanks. Ricker Hill has 13 tanks in total on its brewery production floor, including four 3,370 gallon tanks. Also on the production floor is a small titration lab used to test pH levels in the cider before

Figure 1: Ricker Hill's Crossflow filter

fermentation. Once fermentation is completed, the finished product is pumped to a canning line.

II. Purpose

The purpose of this project is to improve the production and quality of hard cider at Ricker Hill. Ricker Hill is trying to mass produce a consistent and high quality cider throughout the northeast. In order to compete in the hard cider business, they must also have an efficient process. However, as a new brewery they are faced with several problems, such as *Acetobacter* infections, flavor quality and consistency, nutrient deficient fermentation, and crossflow filtration efficiency.

III. Objectives

Our objectives are the following:

1. Determine what species are infecting the cider during production and evaluate the cost and feasibility of this process.
2. Develop a complete apple cider flavor profile by determining the volatile compounds that exist in the apples.
3. Establish a yeast assimilable nitrogen (YAN) procedure for Ricker Hill Orchards and determine the expenses for this procedure.
4. Optimize the cross-flow filter to ensure its longevity.

Bacterial Infection

I. Background

i. Acetobacter Overview

During production of Ricker Hill's hard cider, they experienced frequent problems with spoilage due to bacterial infections. The most common spoilage bacterium in fermented beverages is *Acetobacter*. *Acetobacter*, a gram negative bacterium, produces acetic acid from ethanol. These bacteria are not toxic, and have a long history of being used in the fermentation industry, mostly in the production of vinegars.[1] *Acetobacter* growth during hard cider production is unfavorable, as the bacteria consumes ethanol and produces acetic acid. This alters the alcohol content, as well as the taste of the hard cider. Currently, Ricker Hill does not have a procedure to limit *Acetobacter* infections during production of its hard cider. Developing an anti-bacterial procedure would allow Ricker Hill to subdue infections, which would improve the quality of hard cider. In addition, having this procedure would reduce the risk of having to dump a spoiled batch, which is a cost that can hurt Ricker Hill's business.

The majority of studies done on *Acetobacter* infections have been in the beer production industry. Beer is considered a microbial resistant beverage, as its microbiological stability allows it to be resistant to spoiling. This resistance is mainly due to hops, which has antimicrobial behavior. When infections do occur, brewers must either dump their batch or filter out the bacteria. Having to dump or filter a batch is wasteful and inefficient for brewers during production. Because of this, there has been a lot of attention in the brewing industry historically on aerobic gram-negative bacteria such as *Acetobacter*. Since aerobic bacteria

[1] (Acetobacter aceti Final Risk Assessment)

require oxygen to survive, processes have been designed that limit exposure to the atmosphere. In modern breweries, aerobic bacterial infections are no longer an issue. These types of infections are much more prevalent in small scale operations, which are usually less advanced than large scale operations.[2] Ricker Hill is a small scale operation, and does not have the resources to invest in expensive equipment that limits oxygen exposure. Along with hard cider production, Ricker Hill is interested in producing apple cider vinegars. The same bacteria, *Acetobacter*, that is seen as an infection during hard cider production is the bacteria responsible for producing acetic acid, the main ingredient of vinegar. However, vinegar production is slow, making it not an economically feasible option.

The bacteria involved during hard cider and vinegar production are crucial to the efficiency of each process. Regulating the activity of these bacteria is an efficient and cost-effective method to improve hard cider and vinegar production. To devise a plan of bacterial control, the first step is to identify the species of bacteria involved. For hundreds of years, brewers have identified bacterial infections with a simple smell test. Although the bacterial infection is likely *Acetobacter*, brewers cannot be certain because there are several other bacteria that produce acetic acid, such as *Gluconacetobacter* and *Gluconobacter*.

A common practice utilized by biologists to identify the species of an unknown bacterium is to amplify, sequence, and compare the 16S ribosomal DNA (rDNA). Bacterial ribosomal genes are essential for survival and are therefore highly conserved. This genetic

[2] (Sakamoto and Konings)

material is not as sensitive to environmental factors compared to morphology and metabolic signatures, which help ensure success with bacterial taxonomy.[3]

ii. Polymerase Chain Reaction (PCR) and DNA Sequencing

Polymerase chain reaction (PCR) is the current industry standard when amplifying DNA. PCR uses a thermal cycling process to produce thousands of a certain DNA sequence. For DNA sequencing, several new, cost-effective methods have arisen such as Single-Molecule Real-Time Sequencing, Ion Semiconductor, and Sequencing by Ligation[4]. However, for small projects, the Sanger method of chain termination, which was developed in 1977, is still widely used. For PCR and DNA sequencing, synthetic primers are used to indicate a particular region of DNA and are typically around 20 nucleotides long. Designing primers for an unknown species can be difficult. For both PCR and DNA sequencing, universal primers designed for the 16S rDNA gene are frequently used because this gene is so highly conserved between bacterial genera.

iii. NCBI's BLAST Search

Once the DNA sequence has been determined, comparison to known DNA sequences from databases can determine the species. The National Center for Biotechnology Information (NCBI) website has an extensive DNA sequence database. NCBI's nucleotide Basic Local Alignment Search Tool (BLAST) compares a sample DNA sequence to its database. Results from the BLAST search give scores of how closely the sample DNA sequence matches DNA sequences from specific bacterial strains in the database. Three programs with different speeds and sensitivities are available for nucleotide versus nucleotide sequence comparison: megablast[3], discontinuous

[3] (Graciela, Eugenia and Alejandro)
[4] (Hanlee and Shendure)

megablast[5], and blastn[6]. These programs are based on algorithms designed for a certain task. The megablast program works best for identifying the input query and searching with large genomic query. Discontinuous megablast is best suited for finding related sequences from other organisms. Blastn works better for shorter sequences and cross-species searches. Each program ranks sequences from a database based on how well the input sequences match. The database sequences are given a score for how well they match, and the sequence with the highest score identifies the sample of interest.

II. Methodology

Two sources from Ricker Hill were sampled for their bacterial content. One source was taken from a hard cider batch that had recently developed a bacterial infection. The other source was taken from a vinegar batch that been sitting for several months. An inoculating loop was used to streak these sources onto an agar medium. The composition of the agar medium included 30 g glucose, 5 g yeast extract, 3 g peptone, 20 g peptone, 20 g agar, and 1000 ml of distilled water. The incubation temperature was room temperature, or approximately 20˚C.

After about three days, significant bacterial growth was found. Five different colonies, shown in Table 1, were isolated and placed on their own agar plate. Since colonies begin from one bacterial cell, each colony represented one bacterial species.

[5] (Zhang, Schwartz and Wagner)
[6] (Altschul, Madden and Schaffer)

Colony	Source
Vin-1A	Vinegar
Blb-1C	Infected Hard Cider
Blb-1D	Infected Hard Cider
Blb-1E	Infected Hard Cider
Blb-1F	Infected Hard Cider

Table 1: Colonies of Bacteria Isolated

Following another three days for the five isolates to grow, the next step was to expose the DNA contained in the bacterial cells. This was done using 106 µm glass beads to break open the cells. Before their use, the glass beads had to be washed according to a procedure located in Appendix A. For each isolate, 10 ml of deionized water, approximately 10 ml of glass beads, and a colony from each isolate agar plate were put into an Eppendorf tube. The Eppendorf tubes were then vortexed for five minutes, ensuring DNA exposure.

In order to be sequenced, the exposed DNA had to be amplified. This was done using PCR and universal primers. Two primers were used with each PCR run. Primers 27F, the forward primer, and 1492R, the reverse primer, were used together, while primers 8F and 1391R were used in junction[7][8]. Table 2 shows the different primers and their base sequence.

Primer	Base Sequence (5'-3')
8F	AGAGTTTGATCCTGGCTCAG
27F	AGAGTTTGATCMTGGCTCAG
1391R	GACGGGCGGTGTGTRCA
1492R	ACCTTGTTACGACTT

Table 2: Primer Sequences

[7] (Stackebrandt and Goodfellow)
[8] (Turner, Pryer and Miao)

Two universal primers, an isolate DNA sample, and PCR Master Mix were put into PCR specific Eppendorf tubes. These tubes were designed with thin walls to efficiently allow the transfer of heat during the PCR machine's thermal cycling. With all these three inputs, the tubes were placed into the PCR machine. The specific procedure for conducting PCR can be found in Appendix B.

Product from the PCR was confirmed using gel electrophoresis. A sample from each PCR tube was placed on a gel. Gel electrophoresis uses a current to pull DNA sequences down a gel. Because DNA is negatively charged, the samples were placed on the negative terminal of the gel. Once the current was turned on, the DNA sequences migrated down the gel towards the positive terminal. After approximately 30-45 minutes, the gel was taken to be analyzed under a UV-light. The procedure for gel electrophoresis can be found in Appendix C.

With a confirmed PCR result, the remaining PCR product and four universal primers were sent to be sequenced at Eton Biosciences. Eton Biosciences was consulted because their DNA sequencing services were time-effective and relatively cheap. The resulting DNA sequences from Eton Biosciences were put into NCBI's BLAST search, which compared each sequence to a library of sequenced DNA. The megablast setting was used because the isolates were unknown and this setting has a large genomic query. The best matched sequences were given the highest score, and the species was determined.

III. Results and Discussion

i. Isolation of Bacterial Samples

Five distinctly different colonies were identified from the two cider sources; four colonies were taken from the infected hard cider sample and one colony was taken from the vinegar sample. These results and their characteristics are also shown in Table 3.

Name	Source	Characteristics
Vin-1A	Vinegar	Round, shiny, brown
Blb-1C	Infected Cider	Opaque, yellow center, round, smooth
Blb-1D	Infected Cider	Shiny, brown, small, round
Blb-1E	Infected Cider	Small, round, white, dull
Blb-1F	Infected Cider	Round, white surface, rough

Table 3: Colony Descriptions

Each colony was isolated onto its own agar plate. Figure 2 shows an example of one of these plates.

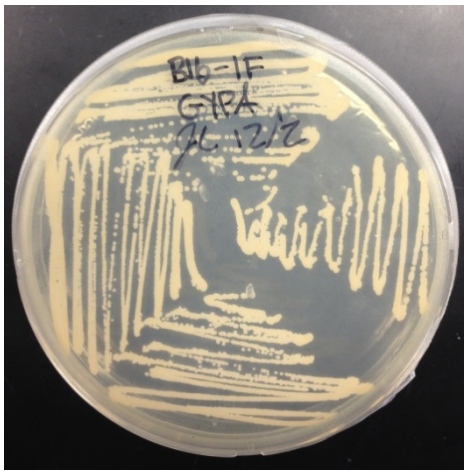

Figure 2: Blb-1F Agar Plate

ii. PCR and Gel Electrophoresis

The DNA of the five isolates was amplified according to the PCR protocol. The PCR product was

The orange bars represent ethidium bromide that was bonded to a DNA fragment.

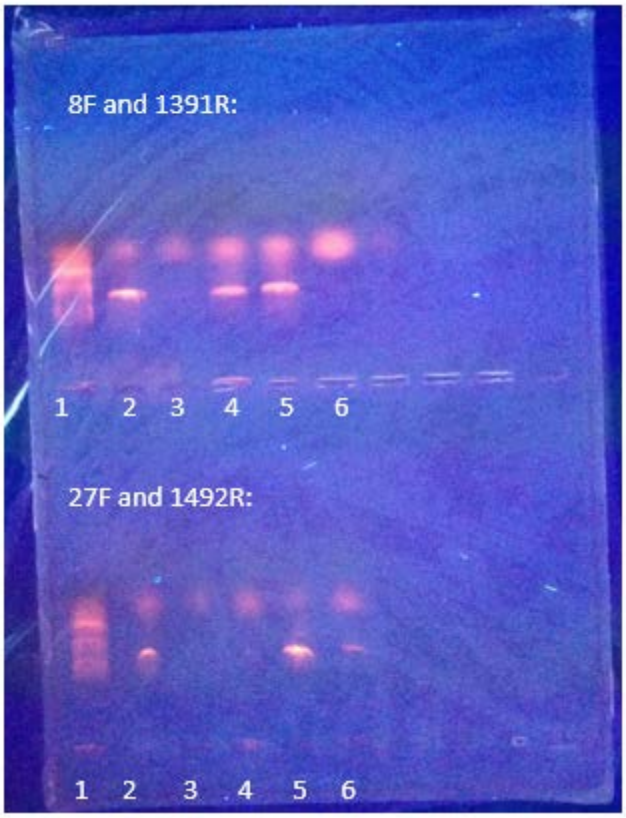

Figure 3: Gel under UV Light

Ethidium bromide luminesces when exposed to UV light. The DNA in the gel migrated in each lane upward. The bottom lanes represented samples that had been amplified with the 27F and 1492R primers and the top lanes represented samples with the 8F and 1391R primers. Tables 4 and 5 show the lane designations for each isolate, with lane 1 representing the lane furthest to the left.

Lane	Isolate	PCR Product Visible
1	Ladder	N/A
2	Vin-1A	Yes
3	Blb-1C	No
4	Blb-1D	No
5	Blb-1E	Yes
6	Blb-1F	Yes

Table 4: PCR Products from 27F and 1492R Primers

Lane	Isolate	PCR Product Visible
1	Ladder	N/A
2	Vin-1A	Yes
3	Blb-1C	No
4	Blb-1D	Yes
5	Blb-1E	Yes
6	Blb-1F	No

Table 5: PCR Products from 8F and 1391R Primers

iii. DNA Sequencing

The ten PCR products along with the four primers were sent to Eton Biosciences for sequencing. The full sequencing results can be found in Appendix D. Tables 6, 7, 8, and 9 summarize the results of each primer. The results for each primer were put into NCBI's BLAST search. The top result for each isolate is included in the corresponding tables.

Isolate	BP Length	BLAST Species Top Result
Vin-1A	1010	*Acetobacter pasteurianus*
Blb-1C	253	None
Blb-1D	535	None
Blb-1E	658	None
Blb-1F	441	None

Table 6: 27F Primer

Isolate	BP Length	BLAST Species Top Result
Vin-1A	1286	*Acetobacter pasteurianus*
Blb-1C	602	None
Blb-1D	567	None
Blb-1E	177	None
Blb-1F	218	None

Table 7: 8F Primer

Isolate	BP Length	BLAST Species Top Result
Vin-1A	237	None
Blb-1C	548	None
Blb-1D	753	*Acetobacter malorum, Acetobacter cerevisiae, & Acetobacter orleanensis*
Blb-1E	662	None
Blb-1F	690	None

Table 8: 1492R Primer

Isolate	BP Length	BLAST Species Top Result
Vin-1A	1071	*Acetobacter pasteurianus*
Blb-1C	354	None
Blb-1D	1260	*Acetobacter cerevisiae*
Blb-1E	1210	*Acetobacter pasteurianus*
Blb-1F	326	None

Table 9: 1391R Primer

Results of the BLAST search show that 1391R was the most effective primer used during sequencing. Sequencing with 1391R produced three results that had significant matches in the BLAST search, while the other three primers only produced one result each. Therefore, it can be assumed that 1391R was the primer best designed out of the four for species identification during this project.

Three isolates from the 1391R primer were identified in the *Acetobacter* genus. This was predicted, as *Acetobacter* infections are common in fermentations. Isolates Vin-1A and Blb-1E were both *Acetobacter pasteurianus*, while Blb-1D was *Acetobacter cerevisiae*. Sequencing of isolates Blb-1C and Blb-1F did not produce significant matches with any of the four primers. A

different set of primers or additional tests are needed to identify what the species of these isolates are. DNA sequences of the *Acetobacter* genus do not deviate significantly between species, so it is possible that the primers were ineffective for isolates Blb-1C and Blb-1F because they are of a different genus.

IV. Conclusion and Recommendations

Identifying the bacterial species involved during Ricker Hill's hard cider and vinegar production was a critical first step in understanding the bacterial environment. Future tests, however, could still be conducted to produce more confident results, as two of the five isolates were not identified. If possible, primers specific to the *Acetobacter* genus are recommended. Several biotechnology companies such as Eton Biosciences offer a custom primer service where a specific DNA sequence can be made. A simple test to identify acetic acid producing bacteria is the addition of $CaCO_3$ to the agar mixture used to grow the bacterial isolates.[9] Acetic acid production could be detected by the dissolution of $CaCO_3$, which would produce a clear ring around the bacterial colony. In Ricker Hill's small laboratory, this test could be a quick and easy way to identify acetic acid bacteria such as *Acetobacter* in a batch of hard cider.

The next step in understanding Ricker Hill's bacterial environment is to categorize the activity of the bacteria. To accomplish this, a proposed project is to track the concentrations of ethanol and acetic acid over the length of fermentation. Separate fermentations with a single bacterial isolate introduced could be conducted to determine each isolate's activity. However, taking several samples of the hard cider during fermentation could lead to contamination

[9] (Asai, Iizuka and Komagata)

because of the frequent air exposure. It is recommended that an apparatus be made with a hole just big enough for a pipette. The hole could have a rubber tube through it, both allowing the pipette to reach the cider and to be pinned off when not in use. Specifically how to measure the concentration of ethanol and acetic acid is unknown, as extraction methods in conjunction with GC-MS were largely unreliable. If these technical restrictions are able to be resolved, the concentration of ethanol and acetic acid during fermentation can be modeled. Modeling these concentrations over time can be directly correlated to the activity of bacteria during production, which is useful in understanding the bacterial environment.

Ricker Hill hopes to decrease bacterial growth during hard cider production, while increasing bacterial activity in vinegar production. Controlling bacterial growth and processes requires an understanding of the bacteria involved. This project took the first step in understanding the bacterial environment by identifying the species of bacteria in Ricker Hill's hard cider and vinegar production. Out of the five bacterial isolates that were found, three were able to be identified. As predicted, all three isolates belonged to the *Acetobacter* genus. With this information, future projects to model the concentrations of ethanol and acetic acid during fermentation are recommended. With a clear understanding on the bacterial activity, Ricker Hill can begin to manipulate associated variables during production to have desirable fermentations.

Cider and Juice Flavor Compounds

I. Background

i. Gas Chromatography

Gas Chromatography (GC) is a widely accepted analytical technique used for separation of volatile compounds over time. The GC column is a hollow tube that is layered with a coating of a stationary phase. The stationary phase is a solid that has chemical properties that affect the mass transfer of sample analytes throughout the column. The column is heated by an oven that separates the less volatile compounds as the temperature is increased to their boiling points. The carrier gas is used to transport the injected sample throughout the column. A common choice for a carrier gas is helium because it is inert with the injected sample and stationary phase.

Chromatography separates components of a sample with physical or physico-chemical methods. The compounds to be separated are distributed between two non-miscible phases; one of the phases is usually stationary and the other phase travels over the stationary phase in a nearly complete divided state. The moving phase transports the components for separation. The three requirements for a gas chromatographic process are as follows:

1. An adsorbent in the liquid phase
2. Substances which are adsorbed or dissolved in the adsorbent
3. A gas current to carry the substances over the adsorbent [10]

All of the components that are separated by chromatography travel in the same direction as the moving current. In addition, all components travel at the same speed. The

[10] (Kitson)

number and duration of rest periods that the compounds take in the stationary phase differs between the compounds. Since each type of molecule has its own unique quality of adsorption through the gas column, the components will reach the detector at different times. A detector is used at the end of the gas column that determines the component and the quantity of the component in the sample by recording the retention time and order of emergence in the other side of the column.

Separation of desired analytes is affected by a variety of factors in the GC. The choice of the stationary phase is necessary to attain different retention times for the desired analytes. The stationary phase chosen should be able to handle temperatures of the boiling points of the less volatile analytes without 'bleeding', or degrading. Typically non-polar coatings for the column are chosen because they are less prone to bleed than more polar coatings. Another factor affecting compound separation is rate of temperature increase.

ii. Mass Spectrometry

Mass spectrometry is an analytical tool that is used to identify compounds from molecular masses. A model of a mass spectrometry is show below in Figure 4. This tool measures the mass-to-charge ratio, $\frac{m}{z}$, of gas phase ions and provides a measure of the quantity of each ionic species.

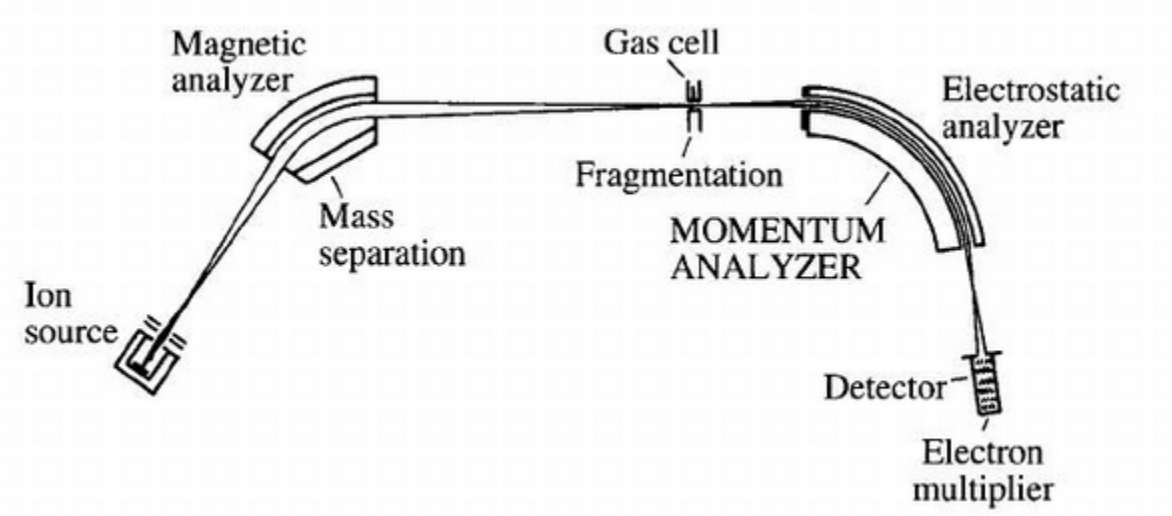

Figure 4: A model of mass spectrometry from Kitson

First, gas phase ions are produced in an ion source by one of several different methods. These charged particles are accelerated from near rest through a potential gradient. The ions travel through a vacuum chamber into a magnetic field at a low pressure, which allows for the gas phase ions to separate. A magnetic field exerts a force perpendicular to the direction of movement by the charged particles according to the equation:

$$\frac{m}{z} = \frac{B^2 r^2}{2V}$$

Where 'r' is the radius of curvature the particles travel through the magnetic field, 'B' is the magnetic field, and 'V' represents the acceleration voltage. As shown by the governing equation, for a constant electromagnetic field and voltage, ions of lower molecular mass will travel a radius of curvature smaller than the heavier ions.[11]

An electrostatic analyzer is used to provide energy resolution and directional focusing of the ion beam. The resolution is capable of separating ions with the same molecular mass but different chemical formula. The electrostatic compartment is composed of two flat curved

[11] (Kitson)

metal plates having opposite electrical potentials. The plates' potential is adjusted so that ions having translational energy will follow the curvature of the path.[12]

The ions are detected at the end of the path with use of an electron multiplier. Electrons with sufficient kinetic energy will emit secondary electrons when they strike a metal surface. A discrete electron multiplier has a series of dynodes that are connected by a resistor chain so that the first dynode has a higher negative potential than the last. Each dynode after the first has an increased voltage gradient to allow for continuous electron emission through the following dynode. This increasing cascade of electrons will provide a sufficient signal to be detected and the results are sent to a computer for processing.[13]

iii. Flavor Profiles of Apples

The flavor profiles that exist in apple juice to the finished hard cider are intricate. The major volatile components of apple cider have been identified as alcohols, esters, fatty acids, and carbonyls. The most aromatic active compounds have been identified alcohols, esters, lactones, phenols, and short to medium chain fatty acids. It has been thought that two of the most important contributing aromatic alcohols are phenethyl alcohol and 3-methyl butanol.[14] Larger alcohols have been thought to contribute the sensory profile as well; however, these have been known to exist in lower amounts and hold lower aroma intensities. These have a honey rosy aroma and fruity nail polish-like odors, respectively. Known aromatic acids in cider were acetic, butanoic, propanoic, hexanoic, octanoic, decanoic acid and 2-methyl-butanoic acid.

[12] (Gross)
[13] (Kitson)
[14] (Wiley)

These have been known to produce rancid, cheesy, and sweat-like odors. On the basis of aromatic intensity, esters have been known to be some of the most important contributors. Esters such as ethyl 2-methyl propanoate, ethyl 2-methylbutanoate, ethyl butanoate, ethyl octanoate, and ethyl decanoate contribute to fruity, sweet, apple, pineapple, and floral odors. Several ketones, such as 3-hydroxy-4-phenyl-2-butanone, 1-octen-3-one, and 1-octen-3-one have been thought to contribute a wide variety of aromas. Phenols have been thought to have an interesting effect on aroma due to some of them having low sensory limits and high to medium sensory intensities.[15] Besides the production of ethanol during fermentation, other flavor compounds are produced throughout fermentation.

Studies done on Fuji apples show that the sensory profiles change when Fuji apple juice was compared with its fermented product. The wine and juice samples had the greatest difference in the concentration of the organic acids, esters, and alcohols. Whereas the juice contained shorter carbon chains of the above these components, the cider had longer carbon chains of the organic components. A solid understanding of the volatile compounds in apple cider can allow the cider maker to control the raw materials and fermentation conditions to produce a finished product of a targeted specific flavor profile.[16]

For chemical analysis of the flavor compounds, gas chromatography coupled with mass spectrometry (GC-MS) is typically used. The apple cider cannot be directly injected into the GC-MS because of the large presence of water in the ciders. Water injections can cause a variety of problems when injection without treatment. Back-flashes can occur from water injections because of the large expansion of water when it is vaporized. In addition, damage to the

[15] (Xu, Fan and Qian)
[16] (Wang, Xu and Zhao)

stationary phase of GC can happen due to the polarity of water. This damage to the stationary phase can alter the column's selectivity and the retention times of these compounds. Thus, a liquid-liquid extraction was chosen that would extract the flavor compounds from the apples while greatly reducing the amount of water injected.[17]

II. Methodology

The Shimadzu GCMS-QP2010 SE with AOC-20i Auto Sampler was used for chemical analysis. The proposed method was adapted from a previous wine analysis.[18]. The goal of this method was to extract the flavor compounds from the juice and hard cider with dichloromethane. The concentrations of these compounds were to be determined with the use of internal standard solutions. The preparation of these internal solutions can be found in Appendix E. The phase of interest was the dichloromethane phase on the bottom; this was extracted with a pipette and placed into a syringe with a filter tip. The plunger, which was removed for the injection of the dichloromethane, was forced down the syringe. The sample was pushed through the filter into a sampling screw-top capsule and put into the GCMS for analysis. The full sample preparation can also be seen in Appendix E with the input GC-MS parameters.

[17] (Richardson)
[18] (Ortega, Lopez and Chacho)

III. Results and Discussion

i. Internal Standard Calibration

The initial goal of determining the concentration of the flavor compounds of Ricker Hill's juice and hard cider with internal standards encountered setbacks. It was expected to observe relatively constant values for the peak heights of the internal standards in the different samples. Although all juice and wine samples were added with the same amount of internal standard solution (.1 mL of 50µg/mL), the peak height observed for these chosen internal standards did not represent any consistency. Thus, the concentration of the flavor compounds could not be determined with confidence. There were different possible causes for this. Thoroughly mixed internal standard solutions could have contributed. In addition, the accuracy of the pipette when developing the internal standard solutions and placing the solution into the juice and wine samples could have caused the difference in internal standard concentration. However, calibration graphs of the internal standard solutions (without the liquid-liquid extraction with dichloromethane) were developed, as shown in Figure 5.

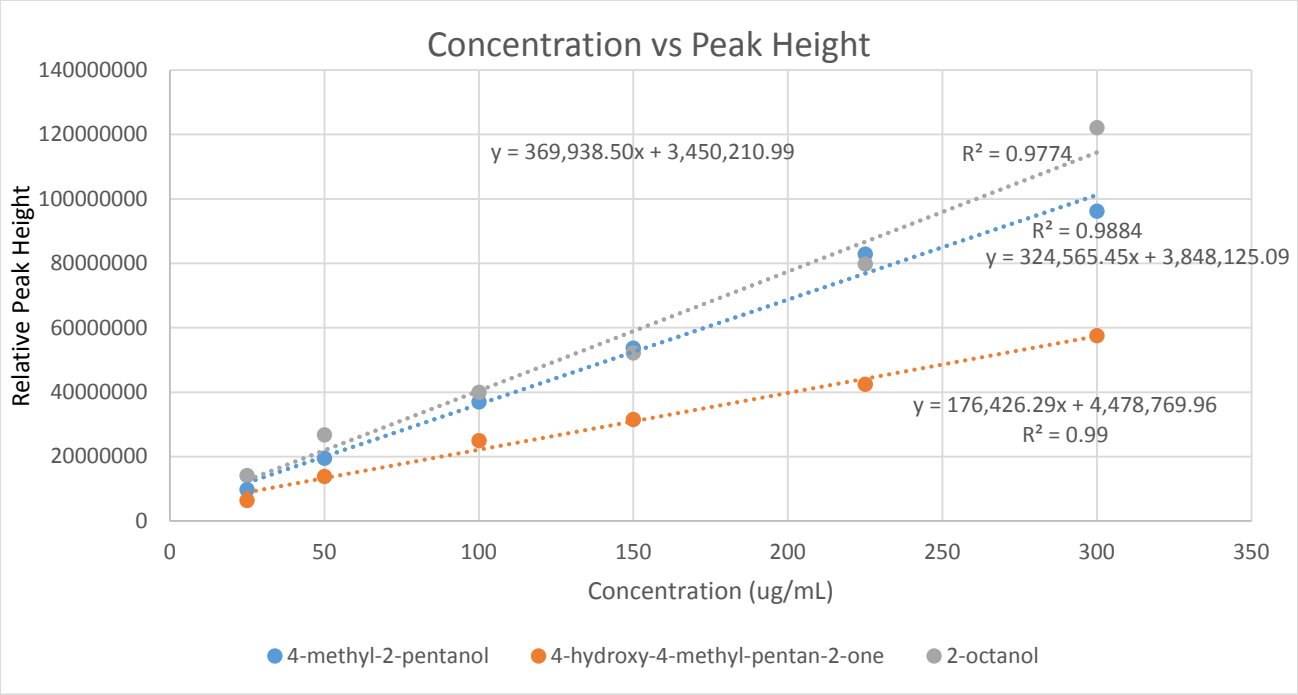

Figure 5: Concentration for internal standard solutions

The graph shows that the concentration of the internal standard solutions produces a linear relationship to peak height. Thus, the peak height could still be used as a relative comparison to concentration among the flavor compounds in the juice and ciders; however, without the use of standards, the absolute concentration could not be known. Results could still be collected to compare the relative concentrations of the flavor compounds and observe the changes of these flavor compounds between batches of juice and cider.

ii. Comparisons between Maniac Gold Batches

From the data collected between Batches 25, 26 and 27, similarities and differences in compounds' concentrations were shown. The mass spectrometer data recorded for Mainiac Gold Batches 25, 26, and 27 are shown in Appendix F. The main flavor compounds are ethyl lactate, ethyl acetate, and ethyl hydrogen succinate.

Some of the large peaks and common medium high peaks shared for all the three Mainiac Gold batches are shown below in Table 10.

Large Peaks	Medium High Peaks
3-methyl-1-Butanol	2, 3 butandiol; 3-(methylthio)-1-propanol
phenylethyl alcohol	tetrahydro-2-methyl- thiopene
ethyl hydrogen succinate	hexanoic acid
1, 3-octandiol	tryptophol
4-hydroxy- benzeneethanol	dibutyl phthalate
propanoic acid, 2-hydroxy-, ethyl ester, (S)- (ethyl lactate)	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione
formic acid, ester ethyl	n-decanoic acid

Table 10: Large and medium high peaks shared by all 3 Mainiac Gold batches

One of the most interesting differences was observed in Batch 27, which had a strong sulfur odor. The large peak shown by sulfuric acid was the most noticeable compound that was present in Batch 27, but not in the other two. Butanedioic acid diethyl ester, better known as succinic acid, 2-methylresorcinol diacetate, and hexadecanamide were only present in Batch 25, and they existed in high quantities. In addition, Batch 25 had significantly higher concentrations of tryptophol than the other two batches. N-nonadecanol-1 was found only in Batch 26 and was present in high quantities. 9-Octadecenamide, (Z)- was not present in Batch 26, but was found in the other two in high quantities.

Because Mainiac Gold is made from Golden Delicious apples, a comparison of the compounds found in the cider were compared to other studies done on Golden Delicious apples. Straight chain esters, branched-chain esters, fewer alcohols and aldehydes were the

most important volatile components in Golden Delicious apples.[19] Some of these compounds were, but not limited to, 2-methylpropyl acetate, butyl acetate, 1-hexanal, 2-methylbutyl acetate, butyl propanoate, butyl butanoate, hexyl acetate and 1-hexanol. Most of these compounds were found in the Mainiac Gold batches, except 1-hexanol. In addition, malic acid was one of the most important volatile organic acids, and it was found in medium amounts in Batches 26 and 27, but was not detected in Batch 25. Furans, nitriles, phenols and benzene rings were detected in some of the Mainiac Gold batches.[20]

iii. Analysis of Maniac Mac Hard Cider and Juice

Data was collected for the Mainiac Mac hard cider and juice, which are made predominantly from McIntosh apples. The changes in the flavor profile are represented in Appendix G.

Overall, the results were consistent of previous research because they show that Mainiac Mac juice has more of the smaller volatile compounds, while the larger volatile compounds were detected in the hard cider. It is most likely that the larger volatiles from the cider came from the breakdown of even larger molecules during fermentation. The results also show that the fermented product has very little overlap in volatile compounds as its juice, as shown in the Table 11 below. The only detected compounds that had similar concentrations in the juice and hard cider include: phenyl ethyl alcohol, nonadecanenitrile, abscorbic acid, hexadecanal, n-tetraconsanol, and n-nonadecanol-1.

[19] (Raffo)
[20] (Raffo)

Common Compounds
Phenyl ethyl alcohol
Nonadecanenitrile
Abscorbic acid
Hexadecanal
Tetraconsonol
n-nonadecanol-1

Table 11: Compounds that overlap for juice and hard cider

Some of the results from the data presented some unexpected outcomes. Observing similar concentration of phenyl ethyl alcohol was unusual because research done with Fuji apples suggested that phenyl ethyl alcohol accumulates in large amounts as a result of the yeast fermentation. Some compounds were found only in the juice but not detected in the hard cider that were not expected such as acetic acid; 1-Butanol, 2-methyl-; 1-Butanol, 3-methyl.[21] According to the research done by Wang, there should be high concentrations of both of these compounds.

From previous studies on apple juice and cider, it was expected to detect some compounds in the juice but not the hard cider. Some compounds that fit this expectation in the Mainiac Mac juice and cider were 2- hexanal; cyclohexaneacetic acid; benzene, 1,3-bis(1,1-dimethylethyl) and several more. Some compounds that were found in either Ricker Hill's juice or cider were unique when compared with other ciders and juices. The unique compounds that had at least medium high peaks included 1,3-octanediol, 9-octadecenamide, (Z)-; ascorbic acid 2,6-dihexadecanoate; nonadecanenitrile; n-nonadecanol-1. Tables 12 and 13 below show both

[21] (Wang, Xu and Zhao)

the compounds that were unique to Ricker Hill's products as well the compounds that were expected to be present in the cider and juice but were not detected.

Flavor Compounds Not Found in Juice	Flavor Compounds Not Found in Cider
1-butanol, 2 methyl (isobutyl alcohol)	acetic acid, butyl ester (n-butyl ester)
acetic acid	acetic acid
cctanoic Acid	heptanol
heptanol	Hexyl acetate
hexyl acetate	ethyl lactate
ethyl lactate	hexanol
hexanol	2-hydroxypropanoic acid ethyl ester (ethyl lactate)
2-hydroxypropanoic acid ethyl ester (ethyl lactate)	diethyl succinate
diethyl succinate	

Table 12: Compounds that were expected to be found but were not found

Compounds Unique to Ricker Hill's McIntosh Products
2-hexen-1-ol *
2,3 Butanediol **
Benzene, 1, 3-bis (1,1-dimethylethyl)-*
1,3-Octanediol
1-dodecanol, 2-hexyl
N-nonadecanol-1
Sulfurous acid, butyl decyl ester *
9-octadecenamide (oleamide)
Hexadecanal
Glycerol 1-palmitate*
Squalene*
n-Tetracosanol
1,5-Hexanediol
*Only found in Mainiac Mac Juice Blend ** Only found in Mainiac Mac Cider

Table 13: Compounds that were unique to Ricker Hill's McIntosh products

iv. Comparisons between Cranberry- Golden Delicious Juice and Cranberry-Mainiac Gold

The data collected for the Cranberry-Golden Delicious juice and Cranberry-Mainiac Gold are shown in Appendix H. Similar to the data collected from McIntosh apple juice blend and the Mainiac Mac, there were larger volatiles present in the hard cider. From comparison of the Mainiac- Cranberry juice and cider with the Mainiac Mac juice and cider, it was shown that the overall changes in compounds were similar. The compounds that were found in the Cranberry Mainiac Mac and not the Mainiac Gold can be attributed to the addition of cranberries.

v. Qualitative Sensory Analysis

The samples that were analyzed in the GC-MS were tasted to develop a qualitative analysis. Table 14 below displays the sensory and tasting characteristics of the analyzed juices and hard ciders.

Flavor Profiles of Samples	Sensory	Taste
McIntosh Blend Juice	sweet, apple-like	slight tartness, medium sweetness, strong apple flavor
Mainiac Gold Batch 22	sulfur-like, no fruit smell	dry, semi- harsh fermentation 'bite'
Mainiac Gold Batch 25	less sulfur smell, hint of sweetness	dry, no apple flavor, Keystone finish
Mainiac Gold Batch 26	rotten pineapple, slight sulfur	harsh sourness, not sweet
Mainiac Gold Batch 27	medium strong sulfur aroma	sulfur taste, not much fruity taste, dry
Mainiac Maple	sweet maple	sweet maple, pleasing soft fermented finish
Golden Delicious Blend with Cranberry	sweet flower	medium sweetness, 2/3 apple, 1/3 cranberry taste
Mainiac Gold with Cranberry	mild apple aroma, hint of sulfur	mild cheese-like flavor, little sweet

Table 14: Qualitative sensory and taste profile of ciders and juice

Unfortunately, the overall harshness of the aroma and taste of the hard ciders juxtaposed with the favorable analysis of the unfermented juices could not be ignored. This is largely due to compounds that were present in the juice that existed below the sensory threshold whereas the cider had large noticeable quantities of unfavorable compounds. From comparisons between the qualitative analysis and the chemical analysis, a range of compounds were detected in the hard ciders that are known to attribute less desirable aromas.

Batch 27 of Mainiac Gold and Cranberry-Mainiac Gold contained relatively large amounts of sulfuric acid, which could have contributed to harsh aromas and sour tastes. In addition,

sulfurous acid derivatives were found in Batches 25 and 27. Another undesirable aroma found was likely acetoin, which was found in all the Mainiac Gold ciders, including the Cranberry-Mainiac Gold cider and juice. Acetoin is a compound that has a rancid smell and can largely be attributed to a large presence of yeast or fast fermentations at high temperatures [22](Hui, 2012).

IV. Conclusion and Recommendations

The results GC-MS detected a variety of alcohols, esters, acids, furans, phenols, amines and sulfuric compounds in the juices and ciders. Comparisons between Mainiac Gold Batches 25 to 27 show that most detected compounds that were found were similar. However, some of the similar compounds were found in varying concentrations. Some compounds were not detected in all batches, and some of these compounds were even detected in high concentrations. Comparisons between the Mainiac Mac juice blend and hard cider show that there is little overlap in flavor compounds. The detected compounds juice blend had a much smaller profile than the hard cider. The cider profile suggests that most of larger esters, alcohols and organic acids as well as other types of compounds are produced after fermentation.

The dichloromethane extraction could be used as a tool to compare the existent compounds between batches. However, the setbacks to this method were exposed, especially for accurately determining the concentration of flavor compounds. The most problematic aspect of using dichloromethane as a solvent to extract all flavor compounds is the difference in polarity of the flavor molecules. The efficacy of the method is greatly decreased for smaller compounds because they are capable of migrating to the water phase during the extraction.

[22] (Hui and Ozgul)

Found in many of the batches of juices and cider were alkanes and alkenes, both of which seemed highly unlikely to exist in the analyzed liquids. In addition, the different functional groups of the organic molecules affect the ability for the compounds to be extracted. Thus, it would be more likely to trust the results obtained for larger non-polar molecules and determine the concentration of those molecules. Another problem with the method was its ability to extract flavor compounds from all cider and juice samples. The Maple Mainiac juice and hard ciders were run through the GC-MS, but the results detected less than 15 compounds for both samples. It is largely unlikely that so many flavor compounds were nonexistent in the cider because the main difference is the addition of the maple.

For future hard cider analysis, it would be beneficial to pursue other extraction methods. In order to analyze the shorter chain volatile compounds accurately, headspace solid-phase microextractions (HS-SPME) could be used. It is a widely known technique that has been used for analysis of wines and some ciders. Unfortunately, for a long period of time during the academic year, the HS-SPME equipment had been under technical maintenance. In addition, high-performance liquid chromatography (HPLC) is also a standard technique for cider analysis. This method would be helpful to find larger non-volatile compounds that also contribute to the overall flavor profile of the cider. We were unable to use this equipment because we were not granted access to the machine.

Yeast Assimilable Nitrogen

I. Background

i. Yeast Assimilable Nitrogen Testing (YAN) Introduction

Several factors and conditions are involved in cider fermentation including temperature, brix, and nutrients. Since there are many components involved, each fermentation batch has its own unique challenges. To ease these challenges, it is crucial for cider makers to evaluate and understand the cider before fermentation begins.

One element that is crucial for fermentation is nitrogen. Nitrogen is the primary growth-limiting nutrient for yeast cell growth[23]. If there is not enough nitrogen for the yeast, a by-product, hydrogen sulfide (H_2S), will be produced. As a result of this nutrient deficiency, the H_2S will leave a sulfur odor in the cider[24]. Lack of nitrogen can also result in a sluggish and unhealthy fermentation[25]. To avoid the lack of nitrogen, additional nutrients are added to the cider to increase nitrogen levels. Yeast assimilable nitrogen, also known as YAN, is a test to evaluate the nutrients that the cider needs to obtain a nourishing fermentation. The concentrations of organic nitrogen known as primary amino nitrogen and an inorganic form known as ammonia are obtained through chemical analysis using a UV spectrometer to determine the total YAN. The total YAN number yields the amount of available nitrogen in juice fermentation. Calculating YAN is essential to cider making because it will regulate nutrient adjustments for the cider.

[23] (Shantanu Kelkar)
[24] (Winemakers)
[25] (Alberti, Vieria and Drilleau)

ii. UV Spectroscopy

The Ammonia Nitrogen and Primary Amino Acid nitrogen enzymatic tests can be carried out to determine YAN. The Ammonia Nitrogen test will produce an Ammonia Nitrogen (AN) number and the Primary Amino Acid test will produce a Primary Amino Acid Nitrogen (PAAN) number. For the Ammonia Nitrogen test, nicotinamide adenosine dinucleotide (NADH) and glutamate dehydrogenase (GIDH) are added to the cider. A UV spectrometer at 340 nm is used to detect the amount of NADH consumed since it is directly related to the ammonia that is present, giving the AN level[26].

For the Primary Amino Acid test, orthophthalialdehyde (OPA) and N-acetyl-L-cysteine (NAC), in the presence of an alkaline buffer, bind with primary amino acids. The reaction forms "coloured complexes"[27] that are measured by a UV spectrometer at 335 nm.

The AN and PAAN can be determined mathematically given the absorbance in the UV spectrometer. These two calculations are added together to obtain the YAN.

YAN tests can be carried out by a third party or done in house. In order to be able to test for YAN in house, special equipment is needed for the lab. These items include a UV-Spectrometer, quartz cuvette, and syringe filters. UV-Spectrometers are expensive pieces of equipment. The HP-Agilent 8453 UV-Spectrometer sells within the range of $1,000 to $6,000 depending on its condition. A front view of the spectrometer is shown below in Figure 6. Conveniently, some systems come with quartz cuvette, which saves around $100 per piece. Target 2 Cellulose Acetate Syringe Filters are also used to filter juice sample before they are mixed with chemicals and placed in the UV-Spectrometer. These filters sell for about $200 for a

[26] (Laboratories, Enzymatic Analysis Kit for Determination of Ammonia in Grape Juice and Wine)
[27] (Laboratories, Analysis Kit for Determination of Primary Amino Acid in Grape Juice and Wine)

pack of 100. Lastly, Ammonia and Primary Amino Acid test kits are required for YAN testing. Vintessential sells these two kits together for $144. This kit comes with enough material for 30 YAN tests. Investing in lab equipment is expensive, however, sending juice samples to a third party can be just as expensive at $45 to $65 per test.

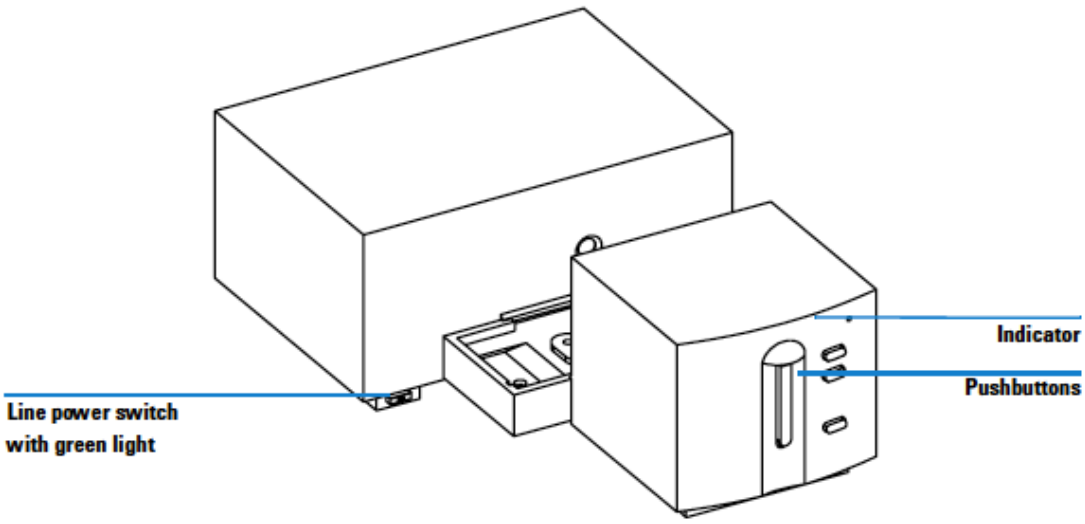

Figure 6: A model of the HP-Agilent 8453 spectrometer

iii. YAN Test Analysis

The range of YAN in raw materials varies depending on crop variety, season, and age of fruit. Despite these variables, the target YAN for a batch of raw cider is 150-200 mg/L[28]. If a batch results in a lower YAN level, it is considered to be nitrogen or nutrient deficient. After determining the YAN levels, there are several things that can be done to ensure a healthy fermentation. More nutrients can be added through a nutrient strategy to increase the nitrogen level of the cider. Table 15, from Scott Laboratories, shows the different nutrients that can be added to the cider to increase YAN levels. Using this table and the desired YAN level, the right amount of nutrients can be added to the cider to optimize fermentation.

[28] (dekramer, Royer and Jones)

YEAST NUTRIENT YAN CONTRIBUTION

Nutrient	Dose 20 g/hL (1.7 lb/1000 gal)	Dose 25 g/hL (2 lb /1000 gal)	Dose 30 g/hL (2.5 lb /1000 gal)	YAN Source
Anchorferm	2 mgN/L	2.5 mgN/L	Not recommended	Organic nitrogen from autolyzed yeast
DAP		50 mgN/L	63 mgN/L	Inorganic nitrogen
Fermaid A		30 mgN/L	36 mgN/L	Inorganic nitrogen (from DAP) and organic nitrogen from autolyzed yeast
Fermaid K		25 mgN/L	30 mgN/L	Inorganic nitrogen (from DAP) and organic nitrogen from autolyzed yeast
Fermaid O		10 mgN/L	12 mgN/L	Organic nitrogen from autolyzed yeast
Go-Ferm		7.5 mgN/L	10 mgN/L	Organic nitrogen from autolyzed yeast
Go-Ferm Protect Evolution		7.5 mgN/L	10 mgN/L	Organic nitrogen from autolyzed yeast
Nutrient Vit End		7 mgN/L	8.5 mgN/L	Organic nitrogen from autolyzed yeast
Phosphate Titres		50 mgN/L	63 mgN/L	Inorganic nitrogen
SIY 33 (Fermaid 2133)		8 mgN/L	10 mgN/L	Organic nitrogen from autolyzed yeast

Table 15: Nutrients that can be added to juice before fermentation to increase YAN levels

iv. Apple Variety

YAN test results are dependent on several environmental and physiological factors. These variables include climate, soil, farming techniques, apple maturity, and apple variety[29]. The amount of nitrogen in apple juices can be between 75 mg/L and 150 mg/L. Whether an apple has high or low nitrogen levels depends on the maturity of the orchard and amount of fertilizer that is used[30]. These factors affect the growth of yeast and fermentation rate[31]. There is no way of controlling how much nitrogen is in an apple or how much nitrogen is in a batch of apple cider juice. However, YAN is a way to monitor the nitrogen level in cider. Thus, YAN tests are essential for cider making to ensure a healthy fermentation.

[29] (dekramer, Royer and Jones)
[30] (Nogueira; Drilleau)
[31] (Garde-Cerdán)

A study was performed in Brazil to observe the effect of apple variety on the growth of yeast and fermentation rate. In their study, three apple varieties were used; Fuji, Gala and Joaquina in order from highest total nitrogen to lowest. The apples were washed, pressed for juice, and fermented. The average nitrogen content in 51 samples was 155.81 mg/L. They found that differences in yeast development were due to the effect of the initial nitrogen levels. They also noticed that consumption by the yeast was highest for Fuji apples followed by Gala, and lowest for Joaquina. As a result, they concluded that nitrogen consumption by yeast was directly related to the initial nitrogen available. The fermentation rate was also observed for Fuji, Gala, and Joaquina apples. Complete fermentation for Fuji was fastest, finishing at 10.50 days, whereas Gala fermentation finished in 12 days, and Joaquina finished in 19 days. Consequently, nitrogen content also effects fermentation rate[32]

II. Methodology

YAN is determined by adding the Ammonia Nitrogen (AN) level with Primary Amino Acid Nitrogen (PAAN). These values were found by running Ammonia and Primary Amino Acid Nitrogen Tests. The test procedures used in this project were adapted from Vintessentials. The YAN test kit from Vintessentials contained an Ammonia Nitrogen test kit and Primary Amino Acid Nitrogen test kit. For each test, there was a blank, a standard, and a juice sample prototype. The blank model served as a reference for the standard and sample. The standard prototype calibrated the analysis. Finally, the sample juice contained a sample of the filtered apple cider which was filtered using a Target 2 Cellulose Acetate Syringe Filter. Juices were filtered in order to obtain a better absorbance and to ensure that the concentration in the

[32] (Alberti, Vieria and Drilleau)

assay solution was no more than 80 mg/L. Dilution may also be required if the sample net absorbance, AN, is greater than 1 absorbance unit. For each of these prototypes, there were two absorbance samples; absorbance 1 and absorbance 2. A HP Agilent 8453 UV-visible Spectroscopy System was used to collect the absorbance of each prototype. The mixtures were pipetted into a 1 cm quartz cuvette cell that was placed inside the UV Spectrometer to collect the absorbance. The collected absorbance were used in a calculation spreadsheet to determine the YAN value in mg/L.

To conserve the chemicals and obtain the most YAN samples, while consuming the least amount of chemicals, the blank and standard models for both absorbance 1 and absorbance 2 were developed and reused for AN and PAAN tests. However, the juice samples were freshly made for each test. Saving and reusing the absorbance 1 and absorbance 2 blank and standard prototypes saved a lot of lab time. Figure 7 shows the total number of vials and models used to create all of the prototypes for each absorbance. When the chemicals were not in use they were stored in a refrigerator to extend its longevity. The procedure for the Ammonia Test and Primary Amino Acid Nitrogen Test is provided in Appendices I and J. In addition, YAN level calculations are provided in Appendix K.

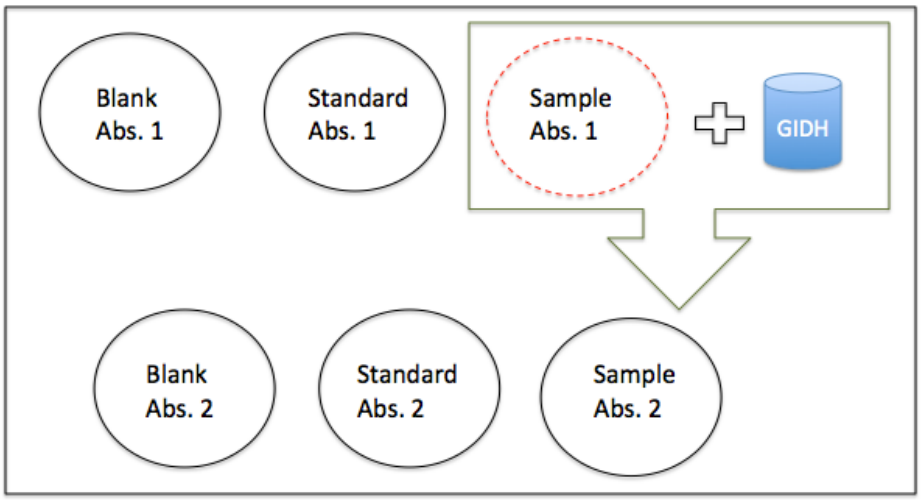

Figure 7: The total number of vials and prototypes for absorbance 1 and 2 for YAN testing

III. Results and Discussion

PAAN, AN, and YAN results of cider samples from Ricker Hill are summarized in Table 16. None of these samples reached the target YAN of 150 mg/L. The greatest YAN level that was achieved was 139.21 mg/L and the lowest YAN was 85.84 mg/L. The average of these samples was 107. 05 mg/L. Because the YAN levels for these samples did not achieve the recommended range of 150 – 200 mg/L, according to Scott Laboratories, the samples were concluded to be nutrient deficient.

Juice Type	PAAN (mg/L)	AN (mg/L)	YAN (mg/L)
Press 2	156.00	-16.79	139.21
Press 1	150.72	-18.68	132.03
Juice 12/1	148.59	-17.09	131.50
Juice 1/5	112.81	-34.26	78.56
2-27(1)	105.85	-20.01	85.84
2-27(2)	108.91	-21.12	87.79
2-27(3)	116.79	-19.48	97.31
2-23(mac)	117.09	-21.43	95.66
2-17(mac)	115.51	-20.03	95.48
3-2-15	148.48	-19.04	129.44
2-9-15	115.46	-18.97	96.49

Table 16: YAN results for cider samples from Ricker Hill

A flavor and odor test was performed on Mainiac Gold; upon review a sulfur smell and taste was noticed. Since the YAN results were relatively low, the sulfur smell and taste may be a result of the lack of nitrogen for yeast to ferment healthily.

No tests were done to observe the fermentation rates of these samples. However, a study in Brazil, as mentioned in the Background section, proved that nitrogen levels directly affected fermentation rates. A greater level of nitrogen in the cider corresponds to a faster fermentation rate. Low nitrogen levels in these samples may cause slow fermentation rates.

An analysis of apple variety was also completed. Table 17 shows the different apple varieties and YAN results. Two McIntosh and one Cortland samples were used to test for YAN in apple variety. As a result of the YAN tests, both varieties had similar YAN levels. Both McIntosh samples averaged 95.57 mg/L in YAN. The Cortland sample resulted in 96.49 mg/L. Unlike the study in Brazil, there was no trend recognized for YAN levels and apple variety.

Apple Variety				
Type	**PAAN**	**AN**	**YAN**	**Details**
2-17(mac)	115.51	-20.03	95.48	McIntosh from the press, no bin tags
2-23(mac)	117.09	-21.43	95.66	McIntosh from the press, Ricker Hill apples
2-9-15	115.46	-18.97	96.49	Cortland, from the packing room

Table 17: Apple variety YAN results for McIntosh and Cortland apples

In addition to apple variety, the YAN level at different stages of fermentation preparation was tested. The results for this analysis are shown in Table 18. The cider sample in this analysis contained a variety of McIntosh, Cortland, Jona Gold, and Gala apples. At the first

stage when the cider was taken, the tank was halfway through pressing. At this stage, the YAN was at its lowest at 85.84 mg/L. The YAN increased to 87.79 mg/L at stage 2 when the cider was duplicated. At stage 3, the added Go-ferm enabled the YAN to reach 97.31 mg/L. Finally, at the last stage, Fermaid-O was added and the YAN level increased to 129.44 mg/L.

Fermentation Preparation Stages				
Stage	**PAAN**	**AN**	**YAN**	**Details**
1	105.85	-20.01	85.84	B37 half way through pressing 1500gal: mac 330Bu, Cortland 21Bu, Jona Gold 42Bu, Gala 58Bu
2	108.91	-21.12	87.79	B37 full tank: mac 537Bu, Cortland 84Bu, Jona Gold 63Bu, Gala 121Bu
3	116.79	-19.48	97.31	B37 after adding Go-ferm and yeast @ 250ppm
4	148.48	-19.04	129.44	B37 after adding Fermaid-O @ 200 ppm

Table 18: YAN results for cider at different fermentation stages

This analysis at different stages proved that as nutrients were added, YAN levels increased. Figure 8 shows the increase in YAN as nutrients are added to the cider blend.

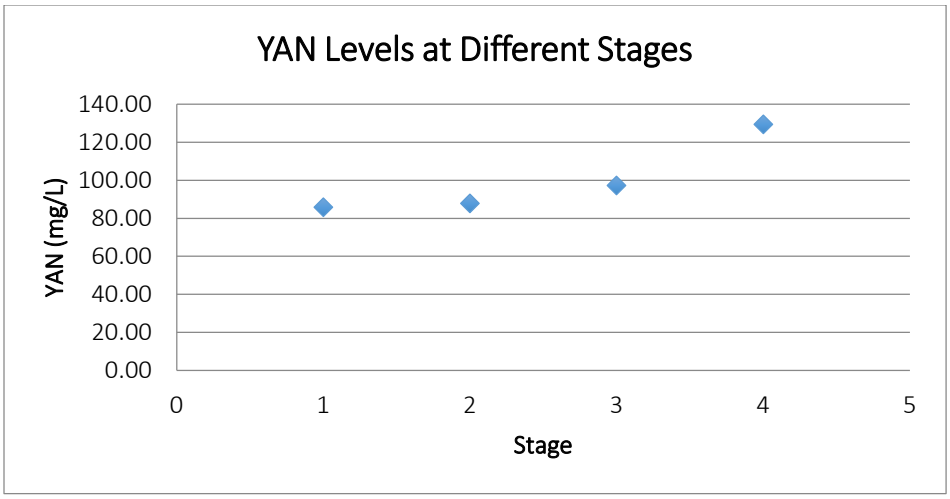

Figure 8: A graph of YAN levels at different stages of fermentation

During YAN testing, there were several errors that may have occurred. YAN testing requires the use of pipettes. The pipettes used in this particular study were old and used for a long time. Therefore, the accuracy of the pipettes may have been off from the actual values needed for YAN sample mixtures. Another source of error that may have contributed to the YAN results is the UV-spectrometer. The spectrometer was an old and used piece of equipment that was given to WPI from a company.

IV. Conclusion and Recommendations

Overall, the samples of juice that were tested were concluded to be nutrient deficient because they did not have YAN levels within the 150-200 mg/L range. The lack of nitrogen in the juice may have also caused a sulfur smell and after taste in Ricker Hill's Mainiac Gold. In addition to performing YAN tests to determine the YAN levels of the cider, an analysis on apple variety and YAN level at different fermentation preparation stages was performed. Nothing was concluded after the analyzing YAN levels for McIntosh and Cortland apples since both YANs

were very similar to each other. However, for fermentation preparation stages, nitrogen levels increased as nutrients were added to the cider.

i. YAN Testing at Ricker Hill

As a result of the YAN tests performed on Ricker Hill's cider, it is recommended to perform YAN tests prior to fermentation. None of Ricker Hill's samples accomplished the target range of 150-200 mg/L in YAN level. By performing YAN tests, the cider maker can measure how much nutrients to add into the cider in order to achieve a healthier fermentation. YAN tests at different stages proved that the addition of nutrients increased YAN values. Table 15 from the Yeast Assimilable Nitrogen background section shows the different nutrients that can be added to accomplish a desired YAN level. From this table, the dosage of nutrient is determined in order to increase the yeast by a certain amount. For example, to increase YAN by 10 mg/L, 2 lbs. of Fermaid-O can be added per 1000 gal of cider. Increasing the yeast assimilable nitrogen will also help eliminate sulfur production during cider fermentation. As a result, this will enhance the aroma and flavor of the cider product.

ii. Apple Variety Study

There was no conclusion drawn on YAN levels and apple variety in this study. However, the apple variety study in Brazil was able to observe the trend with apple varieties and YAN levels. It would be interesting to further this study with a wider variety of apples. With successful results, this data may help Ricker Hill with choosing the right apples for the healthiest cider fermentation.

iii. Financial Analysis

A financial analysis was also done on investing in YAN testing equipment. Table 19 shows the average cost for each piece of equipment necessary for 30 tests, 90 tests, and 120 tests. At

30 tests, the cost per test is $173. 47. This is greater than the average cost of $55 for sending a juice sample to a third party. However, as the number of YAN test increases, the cost per test decreases. At 120 tests, the average cost per test is $48.47, which is less than the average cost of having a YAN test from a third party.

	30 Tests	90 Tests	120 Tests
YAN Test Kit	$144.00	$432.00	$576.00
Filters	$60.00	$180.00	$240.00
Spectrometer	$5,000.00	$5,000.00	$5,000.00
Total	$5,204.00	$5,612.00	$5,816.00
Cost Per Test	$173.47	$62.36	$48.47

Table 19: Average cost for YAN test materials

Another financial analysis was completed on sending samples out to third parties for YAN laboratory testing. Table 20 shows 4 vendors and the price for YAN testing. Texas Wine Lab's YAN test was the most expensive followed by Virginia Tech, Vinmetrica, and Vintessentials. The average YAN test cost was $55.00 per test. Throughout time, more and more YAN tests are necessary. Therefore, the cost for 30, 90, and 120 tests were analyzed. For YAN testing through a third party, the costs increases, as there are more tests.

Companies	Price/Test	30 Tests	90 Tests	120 Tests
Vinmetrica	$50.00	$1,500.00	$4,500.00	$6,000.00
Vintessentials	$45.00	$1,350.00	$4,050.00	$5,400.00
Virginia Tech	$60.00	$1,800.00	$5,400.00	$7,200.00
Texas Wine Lab	$65.00	$1,950.00	$5,850.00	$7,800.00
Avg	$55.00	$1,650.00	$4,950.00	$6,600.00

Table 20: Third party YAN test costs

As a result of the financial analyses, it is recommended to invest in YAN testing equipment. After 120 YAN tests, investing in YAN testing lab will save the company $6.53 per test which equates to $784 total for 120 tests. For testing in house, as the number of tests increased, the cost per test decreased. Therefore, throughout time, the company would save more by purchasing equipment and materials for YAN testing. In addition, having the materials necessary to perform YAN in house will allow testing to be done immediately. Shipping samples to vendors for YAN testing will take time depending on the vendor location. In this case, investing in YAN testing equipment will save the company time and money in the long run.

Crossflow Filtration

I. Background

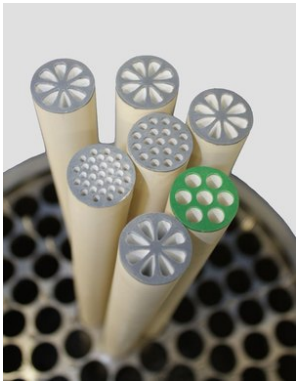

Figure 9: Ceramic Membranes for Crossflow Filtration

Crossflow filtration was first developed for wine clarification but the high quality performance of filtration made other industries, like hard cider production, begin to use it as well.[33] Crossflow filtration uses membranes that are either hollow-fiber, tubular, plate-and-frame or spiral. A number of parallel cylindrical ceramic membranes that are similar to the ones Ricker Hill Orchards uses are shown in Figure 9.[34] The membranes are barriers that allow separation of particles in the fluid by means of pores. Pore size can range from nanometers to micrometers. The Ricker Hill crossflow filter uses a membrane with a pore size of 0.2 μm. If pore size is extremely small, a specific fluid is required to drive the liquid through the pores.[35] This system of filtration uses tangential flow filtration, in which an incoming feed stream passes across the surface of a membrane at high velocities; this decreases solid build-up instead of using a dead-end filter operation. Dead-end filter operations filter by allowing the feed to flow directly perpendicular to the membrane's surface. The difference between crossflow and dead-end filters is demonstrated in Figure 10.[36] Specifically, this figure shows that the dead-end filter's feed flows parallel to the membrane surface while the crossflow's feed flows perpendicular. After the flow goes through the membrane, two exiting streams are generated. One of the streams, the

[33] (Royer, Jones and Howard)
[34] (Novasep)
[35] (Hammel and Young)
[36] (KOCH Membrane Systems)

permeate stream, is the filtered product. The second stream, the outlet retentate stream removes larger particles that cannot go through the membrane, and it also recycles back into the filtration system to further filtrate before exiting as waste.[37] Figure 11, shows the process of the whole crossflow filtration system being used currently at Ricker Hill Orchards.

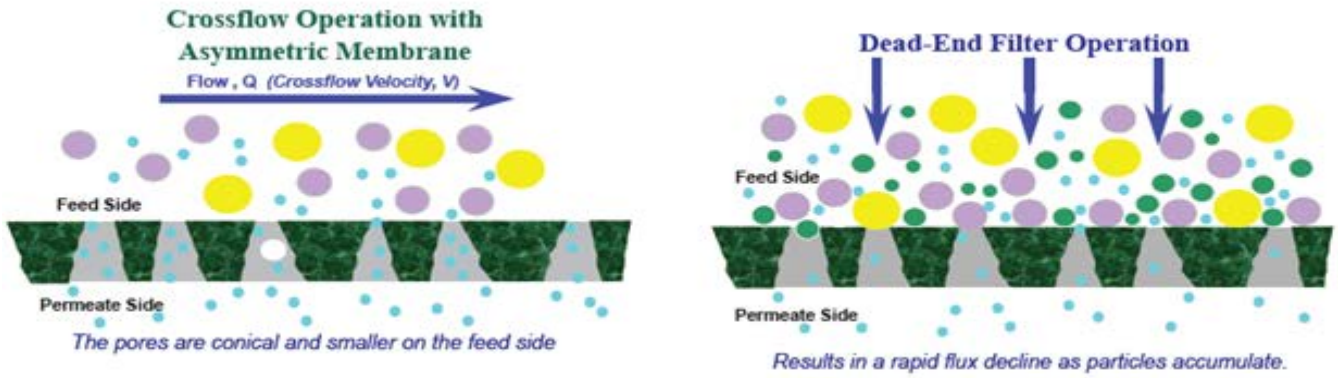

Figure 10: Crossflow vs Dead-end Filtration

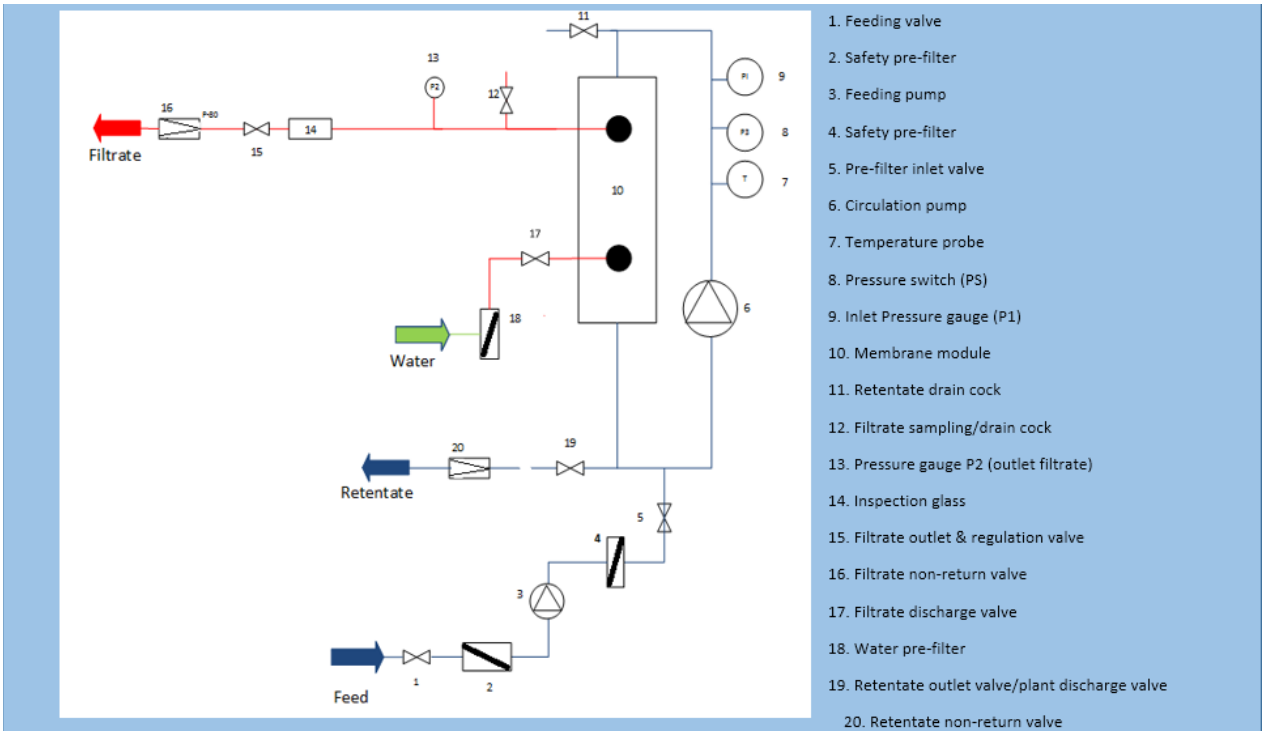

Figure 11: Ricker Hill Orchards' Crossflow Filtration System

[37] (Pall Corporation)

Crossflow filtration is becoming very popular to use because it has many advantages that other filtration systems do not have. The concept behind crossflow filtration is "Set and Forget" because the system is automated and does not require an operator during the entire filtration cycle.[38] Once the system is turned on, the crossflow can run on its own until the filtration is completed. The crossflow virtually cleans itself; all that is needed is the connection of water to the feed as well as adding NaOH (2.0 kgs powder NaOH) inside the buffer tank.[39] The wheels on the crossflow system, like most systems, enable it to be easily moved to different vessels and tanks that are out of reach.

Another benefit of crossflow filtration is the use of tangential flow filtration, as mentioned earlier. Tangential flow filtration produces a constant flow rate and minimizes the solid build-up on the surface of the membranes. This reduces the need to clean or replace the membranes, making it more affordable to maintain than other filtration systems.[40]

A disadvantage of crossflow filtration is that in order to optimize the filtration system, much research must be done on the membrane to ensure that the correct membrane is used for the intended purpose.[41] If the correct membrane is not used for the specific task, the filtration time and clarity will vary. Another disadvantage is fouling of the membrane, which causes the system to stop the production line. When this occurs, cleaning must be done in order for the filter to work properly again.

[38] (Royer, Jones and Howard)
[39] (Winetech)
[40] (Hammel and Young)
[41] (Hammel and Young)

II. Methodology

The goal of this project was to optimize the crossflow filtration system that Ricker Hill Orchards is currently using. Currently, the time required for the crossflow filter to complete a batch of hard cider is varying. A pressure drop versus flux analysis will show the time required for each filtration run. This pressure drop versus flux analysis can be determined by collecting current data from Ricker Hill's crossflow filter. An ASPEN simulation will show the optimal inlet pressure and flowrate for the crossflow filter.

i. Pressure Drop versus Flow Analysis

In order to determine when the filtration system was starting to not perform effectively data was collected to pin point at what time the filtration cycle started to decline in performance. The crossflow was fed with water to see what affects the filter had over several runs of filtration and then runs were done with hard cider. The methods for this procedure are in Appendix L.

ii. ASPEN simulation

With the data collected from previous filtrations completed at Ricker Hill, a simulation on ASPEN PLUS V8.2 was completed. Figure 12 shows the ASPEN simulation of Ricker Hill's crossflow filter.

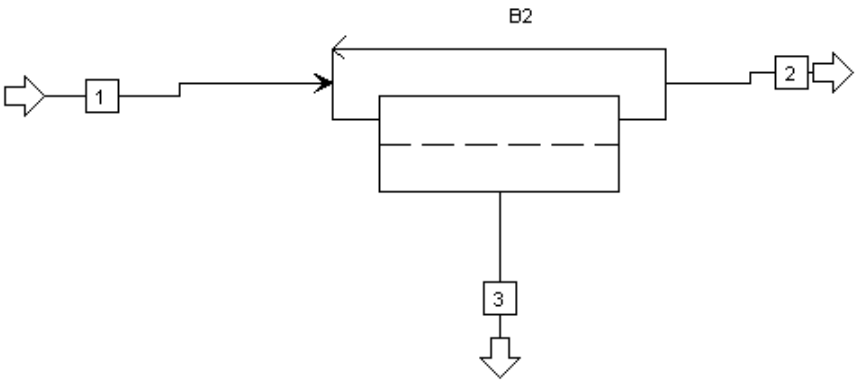

Figure 12: ASPEN crossflow filtration simulation

Feed and specifications of the Ricker Hill crossflow filter was entered into ASPEN before starting to manipulate the variables to give the best optimization of the crossflow filtration system. The pressures and flux collected from the crossflow filter were used as guidelines in ASPEN, as shown in Appendix M. Pressures and flowrates were manipulated to find the most efficient filtration. A trial and error method was used in order to determine the impact of one variable on the flowrate and time of filtration.

III. Results & Discussion

i. Pressure Drop versus Flux Analysis

Due to lack of time, a pressure drop versus flux analysis could not be performed on the Ricker Hill Cross flow filtration system. Not enough data was collected to establish a trend for the pressure drop and flux of the crossflow. If enough data was collected, the trend would show the time that the filtration system began to decline in performance. This experiment should be performed in the future so that Ricker Hill can identify the approximate time the crossflow needs to be cleaned, to ensure efficient filtration cycles.

ii. ASPEN Evaluation

An increase in inlet pressure and flowrate resulted in better filtration. Improved filtration was likely due to a greater pressure drop, which caused minimal fouling in the membrane. A flowrate of 970 L/hr gave the greatest allowable inlet pressure without exceeding equipment restrictions, which were 1.2 – 2 bar. Table 21 gives a summary of the flowrate and pressure that the crossflow filter can efficiently run. All ASPEN input files be found in Appendix N.

	Flow Rate (L/hr)	**Inlet Pressure (bar)**	**Filtration %**
1	370	1.30	90
2	470	1.37	93.5
3	570	1.53	94.6
4	970	1.76	98

Table 21: Most efficient flowrates and pressure drops for crossflow filter

IV. Conclusion and Recommendations

Running the filter at 1.76 bar for the inlet stream pressure and 970 L/hr for the flowrate will optimize the crossflow filtration system. This will ensure that the filter is performing consistently. The new batch data sheet should be used in order to maintain consistency in each batch. This will make it easier to analyze the data and improve organization.

Another recommendation is to collect pressure drop and flux data for the hard cider juice to further optimize the crossflow filter. This will show how the pressure and cleaning time vary with different blends of hard cider and if alterations need to be done. Knowing how long each batch takes to filter will improve Ricker Hill's organization.

Overall Conclusion

As a new brewery, Ricker Hill is encountering many challenges. The purpose of this project was to improve the brewery's efficiency and product quality. A big problem for Ricker Hill during hard cider production is the organisms involved with fermentation. After conducting YAN tests, it was concluded that YAN levels for the cider samples were low, meaning that the juices were nutrient deficient. When the cider lacks nutrients for the yeast, it produces sulfur byproduct. From the flavor compounds analysis in the cider, it was confirmed that this sulfur byproduct existed in high levels. Increasing the nutrient levels in the juice will improve fermentation and prevent sulfur byproduct production, thus enhancing the aroma and taste of Ricker Hill's product. Ricker Hill also wants to understand the activity of bacteria during hard cider and vinegar production. The first step in furthering this study was to identify the species of bacteria involved which were determined to be all in the *Acetobacter* genus. Finally, with an increase in demand Ricker Hill must increase the efficiency of their production. Ricker Hill's crossflow filtration unit varies in performance due to cider type. An Aspen simulation was done to mimic Ricker Hill's crossflow filtration. The results of this simulation gave the optimal time and pressure specification for the crossflow filter.

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Appendix

A. Acid Washed Glass Beads

1. Weigh 50 g of 0.5 mm glass beads into a 100 ml-orange cap Pyrex bottle. The volume of glass beads should be no more than 1/5 of the bottle used for washes.
2. Add 5.8 M HCl to cover class beads. Alternatively, use concentrated nitric acid. To prepare 5.8 M HCl, add 1 volume of concentrated HCl to 1 volume of dH_2O with moderate stirring. Dilute in a fume hood.
3. Incubate at room temperature for 1 hour.
4. Carefully pour 5.8 M HCl down drain. Some HCl will remain with beads. Rinse HCl down drain with tap water.
5. Add dH_2O to the 80 ml mark on the 100 ml bottle. Swirl bottle for 10 seconds to stir up beads. The dH_2O wash volume should be at least 5X the volume of the beads in the bottle.
6. Pour off the dH_2O wash in the sink. Rinse wash volume down the drain with tap water.
7. Repeat steps 5 and 6 for a total of 10 washes to reduce the HCl concentration below 10 mM.
8. Autoclave beads for 20 minutes.
9. Dry beads in oven at 50˚C overnight.
10. Store beads at room temperature. Spoon out beads with sterile “dipper” made with tip cut from 1.5 ml tube glued to end of plastic transfer pipette. Use gloves that have been washed and dried.

B. PCR Protocol

1. Centrifuge Eppendorf tubes with the DNA samples to get glass beads on the bottom.
2. Transfer 1 µl from DNA sample tube to a PCR specific Eppendorf tube. Make sure to take from the top of the sample tube to limit the amount of glass beads taken up.
3. To each PCR tube, add 10 µl of Promega GoTaq® Green Master Mix.
4. Add 2 µl of primers, 1 µl each of forward and reverse, to each PCR tube. Use primers 27F and 1492R together, or 8F and 1391R together.
5. Add 7 µl of ultra-pure water, bringing the volume of each tube to 20 µl.
6. Place PCR tubes in PCR machine.
7. Run PCR machine based of the following protocol:
8. Initial denaturation step for 3 min at 94˚C.
9. 30 additional cycles, each consisting of a denaturation for 1 min at 94˚C; an annealing for 30 s at 55˚C; and an extension for 2 min at 72˚C.
10. One-step extension for 7 min at 72˚C.
11. Store products at 4˚C.

C. Gel Electrophoresis

1. Mix 0.45 g of agarose and 50 ml of 1X TAE together in an Erlenmeyer flask.
2. Swirl flask until agarose powder dissolves.
3. Microwave flask on high for 1 min.
4. Add 3 µl of ethidium bromide to flask.
5. Pour contents of flask into gel system.
6. Place lane template in gel.

7. Allow gel to fully cool.

8. Remove lane template and add 1X TAE until liquid covers gel.

9. Add 10 µl of each PCR product in separate lanes.

10. For the ladder, add 9 µl of water and 1 µl of 100 bp ladder to a lane.

11. Run gel at 80 V for approximately 45 min.

12. When finished, analyze gel under UV light to confirm PCR product.

D. Full Sequencing Results:

<u>27F Primer</u>

Vin-1A:
NNNGTNCTATTACACATGCTAGTCGTACAGGTATACCACCTTAGTGGGGACGGGGAGTAACGCGA
GGAATCTATCCATGGGTGGGGGATAACACTGGGAAACTGGTGCTAATACCGCATGACACCTGAGG
GTCAAAGGCGCAAGTCGCCTGTGGAGGAGCCTGCGTTTGATTAGCTAGTTGGTGGGATAAAGGCCT
ACCAAGGCGATGATCAATAGCTGGTTTGAGAGGATGATCAGCCACACTGGGACTGAGACACGGCC
CAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGGGCAACCCTGATCCAGCAATG
CCGCGTGTGTGAAGAAGGTCTTCGGATTGTAAAGCACTTTCGACGGGGACGATGATGACGGTACCC
GTAGAAGAAGCCCCGGCTAACTTCGTGCCAGCAGCCGCGGTAATACGAAGGGGGCTAGCGTTGCT
CGGAATGACTGGGCGTAAAGGGCGTGTAGGCGGTTTGTACAGTCAGATGTGAAATCCCCGGGCTT
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ATGGCCCCGGTTGGCTGTATATCGCGAG

Blb-1C:
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GGGAACGTCACCCAATATGTTTTAATTTGGATATTTTTTAGCCCCCAGTTTTTTTCTGAATATATTCTA
TTATACTGCATAAAATATGCACGGAATTGCCATGATACGGACGCCTAAATGG

Blb-1D:
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GCCTCATAATATTACGTAGGGAATTTCCTTGTTGCCTTGGCGGAGGAATTCTTTACCT

Blb-1E:
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Blb-1F:
NNNCATCTATTCGTCTATATCTCCACAGCGAGTGTAGAGAAAAAAAATTGAGAAAACTTATTCAGAG
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AAGCACCCAAAAAAAGAGATATTTTTTTTATTGTTTTTTTTTCGTCATGATTTATATACGCCTTTTATTA
TTGTGATGTTCCTGTTTGGTGATTCTTACCATTTA

<u>8F Primer:</u>

Vin-1A:
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ACGCGTAGGTATCTATCCATGGGTGGGGGATAACACTGGGAAACTGGTGCTAATACCGCATGACAC
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ACTCTGGCAACCTCGGAGGTGTNCATNANAGGGTTGGGGA

Blb-1C:
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Blb-1D:
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Blb-1E:
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Blb-1F:
GGGGNNNNNNNNNNNANNANNNNANNATACGGNATAATTTCAATAGACCTCAGGAGCAGTTAG
GGGGGGGGAAAGAGACTCCCCCCCCTTAGGAAGGGGTTTGTCCCCAAAGAGGCTTCCCCTGTACTT
CAAATGTAAACTTGAATGCCCCACTGTTTCTTTTTTATATTTTATTTGTCCTCATCCACATTCACCTCAT
TTTCATAGAATCCCAAATG

<u>1492R Primer:</u>

Vin-1A:

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GAGTTAGGGGGGGCAACAACTAACGGCCCACGACCATAGGGATAGGGGGCGCCCTCCAGAGGGG
CCTCCCAATGTGTGCCAATTGTCGTACTTGAATGCCCCACTGATTGTTGGTTAATATTTTTCCGCGTC
GATGCAACTTGCCACATGCTTTTCATAAAAGTATGCCAAATA

Blb-1C:
NATGCACGCACTTCGATGTTTGTGCGTATACGGGTATTGTCGTACTAGTACCATACGACGAGGCTAC
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AGATAGACGCTCGGCTGTCTCGGTGGAAGAATGAGGGGGCAGGGAACAGGTGGGCGGGCTGGGA
CAGCAGGCGACCAGGAGATGGTATACTTCTTGTGAGGTGGTCTTAGGCTTCGGCATATTTATTCCGC
CGTATTTTTGTCTTGCGTGCATGTGGTGCACCGGAATGGAAAGCGTGATGGTGCGGAGATGTCCGG
CGACAGGTTGTTTTCTTCATAG

Blb-1D:
NNNCATGGTTCGGTACAGCCTCCGTCTATGATGTATACTACGTAGTCATATAGGCAACAGCTCGAG
GGGGACGGGCGGGGTGTACAAGGCCGGGACGTATTCACGGCGGCATCTGATCCGCGATAACTAGC
GATTCCACCTTCGTGCACTCGAGGAGCAGAGTGCTATCCGCCTGAGACGACTTTTTGAGATCAGCAC
GATGTCGCCATCTAGCTTCCATTGTCATCGCCATTGTAGCACGGTGTAGCCCAGGACATAAGGGCCA
TGAGGACTTGACGTCATCCCCACCTGCCTCCGGCTTGTCACCGGCAGTCTCTCTAGAGTGCCCACCC
AAACATGCTGACAACTAAGGATAGGGTTGCGCTCGTTGCGGACTTAACCAACATCTCACGACGCGA
GGTGAGGACACCCATGGGCACCTGGGCGGTAGTGCCGTGCGGGAAATGCGCTTCTCTGGAACGGA
CTACCATAAAGCCTGGGAGGGTTCTGGGCGTGGGTCGAATAAAGCACGTGTTAACCGGTTTGCGGC
GGGGTCATGTCTTTTGAGTTTCAATTTGGGGCCGGACTCGCAGGTGAGGGGGTATGGGGGTCGTCC
GAACTGAGGAACAGGGTACCAAAAACCCAGCACACAGCGGTTAAGCGAGGACTTACCAGGGGATC
TAATTCTTTGTTCTCCCCACGGTTTCGCGCGTCAGCTCAGGATTAACCAAGTGGCCGCTTGCCCCCCC
GGGTGCCTTCCAAAAATTGAAAAAT

Blb-1E:
AAGATTGGTGGTGGTTCTTCTTGTTCCGTATGGGAAATTGTCCTCATGATTCCCTGTGCTGTCGCGG
GGGGAAAAACGGTGGCTAATTAAGATCAGAGGCTAGAGAAGTGAAGACCACTCACTATAGGCTCG
CCTGAGCACTTCTCTGCATCCCTAGTTGTTCTCCCCTTCTCTTCCACCACGTATAACTTGCAACAGCAA
TTGATCTGTCCCATCTGCCTAGATATGTCACGAAGCCTGTGCCCCCTCTCTCTTGTCAGAACTGGCCG
TGCTGGAACTGTGCCAGCGACGTTTTCCCTGCCTCTAACCCGTCCTTACCCACAAAAGGAGGCACCG
CCGCGGCATCAGGGTGGCGGTTTTGCTGTCCCCCCTGAAGACATGACTTGGTGGTCTACAGACAAG
GCGGCTATGGTCTGCCAAGGAGCCTGCCCGTGGCACATTTTATTGATATCTACTATTATGATTCCACC
ATAACAGAAACAAAATCGCACCATCCCATGTGTGATTAGATGAATGGAACGCATCAACACTTCTGTC
TGAACTGCGAACCGCCTCCTGCTCCTCGTGCACGGCTTTTGGCGCTCATGAGGGTGCTGGCGGCGC
ACTGAATTGGTGATCCGAGATAGAAGTCTCAGCTCATTGATGGCATTCCAGTATTGTGG

Blb-1F:

TNNNGCCTCTGTGTCAGCTACTCTATCTCGAGTCATAGATTCTCAGAGCATATGAGCCATGATCGTC
CTACTACCTTCTCGCACAATAATTAGAAAGAGGGATCTGAGCCATGATCAAACTCTAGGGGTTGGG
GTGGGATTTATGCGTTGTTGTCTGCAGCGGCCCGAGTTGCTGACTAGAATCGGATGAGTATGACTT
GGAAAGGATATTGTCATGACCCGTCTGCCCTCGATTAGGCGAAGCCGGGGGCAATGTCTCTGGATA
CAACCAACAGCGCCGGAACTGTGCCGCCGATTGCCCCCGGGCCTAAAGCTCGTCATTACCAGTCTGA
GGAGGCACCCCCGCGGCATCAGGGGGACGGTTTGAATGTGGTTCTGGAAATGATGAGGGGGAGG
GAAACGGACTGGGCGGCAGTGGAGCGGAAAGGAGCGAGGCCTTGGAAAATTTCACTGAAAGCTAC
CCTTCTGTTGCTTTCGGGATTTAAACAATCTTGGCCTCTGCCAGTGTGCCGAGTGGAAGAGGATTGC
TCACCACCTTAGCCCTGCTGGGCAGCGCCTAATTCTTCTCGAGAAAAGTTTTGGGCGGATGGGAAGC
GGGGGTGACTGGAGGGGGCCGGAGGCGGAAGCGACCTCGCGTGTCTACTGTATTGCGCAATGACG
TACAAATTTTTGTTAACCCCACCTGTTTAC

1391R Primer:

Vin-1A:
CTCATCACGTAATGCTGATCAGCAATAACTACTAGATACCACCTTCATGCACTCGAGTTGCAGAGTG
CAATCCGAACTGAGACGGCTTTTAGAGATCAGCATGGTGTCACCACCTAGCTTCCCACTGTCACCGC
CATTGTAGCACGTGTGTAGCCCAGGACATAAGGGCCATGAGGACTTGACGTCATCCCCACCTTCCTC
CGGCTTGTCACCGGCAGTCTCTCTAGAGTGCCCAGCCCAACCTGATGGCAACTAAAGATAGGGGTT
GCGCTCGTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAGCCATGCAGCACCTGT
GTTAGAGGTCCCTTGCGGGAAACAAACATCTCTGCTTGCAGCCTCTACATTCAAGCCCTGGTAAGGT
TCTGCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCCTTTGAG
TTTCAACCTTGCGGCCGTACTCCCCAGGCGGTGTGCTTAACGCGTTAACTGCGACACTGAATGACTA
AGTCACCCAACATCTAGCACACATCGTTTACAGCGTGGACTACCAGGGTATCTAATCCAGTTTGCTCC
CCACGCTTTCGCGCCTCAGCGTCAGTAATGAGCCAGGTTGCCGCCTTCGCCACCGGTGTTCTTCCCA
ATATCTACGAATTTCACCTCTACACTGGGAATTCCACAACCCTCTCTCACACTCTAGTCTGCACGTATC
AAATGCAGCTCCCAGTTAAGCCCGGGGATTTCACATCTGACTGTACAAACCGCTACACGCCCTTTAC
GCCAGTCATTCGGAGCAACGCTAGCCCCCTCGTATTACGCNGCTGCTGCACGAAGTAGCGGGCTTCT
TCTACGGGTACGTCATCATCGTCNTCGAGTGCTTACATCGAGACCTTCTCCACAACCCGCATGCTGAT
CAGTGCCCAATGGCAATCCCACTGCTGCTCCGTGAGNTNNCGTTTCAAGTCCAGTGGCGTATCTTCT
TCCAACACGATGAANNNNNTGAAGCTAACNCNATGCATACGGTCCTCCAGGACTTGCCTGACT

Blb-1C:
NNNCATTGCTTCTTCGTATGATTATACTCCTACAATTATGCGCTAAAGGACATAATTACATTCACTCA
CCTTCAGCACGACGGAGCTTGGGAGTATTACCTGACCTCCCGGCCAAGGTTATACTTGCTGGCTCCG
TCAGTGTAGCGCGCGTGCGGCCCAGAACGTCTAAGGGCATCACCGACCTGTTGTTGCCTCAAACTTC
CATCTGTTTGATACCGATAGTCCCTCTTAGAAGAGCAATCCAGCAGGCGCTAGCTGCTTTATTTAGGT
GGTTAAGGGGGCGTCCGTTAAAGCAAATAAGCAAACTTTTTTTGGGAAAGAAAAGGGGGCGGGTA
ACCCCCCCCAAAAAAAAAA

Blb-1D:
TNNATCTTCGGTCTCTCTATCTGCGATACTAGCGATTCCACCTTCATGCACTCGAGTTGCAGAGTGCA
AACCGAACTGAGACGACTTTTTGAGATCAGCACGATGTCGCCATCTAGCTTCCCATTGTCATCGCCAT
TGTAGCACGTGTGTAGCCCAGGACATAAGGGCCATGAGGACTTGACGTCATCCCCACCTTCCTCCGG

CTTGTCACCGGCAGTCTCTCTAGAGTGCCCACCCAAACATGCTGGCAACTAAAGATAGGGGTTGCGC
TCGTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAGCCATGCAGCACCTGTGCGG
TAGGTCCCTTGCGGGAAATGCCCATCTCTGGACACAGCCTACCCATACAAGCCCTGGTAAGGTTCTG
CGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTC
AACCTTGCGGCCGTACTCCCCAGGCGGTGTGCTTATCGCGTTAGCTACGACACTGAGTAACTAAGTT
ACCCAACATCCAGCACACATCGTTTACAGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCAC
GCTTTCGCGCCTTAGCGTCAGTAATGAGCCAGGTTGCCGCCTTCGCCACCGGTGTTCTTCCCAATATC
TACGAATTTCACCTCTACACTGGGAATTCCACAACCCTCTCTCACACTCTAGTCTGCACGTATTAAAT
GCAGCTCCCAGGTTAAGCCCGGGGATTTCACATCTAACTGTACAAACCGCCTACACGCCCTTTACGC
CCAGTCATTCCGAGCAACGCTAGCCCCCTTCGTATTACCGCGGCTGCTGGCACGAAGTTAGCCGGG
GCTTCTTCTGCGGGTACCGTCATCATCGTCCCCGCCGAAAGTGCTTTACAATCCGAAAACCTTCTTCA
CACACGCGGCATTGCTGGATCAGGGTTGCCCCCATTGTCCAATATTCCCCACTGCTGCCTCCCGTAG
GAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCTGATCATCCTTCTCAGACCAAGCTATGATCATCGC
CTTTGGTAGGGCCATTACCCCCACCAACAGCTAATCAAACGCAGGCCTCCTTCCACAGGCGACTTGC
NCTTTGACCCTCAGGTATTCATGCGTATAGCTCAGTTTCCGGGAAGTTATCCCCAACNATGATAGAT
TCTTACGCGTTACTTCACCCGNCCGGCCAACATAAGGNCCGCGAAAGAG

Blb-1E:
TNNCNATCGTAGATTGATGATCCGCGATTACTAGCAGATTACCACCTTCATGCCTCGAGTTGCAGAG
TGAAATCCGAACTGAGACGGCTTTTAGAGATCAGCATGGTGTCACCACCTAGCTTCCCACTGTCACC
GCCATTGTAGCACGTGTGTAGCCCAGGACATAAGGGCCATGAGGACTTGACGTCATCCCCACCTTCC
TCCGGCTTGTCACCGGCAGTCTCTCTAGAGTGCCCAGCCCAACCTGATGGCAACTAAAGATAGGGG
TTGCGCTCGTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAGCCATGCAGCACCTG
TGTTAGAGGTCCCTTGCGGGAAACAAACATCTCTGCTTGCAGCCTCTACATTCAAGCCCTGGTAAGG
TTCTGCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCCTTTGA
GTTTCAACCTTGCGGCCGTACTCCCCAGGCGGTGTGCTTAACGCGTTAACTGCGACACTGAATGACT
AAGTCACCCAACATCTAGCACACATCGTTTACAGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTC
CCCACGCTTTCGCGCCTCAGCGTCAGTAATGAGCCAGGTTGCCGCCTTCGCCACCGGTGTTCTTCCCA
ATATCTACGAATTTCACCTCTACACTGGGAATTCCACAACCCTCTCTCACACTCTAGTCTGCACGTATC
AAATGCAGCTCCCAGGTTAAGCCCGGGGATTTCACATCTGACTGTACAAACCGCCTACACGCCCTTT
ACGCCCAGTCATTCCGAGCAACGCTAGCCCCCTTCGTATTACCGCGGCTGCTGGCACGAAGTTAGCC
GGGGCTTCTTCTACGGGTACCGTCATCATCGTCCCCGTCGAAAGTGCTTTACAATCCGAAGACCTTCT
TCACACACGCGGCATTGCTGGATCAGGGTTGCCCCCATTGTCCAATATTCCCCCACTGCTGCCTCCCG
TAGAGTCTGGGCCGGTGTTCTCAGTCCAGTGTGCTGATCATCCTCTCAAGCAGCTATGATCATCGCTT
GGTAGGCNTTACCCAACAACTAGCTATCAANNCAAGCTCCTCACAGCGACCTGCGCCNTGGACCTC
AGGGTCATGCCGTAATAGCACAGTTCANNNTATCCCANCATGATGATCCCTACGCCGTAACCTGA

Blb-1F:
CNTNNNGNNANNACTGCGGCTTTTGTATAGAGAGAAAAGGGGGAAAAAGGATACCCTTCAGGGG
GAACTAGAAGGGGAACCCCACATAGACTTAGGAAAGGGGCAACACAAGAAAAGGAACAGATAGA
GACAATGGGGACACTGGAAGGCCCAGGATAAAGGCCCGAAAGATTTCATCGGACCCCCCCGCCAC
AGGATTGGACGACATCACGCTAAGATAGCAGCGTGCCTGGGCCTGCTGTTTATATTAGGAGATAGC
GGTTTGGGAAGTGTGCTAGCGATTTTGGACAGGGTTTAAGGAAGTATAACCAGAAGCAGGAAAAA
GA

E. Cider and Juice Flavor Compounds Procedure:

The sampler injected 1 μL of analyte in split mode with the injection port at 230 ˚C. The total flow was set at 9.5 mL/min and the carrier gas was set at 1.0 mL/min. The oven temperature was set at 40 ˚C, held at 40 ˚C for 3 minutes, ramped at 12 ˚C/min until 250 ˚C, and held at 250 degrees for 3 minutes. The detection window started at 3 minutes and ended at 21 minutes.

The prepared internal standards were made with 50 $\frac{\mu g}{mL}$ of 4 hydroxy-pentan-2-one, 2-octanol, and 4-methyl-2-pentanol. Calibration curves were made by recording the peak heights of the internal standards in ethanol. Concentrations of 100, 75, 67.5, 50, 37.5, 25, and 12.5 $\frac{\mu g}{mL}$ of standard were injected and the heights were recorded.

F. Mass Spectrometer Data Recorded for Mainiac Gold Batches 25, 26, and 27

Compound	**Relative Peak Height**		
	Batch 25	**Batch 26**	**Batch 27**
1-Butanol	297735	225102	415447
Acetoin	176821	ND	20626
1-Butanol, 3-methyl-	46106248	27755754	27974916
Sulfuric acid dibutyl ester	ND	ND	4921633
2,3-Butanediol, [R-(R*,R*)]-	683195	291229	282323
2,3-Butanediol, [R-(R*,R*)]-	142037	108977	121313
Butanoic acid, ethyl ester	72833	32376	269383
Propanoic acid, 2-hydroxy-, ethyl ester, (S)- (Ethyl Lactate)	21603774	1432021	433718
Butanoic acid, 3-methyl-	172899	ND	146180
Pentanoic acid	ND	105107	ND
Butanoic acid, 2-methyl-	135214	106131	128744
Butanoic acid, 4-hydroxy-	351318	68402	ND
1-Pentanol, 3-methyl-	71056	ND	ND
Formic acid, hexyl ester	1719164	679782	1144343
1-Butanol, 3-methyl-, acetate	98777	91903	288741
Butanoic acid, 3-hydroxy-, ethyl ester (Ethyl Lactate)	51536	ND	50944
Ethyl Acetate	ND	168652	111163

Butanoic acid, 2-hydroxy-3-methyl-, ethyl ester	54006	ND	ND
1-Propanol, 3-(methylthio)-	929505	713029	454837
Hexanoic acid	213922	352567	208806
(S)-(-)-1,2,4-Butanetriol, 4-acetate	339488	16021	ND
Thiophene, tetrahydro-2-methyl-	315264	52339	51741
1,3-Dioxol-2-one,4,5-dimethyl-	130071	ND	ND
Isoamyl lactate	273400	ND	ND
2-Isopropyl-5-methyl-1-heptanol	105646	ND	87218
Phenylethyl Alcohol	29602955	6493461	10236412
Butanedioic acid, diethyl ester	2006957	ND	ND
Ethyl hydrogen succinate	12470302	1912703	646197
Octanoic acid	ND	ND	1406775
Octanoic acid, ethyl ester	ND	120108	115144
Benzofuran, 2,3-dihydro-	254306	ND	68319
Sulfurous acid, 2-ethylhexyl undecyl ester	38767	ND	ND
Sulfurous acid, 2-ethylhexyl nonyl ester	32648	ND	ND
2-Allylphenol	228901	ND	ND
Benzene, 1,3-bis(1,1-dimethylethyl)-	355071	ND	ND
1,3-Octanediol	2625912	2043833	6116054
Malic Acid	ND	165653	322258
Benzeneacetic acid, hexyl ester	97504	ND	ND
Hexanedioic acid, monomethyl ester	113696	ND	ND
Glycerol 1,2-diacetate	65232	ND	ND
Benzaldehyde, 4-propyl-	ND	44278	ND
n-Decanoic acid	205143	46789	196468
Eugenol	161746	ND	ND
Benzeneethanol, 4-hydroxy-	1140082	1113192	1222296
Dodecanoic acid, 3-hydroxy-, ethyl ester	ND	ND	41021
1,3-Cyclohexadiene, 1,3,5,5,6,6-hexamethyl-	157488	ND	ND
Sulfurous acid, dodecyl hexyl ester	ND	ND	52728
Tryptophol	1036583	253328	164948
2-Methylresorcinol, diacetate	1584830	ND	ND
9-Octadecenamide, (Z)-	1988941	ND	1674999
p-Hydroxycinnamic acid, ethyl ester	252668	33305	158311
Octadecanamide	122623	ND	ND
7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	58256	102399	141017
l-(+)-Ascorbic acid 2,6-dihexadecanoate	203556	ND	222384

Dibutyl phthalate	369237	195375	220541
Hexadecanamide	458409	ND	ND
Benzaldehyde, 4-propyl-	ND	44278	ND
Phenol, 2-methoxy-3-(2-propenyl)-	ND	49592	ND
3-Hydroxy-.beta.-damascone	63894	74110	ND
n-Nonadecanol-1	ND	881269	ND
2,5-Diacetoxytoluene	153566	ND	ND
Nonadecanamide	ND	232825	ND
Hexadecanal	ND	110517	43568
Nonadecanenitrile	ND	97628	ND
4-Cyanobenzoic acid, undecyl ester	ND	ND	186308
Tyramine, N-formyl-	63960	ND	175154
Butanoic acid, cyclopentyl ester	ND	ND	40117

G. Mainiac Mac Changes of the Flavor Profile

Compound	**Relative Peak Height**	
	Man Mac Juice	**Man Mac Hard Cider**
1-Butanol, 3-methyl-	258415	ND
1-Butanol, 2-methyl- acetate	177524	ND
1-Butanol	ND	195005
Acetoin	ND	350548
1-Pentanol	ND	23865019
Hexanal	28084	ND
Acetic acid, butyl ester	85697	ND
2-Hexenal, (E)-	163299	ND
2-Hexen-1-ol, (E)-	56690	ND
Formic acid, hexyl ester (Hexyl Acetate)	261876	746773
2,3-Butanediol, [R-(R*,R*)]-	ND	398679
2,3-Butanediol	ND	109392
Phenylethyl Alcohol	6241440	6006622
Sorbic Acid	25924284	351132
Benzene, 1,3-bis(1,1-dimethylethyl)-	1056500	ND
4-Ketopimelic	19928	ND
1,3-Octanediol	589511	3893463
Benzaldehyde, 4-propyl-	82577	ND
Benzene, (1-butylhexadecyl)-	141733	ND
1-Dodecanol, 2-hexyl-	139482	185648
Dodecanoic acid, 1-methylethyl ester	32999	ND

(isoamyl laurate)		
1-Dodecanol, 2-hexyl-	150484	84322
n-Nonadecanol-1	1759546	2140295
Sulfurous acid, butyl decyl ester	246429	ND
9-Octadecenamide, (Z)-	3626593	1425581
7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	197811	ND
l-(+)-Ascorbic acid 2,6-dihexadecanoate	722856	886111
Hexadecanal	349306	377392
Nonadecanenitrile	174628	242479
Sulfurous acid, octadecyl 2-propyl ester	241586	ND
Glycerol 1-palmitate	492926	ND
Squalane	334038	ND
n-Tetracosanol-1	2066365	2923719
Dodecanoic acid	ND	61749
Octanoic acid	ND	917140
Octanoic acid, ethyl ester	ND	51159
Hexanoic acid	ND	327219
3-Hexenoic acid, (E)-	ND	83995
Butanoic acid	ND	55322
(S)-Isopropyl lactate	ND	111905
Bis(tridecyl) phthalate	ND	304774
1-Propanol, 3-(methylthio)-phen	ND	402625
2,5-Cyclohexadiene-1,4-dione, 2,6-bis(1,1-dimethylethyl)-	ND	100944
1-Butanol, 3-methyl-, acetate (isoamyl actetate)	ND	98664
2-(2-Methoxyethoxy)ethyl acetate	ND	25986
Ethyl Acetate	ND	200499
Thiophene, tetrahydro-2-methyl-	ND	75089
1,5-Hexanediol	ND	372770
Malonic acid, 2-ethylbutyl octyl ester	ND	38675
Malic Acid	ND	526148
Benzaldehyde, 4-propyl-	ND	102832
Oxalic acid, ethyl 2-phenylethyl ester	ND	306441
Oxalic acid, 2-phenylethyl tetradecyl ester	ND	370297
p-Hydroxycinnamic acid, ethyl ester	ND	148628
s-Triazine, 2-amino-4-(piperidinomethyl)-4-piperidino-	ND	293759

E-2-Tetradecen-1-ol	ND	273062
2-(1,1-Dimethylethyl)-5-oxohexanal	ND	227103

H. Cranberry-Golden Delicious juice and Cranberry-Mainiac Gold

Compound	Relative Peak Heights	
	Cran-MG Juice	Cran-MG Hard Cider
1-Butanol, 2-methyl-	73790	ND
Acetoin	776812	176821
1-Butanol	ND	297735
1-Butanol, 3-methyl-	ND	46106248
2,3-Butanediol, [R-(R*,R*)]-	ND	683195
2,3-Butanediol, [R-(R*,R*)]-	ND	142037
2-Hexenal, (E)-	153957	ND
Propanoic acid, 2-hydroxy-, ethyl ester, (S)- (Ethyl Lactate)	ND	21603774
Butanoic acid, ethyl ester	86717	72833
Butanoic acid, 3-methyl-	ND	172899
Butanoic acid, 4-hydroxy-	ND	351318
Butanoic acid, 3-hydroxy-, ethyl ester	ND	51536
Butanoic acid, 2-hydroxy-3-methyl-, ethyl ester	ND	54006
Formic acid, hexyl ester (hexyl acetate)	332524	1719164
1-Butanol, 3-methyl-, acetate (isoamyl acetate)	ND	264439
1-Butanol, 2-methyl-, acetate	70392	ND
(S)-(-)-1,2,4-Butanetriol, 4-acetate	ND	339488
Sorbic Acid	25324406	37729570
1-Propanol, 3-(methylthio)-	ND	929505
Hexanoic acid	ND	430534
4-Hexenoic acid	ND	94003
Benzoic acid	1985448	ND
Thiophene, tetrahydro-2-methyl-	ND	315264
1,3-Dioxol-2-one,4,5-dimethyl-	ND	130071
2-Isopropyl-5-methyl-1-heptanol	ND	30053
Phenylethyl Alcohol	ND	29602955
Isoamyl lactate	ND	273400
Thiophene, tetrahydro-2-methyl-	ND	315264
Butanedioic acid, diethyl ester	ND	2006957
Ethyl hydrogen succinate	ND	12470302
Benzene, 1,3-bis(1,1-dimethylethyl)-	1175231	355071
1,3-Octanediol	2188913	2625912
Benzofuran, 2,3-dihydro-	ND	254306

2-Allylphenol	ND	228901
Benzaldehyde, 4-propyl-	96055	ND
Benzeneacetic acid, hexyl ester	ND	97504
Hexanedioic acid, monomethyl ester	ND	113696
Sulfuric acid dibutyl ester	ND	5637635
1-Propanol, 2-methyl-	ND	84329
Acetic acid, hydrazino-, ethyl ester	ND	93615
Ethyl Acetate	ND	88689
Formic acid, 2-methylpentyl ester	ND	41421
Benzyl alcohol	ND	44945
1-Dodecanol, 3,7,11-trimethyl-	ND	364106
m-Toluic acid, phenyl ester	ND	388889
Phenol, 4-ethyl-	ND	179110
Dodecanoic acid, 1-methylethyl ester (isoamyl laurate)	18202	37137
Malic Acid	ND	269236
5-Isopropyl-3,3-dimethyl-2-methylene-2,3-dihydrofuran	ND	122883
2-Isopropyl-5-methyl-1-heptanol	ND	190009
1-Decanol, 2-hexyl-	ND	195297
1,4-Diacetyl-3-acetoxymethyl-2,5-methylene-l-rhamnitol	ND	85599
n-Decanoic acid	ND	196759
Benzene, [(methoxymethoxy)methyl]-	ND	57473
n-Nonadecanol-1	1842111	ND
9-Octadecenamide, (Z)-	1001088	683134
Tryptophol	ND	200119
11-Methyldodecanol	ND	118455
Hexadecanal	320694	ND
Squalane	266966	ND
n-Heptadecanol-1	402387	ND
Nonadecanenitrile	167493	ND
Sulfurous acid, pentadecyl 2-propyl ester	243495	ND
n-Tetracosanol-1	2123439	ND
n-Decanoic acid	ND	196759
Decanoic acid, ethyl ester	ND	15978
Benzeneethanol, 4-hydroxy-	ND	256049
Carbonic acid, ethyl cyclohexyl ester	ND	74971
Phenol, 2,4-bis(1,1-dimethylethyl)-	ND	177482
11-Methyldodecanol	ND	99661
1-Dodecanol, 2-hexyl-	ND	155158

Tyramine, N-formyl-	ND	70077
4-Methyl-2,5-dimethoxybenzaldehyde	ND	49179
Dodecanoic acid, 1-methylethyl ester (isoamyl laurate)	ND	37137
3-Hydroxy-.beta.-damascone	ND	64070
Sulfurous acid, octadecyl 2-propyl ester	ND	42139
1-Ethyl-7-methyl-4-oxo-1,4-dihydro[1,8]naphthyridine-3-carboxylic acid, methyl ester	ND	72145
Tryptophol	ND	200119
11-Methyldodecanol	ND	118455
p-Hydroxycinnamic acid, ethyl ester	ND	75870
Decanoic acid, 9-oxo-, methyl ester	ND	77278
l-(+)-Ascorbic acid 2,6-dihexadecanoate	ND	415698
Dibutyl phthalate	ND	304533
1-Dodecanol, 2-octyl-	ND	110124
Disulfide, di-tert-dodecyl	ND	241283
Ditetradecyl ether	ND	109055
Formamide, N-{4-[2-(1,1-dimethylethyl)-5-oxo-1,3-dioxolan-4-yl]butyl}	ND	147633
Tricyclo[4.3.1.1(2,5)]undec-3-en-10-one, 3-methyl-, (1.alpha.,2.beta.,5.beta.,6.alpha.)-	ND	242268
Octadecanamide	ND	405216

I. Ammonia Nitrogen (AN) Test Procedure

Materials:

- UV Spectrometer - HP Agilent 8453 UV-visible Spectroscopy System
- Yeast Assimilable Nitrogen Test Kit from Vintessentials (30 tests)
- Ammonia Test
- Primary Amino Acid Nitrogen
- 1 cm cuvette (quartz cell)
- Juice samples
- *Target 2 Cellulose Acetate Syringe Filter*
- 5-dram vials

Ammonia Test Kit

- Buffer
- Nicotinamide adenosine dinucleotide (NADH)
- Glutamate dehydrogenase (GIDH)
- Standard

Operation Parameters:

Samples for ammonia are tested under a wavelength of 340 nm in the UV spectrometer. The tests are conducted at room temperature (20-25C).

Safety Precautions:

Wear Safety goggles

Do not ingest buffer or standard (contains sodium azide as a stabilizer)

Procedure:

1. Filter cider juice with Target 2 Cellulose Acetate Syringe Filter
2. To prepare for absorbance 1 prototypes pipette the following volumes of reagents into the vials:

Reagent	**Blank**	**Standard**	**Juice Sample**
Buffer	1 mL (1000 µl)	1 mL (1000 µl)	1 mL (1000 µl)
NADH	0.10 mL (100 µl)	0.10 mL (100 µl)	0.10 mL (100 µl)
Distilled Water	2 mL (2000 µl)	1.90 mL (1900 µl)	1.90 mL (1900 µl)
Juice	None	None	0.10 mL (100 µl)
Standard	None	0.10 mL (100 µl)	None

3. Mix well and wait about 5 minutes
4. Pipette blank mixture into 1 cm cuvette and place into spectrometer
5. Set wavelength to 340 nm in spectrometer and click on “blank” in Chem Station software
6. The absorbance is not given when you click blank therefore click on “sample” to obtain the absorbance 1 value (A_1) of the blank sample
7. Place blank mixture back into its vial
8. Clean cuvette with Kim wipes and distilled water
9. Pipette standard mixture into cuvette and place into spectrometer

10. Click on “sample” and collect standard absorbance value (A_1)
11. Repeat with sample juice mixture
12. To prepare absorbance 2 samples, recreate the blank and standard prototypes from the table above (absorbance 1).
13. Add GIDH amounts (from table below) into appropriate recreated blank and standard vials– these are made in separate vials to be reused
14. Prepare juice sample model absorbance 2 by adding 0.02 mL of GIDH to the vial with mixture from absorbance 1

Reagent	Blank	Standard	Juice Sample
GIDH	0.02 mL (20 µl)	0.02 mL (20 µl)	0.02 mL (20 µl)

15. Mix well and wait for about 20 minutes before recording absorbance 2, A_2
16. Pipette mixtures into cuvette and place cuvette into uv spectrometer to record the absorbance, A_2

J. Primary Amino Acid Nitrogen (PAAN) Test Procedure:

Materials:

- UV Spectrometer - HP Agilent 8453 UV-visible Spectroscopy System
- Yeast Assimilable Nitrogen Test Kit from Vintessentials (30 tests)
- Ammonia Test
- Primary Amino Acid Nitrogen
- 1 cm cuvette (quartz cell)
- Juice samples
- *Target 2 Cellulose Acetate Syringe Filter*
- 5-dram vials

Primary Amino Acid Nitrogen

- Buffer
- N-acetyl-L-cysteine (NAC)
- Ortho- phthaldialdehyde (OPA)

Operating Parameters:

Samples for primary amino acid nitrogen are tested under a wavelength of 335 nm in the UV spectrometer. The tests are conducted at room temperature (20-25C).

Safety Precautions:

Wear safety glasses
Be careful handling the buffer because it is alkaline
Reagent 4, ethanol, is flammable
Do no ingest the standard (contains sodium azide as a stabilizer)

Procedure:

1. Pipette the following volumes of reagents into cuvettes:

Reagent	**Blank**	**Standard**	**Sample**
Buffer	2 mL (2000 µl)	2 mL (2000 µl)	2 mL (2000 µl)
NAC	0.90 mL (900 µl)	0.90 mL (900 µl)	0.90 mL (900 µl)
Distilled Water	0.05 mL (50 µl)	None	None
Juice Sample	None	None (50 µl)	0.05 mL (50 µl)
Standard	None	0.05 mL	None

2. Pipette blank mixture into 1 cm cuvette and place into spectrometer
3. Set wavelength to 335 nm in spectrometer and click on “blank” in Chem Station software
4. The absorbance is not given when you click blank therefore click on “sample” to obtain the absorbance 1 value (A_1) of the blank sample
5. Place blank mixture back into its vial
6. Clean cuvette with Kim wipes and distilled water
7. Pipette standard mixture into cuvette and place into spectrometer
8. Click on “sample” and collect standard absorbance value (A_1)

9. Repeat with sample juice mixture
10. Add bottle No.4 (Ethanol) to OPA and mix this to dissolve the solution
11. To prepare absorbance 2 samples, recreate the blank and standard prototypes from the table above (absorbance 1).
12. Add OPA amounts (from table below) into appropriate recreated blank and standard vials– these are made in separate vials to be reused

Reagent	**Blank**	**Standard**	**Sample**
OPA	0.10 mL (100 µl)	0.10 mL (100 µl)	0.10 mL (100 µl)

13. Prepare juice sample model absorbance 2 by adding 0.10 mL of OPA to the vial with mixture from absorbance 1

14. Mix well and wait for about 10 minutes before recording absorbance 2, A_2
15. Pipette mixtures into cuvette and place cuvette into uv spectrometer to record the absorbance, A_2

K. YAN Value Calculations

Ammonia Test:

Net Absorbance, $A_N = A_1 - A_2$ (calculate this for blank, standard, and sample)

Sample Corrected Absorbance, A_{C1} = Sample A_N – Blank A_N

Standard Corrected Absorbance, A_{C2} = Standard A_N – Blank A_N

Add: $A_{C1} + A_{C2} = A_c$

Ammonia concentration:

Ammonia (mg/L) = A_C x 84.3 x Dilution Factor [1]

Ammonia Nitrogen (AN):

AN (mg/L) = Ammonia (mg/L) x 0.82

Primary Amino Acid Nitrogen Test:

Net Absorbance, $A_N = A_1 - A_2$ (calculate this for blank, standard, and sample)

Sample Corrected Absorbance, A_{C1} = Sample A_N – Blank A_N

Standard Corrected Absorbance, A_{C2} = Standard A_N – Blank A_N

Add: $A_{C1} + A_{C2} = A_c$

Primary Amino Acid Nitrogen (PAAN):

PAAN (mg N/L) = A_C x 130 x Dilution Factor [1]

YAN: YAN = PAAN + AN

L. Pressure Drop versus Flux Analysis

1. Record the inlet pressure (P1) and outlet filtrate pressure gauge (P2) readings and the time the pressure readings were taken at.
2. In order to calculate the flux, Hagen-Poiseuille equation was used, since the membrane has a number of parallel cylindrical pores. Hagen-Poiseuille equation is defined as:

$$J = \frac{\varepsilon \cdot r_p^2}{8 \cdot \mu \cdot \tau} \cdot \frac{\Delta P}{\Delta x}$$

where:
- ε is the surface porosity
- τ is the tortuosity
- μ is the viscosity
- r_p is the pore radius
- ΔP is the pressure difference across the membrane
- Δx is the membrane thickness (pore length)

The membrane thickness was 0.7 meters and the surface porosity was 0.2 microns.

3. A data sheet on Excel was used to collect the pressures, flux, and time as seen in the table below. In the Excel file the Hagen-Poisuille equation was inserted to calculate the flux at a certain pressure drop and time.

	A	B	C	D	E
1	P1	P2	ΔP	Flux	Time(min)
2			0		
3			0		
4			0		
5			0		
6			0		
7			0		
8			0		
9			0		
10			0		
11			0		
12			0		
13			0		
14			0		
15			0		
16			0		
17			0		
18			0		
19			0		
20			0		
21			0		
22			0		
23			0		
24			0		
25			0		
26			0		
27			0		
28			0		
29					

A data graph of pressure drop versus flux was constructed to better analyze the data. First, water was used in the crossflow filter to obtain a better understanding of the filter's performance. After using water, hard cider was fed into the system. This was used to determine when the filtration performance declined while running hard cider. The only variable that changed between the water and hard cider was liquid viscosity.

M. Aspen Crossflow Filter Model Procedure

1. A crossflow filter was specified in ASPEN V.8.2.
2. Information on the inlet pressure and flowrate were specified. Inlet pressure ranged 1.2 - 2.0 bar.
3. Specifications of the Ricker Hill crossflow were entered into ASPEN.
4. Pressures and flowrates were the variables manipulated to see what was the most efficient and rapid filtration.
5. Simulation was run in order to obtain results on how the crossflow filter performed.

N. Aspen Crossflow Input Files

This is only for one simulation but every other simulation is basically the same all that was changed was the flowrate and pressure to see how efficient the filter could be.

```
O.  ;
P.  ;Input Summary created by Aspen Plus Rel. 28.0 at 14:35:43 Mon Apr 27, 2015
Q.  ;Directory R:\CrossfloFilter_1 Filename
    C:\Users\DGONZA~1\AppData\Local\Temp\48\~ap4cde.txt
R.  ;
S.
T.
U.  PROP
V.
W.
X.  IN-UNITS MET PRESSURE=bar TEMPERATURE=C DELTA-T=C PDROP=bar  &
Y.          INVERSE-PRES='1/bar'
Z.
AA. SIM-OPTIONS MASS-BAL-CHE=YES
BB.
CC. DATABANKS 'APV82 PURE28' / 'APV82 AQUEOUS' / 'APV82 SOLIDS' /  &
DD.         'APV82 INORGANIC' / 'APV82 ASPENPCD' / 'APV82 BIODIESEL' &
EE.          / 'APV82 COMBUST' / 'APV82 ELECPURE' / 'APV82 EOS-LIT' &
FF.          / 'APV82 ETHYLENE' / 'APV82 HYSYS' / 'APV82 INITIATO' &
```

GG. / 'APV82 NRTL-SAC' / 'APV82 PC-SAFT' / 'APV82 POLYMER' &

HH. / 'APV82 POLYPCSF' / 'APV82 PURE11' / 'APV82 PURE12' &

II. / 'APV82 PURE13' / 'APV82 PURE20' / 'APV82 PURE22' / &

JJ. 'APV82 PURE24' / 'APV82 PURE25' / 'APV82 PURE26' / &

KK. 'APV82 PURE27' / 'APV82 SEGMENT' / 'FACTV82 FACTPCD' / &

LL. 'NISTV82 NIST-TRC'

MM.

NN. PROP-SOURCES 'APV82 PURE28' / 'APV82 AQUEOUS' / 'APV82 SOLIDS' &

OO. / 'APV82 INORGANIC' / 'APV82 ASPENPCD' / &

PP. 'APV82 BIODIESEL' / 'APV82 COMBUST' / 'APV82 ELECPURE' &

QQ. / 'APV82 EOS-LIT' / 'APV82 ETHYLENE' / 'APV82 HYSYS' &

RR. / 'APV82 INITIATO' / 'APV82 NRTL-SAC' / 'APV82 PC-SAFT' &

SS. / 'APV82 POLYMER' / 'APV82 POLYPCSF' / 'APV82 PURE11' &

TT. / 'APV82 PURE12' / 'APV82 PURE13' / 'APV82 PURE20' / &

UU. 'APV82 PURE22' / 'APV82 PURE24' / 'APV82 PURE25' / &

VV. 'APV82 PURE26' / 'APV82 PURE27' / 'APV82 SEGMENT' / &

WW. 'FACTV82 FACTPCD' / 'NISTV82 NIST-TRC'

XX.

YY. COMPONENTS

ZZ. DEXTR-01 C6H12O6 /

AAA. SUCRO-01 C12H22O11 /

BBB. POTAS-01 K /

```
CCC.          MALIC-01 C4H6O5-D1 /

DDD.          WATER H2O /

EEE.          ETHAN-01 C2H6O-2

FFF.

GGG.       PROPERTIES UNIFAC

HHH.          PROPERTIES UNIQUAC

III.

JJJ. PROP-DATA UNIQ-1

KKK.          IN-UNITS MET PRESSURE=bar TEMPERATURE=C DELTA-T=C PDROP=bar  &

LLL.             INVERSE-PRES='1/bar'

MMM.       PROP-LIST UNIQ

NNN.          BPVAL WATER ETHAN-01 -2.493600000 756.9477000 0.0 0.0  &

OOO.             24.99000000 100.0000000 0.0

PPP.          BPVAL ETHAN-01 WATER 2.004600000 -728.9705000 0.0 0.0  &

QQQ.             24.99000000 100.0000000 0.0

RRR.       ;

SSS.       ;

TTT.       ;

UUU.       ;

VVV.       ;
```