

EASTERN U.S. WINE AND YEAST STUDY

Kinetics and Composition

A Major Qualifying Project
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Abstract:

Eastern US wine grapes vary considerably from old world styles and thus new methods must be mastered to produce a new and distinct eastern style. Flavor active compounds produced by yeast during fermentation leave a unique chemical signature and helps to determine the flavor and aromatic profile in the finished wine. Identifying yeast strains that are compatible with these grapes is a challenge to winemakers seeking to create commercially successful enterprises. This study developed methods for evaluating commercially available strains of *saccharomyces cerevisiae* in representative grape varieties grown in Eastern US vineyards. Gas chromatography, acid chemistry as well as dynamic mass balance were used as analytical chemistry techniques to support the subjective sensory descriptions taken of each wine. This research was sponsored by Zoll Cellars of Shrewsbury Massachusetts.

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Section I

Wine is sunlight, held together by water

-Galileo

1. Purpose

Wine is fun. Wine is to be closer to friends. Wine is a bonding element which serves to hold the fabric of community a little tighter. That is why it's worth making it better with engineering.

The purpose of this project is to learn how to produce better wines. This knowledge will be shared among three principal groups; 1) Zoll Cellars, the sponsor of the project 2) the principal investigator, namely myself and my palate, and 3) WPI's wine project groups in the near and far future.

2. Background

A primer on the winemaking process, history and science is provided in Section I for context.

2.1 Process

The process of winemaking is at once complex and simple. Wine is fermented grape juice, but dozens if not hundreds of steps may be used to achieve the desired product. Philip Jackisch posited that is helpful to think of the process as four essential stages in a continuous transformative process (Jackisch 1985). The first stage is botanic, where vines catalyze the transformation of water, carbon dioxide and nutrients under the power of the sun into fruit with the correct molecular balance of acids, sugars, and flavonoids. Following the fruit harvest the second stage takes place at the microbial level, where the microbiome transforms fresh juice into wine during the process of alcoholic fermentation. A physical separation stage clarifies the wine as the skins, particulate, and yeast are separated. The final stage is dominated by chemical reactions that mature and define the character of the wine as it ages. These stages are often operating simultaneously and often defy simple linear relations, but the model serves to inform the process engineering involved in improving the final product.

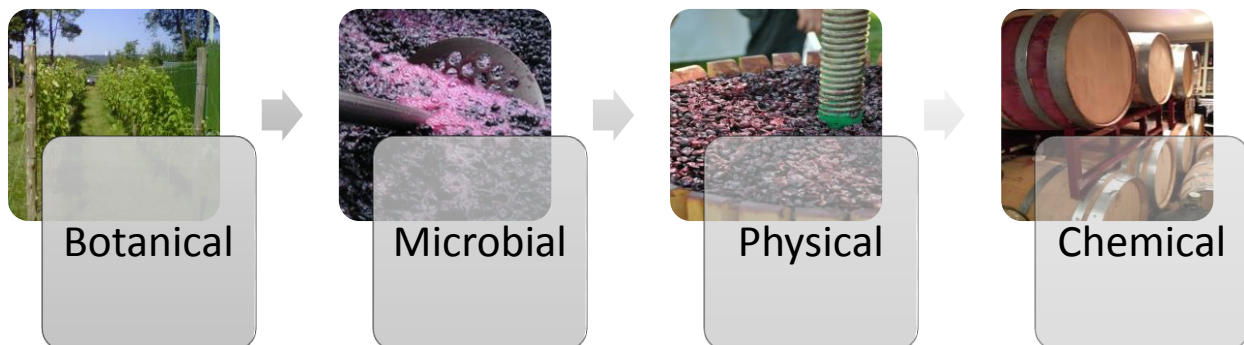


Figure 1: Process flow chart for the four major stages of wine production. Images: Frank Zoll (Zoll 2014)

Botanical

It is accepted almost universally that great wine is not made in a day. The process starts in planting a vineyard of the right varietal in a hospitable climate with good soil (Phillips 2003). Add to the initial site selection a trellis, a pruning regime, irrigation, nutrients, elbow grease, plenty of love, a touch of luck and perhaps three to five years before vines yield serviceable fruit (*Improved Grape and Wine Quality*, 2013). An axiom of engineering is that mistakes compound over time, and this especially applies to viticulture. Wines can only be as good as the fruit that are used to produce it, and it is important that the winemaker is able to work with the vineyard manager to fully express the vision of the wine in the raw ingredients.

Microbial

Once the fruit is harvested from the vineyard and brought to the winery the process enters the vinification phase. Strains of *Saccharomyces Cerevisiae* are the yeast most commonly utilized by winemakers, but the results of fermentation are a complex interaction between yeast, bacteria and other microbial species that may be present (Fleet 2003). Yeasts and other microbes metabolize the compounds in the grape must, producing not just ethyl alcohol and carbon dioxide, but many of the flavor compounds found in wine as well (Nykänen 1985), (Fuselsang & Edwards 2007). The results of these metabolic reactions can add a tremendous array of chemical compounds including higher alcohols, ethyl esters, acetate esters, phenols, volatile fatty acids, sulfides, monoterpenes, and thiols (Cordente, et al. 2012). All of this adds complexity and character to the wine, further differentiating it from the simple juice of grapes.

Physical

The physical separation processes start with removing the stems and leaves from the berries to reduce vegetal flavors in the finished wine (Phillips 2003). Crushing the berries to release the juice and bring the pulp into contact with the microbiome is another important physical step. The press is where juice and skins are separated. This may be done before fermentation to achieve a white wine or after fermentation with dark skinned grapes to get a red wine (Sacchi, Bisson & Adams 2005). The final separations serve to clarify the wine as particulates drop out by gravity or during filtration (Jackisch 1985).

Chemical

Wines are typically aged between 6 months and 10 years before bottling to allow undesirable flavors to dissipate (Tao 2014). During this period the winemaker may make minor adjustments to the wine by acidifying/deacidifying, micro oxygenating, or adding sulfite to achieve a final balanced product. Once the wine is bottled it continues to age and the slower kinetics take over.

2.2 Historical Knowledge

Although the wine process has not fundamentally changed since humans discovered wine, the techniques and methods have seen many improvements over the centuries. This has served to increase quality, reproducibility and affordability, all to the benefit of the consumer. Wine making is likely the oldest chemical process, with direct evidence of wine stored in pottery sealed with resins dating to 5,000 BCE (McGovern 1998). Knowledge of the processes necessary to turn soil, sunlight and water into delicious nectar has passed from master to pupil in family tradition, regional styles, government regulation and academic study. Not that the wine world is static; each year is a new canvas and winemakers must adapt to variable consumer preferences, weather and fruit harvests just to stay

relevant. The challenge is thus to take everything the past has taught and combine it with a creative vision of the future to make something worth doing in the present.



Figure 2: Oldest known winery site. Pictured is the press and a basin hypothesized to hold the wine during fermentation. Photo credit: Gregory Areshian (Barnard, et al. 2011)

Noah's Vine; The Origin Story of Wine

The importance of wine in ancient culture is such that when ancient Jewish scholars were writing the biblical story of Noah they claimed the first thing he did upon landing the arc was to plant a vineyard (McGovern 2013). Anthropologists believe that Transcaucasia, an area today comprised of Armenia, Georgia and Azerbaijan, was the birthplace of wine culture and where humanity first domesticated the grape vine. There is direct evidence of winery dating to 4,000 BCE discovered in Armenia (Barnard, et al. 2011). These techniques then traveled south to the Middle East and Egypt, throughout the Greek peninsula and eventually to every corner of the Roman Empire (McGovern 2013).

Ancient Process

In ancient history the winemaking process was rather crude. Grapes were crushed by stomping on them to release the fermentable juice. The must was then pressed by spreading on limestone basins with channels allowing the free run juice to flow into containers. Fermentation was left to naturally occurring yeast present on the skins of the grapes. The finished wine was then stored in earthen pots sealed with olive oil and resins (McGovern 1998). These limited processes did not allow ancient winemakers much control in the process because they were unable to control the microbiome or introduce their own yeast cultures.

2.3 Modern Science Meets Enology

Winemakers today have access to specialized equipment for all aspects of winemaking, including crushers, several styles of wine presses, stainless fermentation tanks, aging barrels, purpose built filtration systems and high speed bottling lines (Phillips 2003). These systems and the process

engineering to link each step in the process significantly reduces the time and labor required to produce wines while greatly increasing the quality and availability.

Commercially Available Yeast

Historically natural yeast present on the grape skins at harvest were the only microbes available to induce alcoholic fermentation and thus winemakers had very little control over the process (McGovern 2013). Eventually winemakers discovered that yeast could be introduced by addition of must from previous fermentation or the yeast could be isolated and grown from single colony cultures at the winery. Difficulty in starting and growing yeast starter cultures led to the development of commercially available dry yeast in 1963 (Fugelsang 2007). This development has greatly increased the choices available to the winemaker in inoculating must with a specific strain to reach a targeted style and flavor profile (Romano, et al. 1998). During fermentation yeast produce a wide array of flavor active compounds that can affect the taste and aroma of the wine (Nykänen 1985). While this fact was discovered 30 years ago, yeast are now credited with production of a far greater array of compounds than originally believed (Cordente 2012). Targeting specific flavor profiles for individual wines by using yeasts specific to that effort has thus become an important choice for the winemaker in crafting their wine (Romano 2003).

Gas chromatography

With the invention of gas chromatography in the 1950's a new analytical tool was added to enologist's arsenal (Kaiser 1963). Since then procedures for analyzing wine by GC have been well documented by several groups (Skoog 1998). Typically an extraction is performed to move the analyte into an organic solvent prior to injection into the column due to concerns regarding water contaminating the column or associated detectors. One group has developed a method to directly inject wine into their column without an extraction step (Villen 1995).

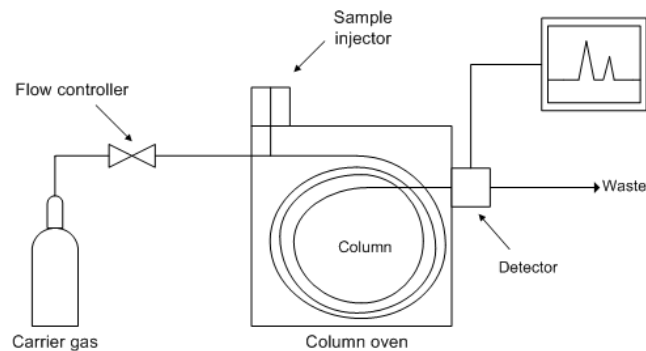


Figure 3: Diagram describing basic components present in all gas chromatography systems. Image credit: (rune.welsh 2005)

2.4 Eastern Frontier

Local Demand

The demand for local artisanal products has been strong enough to support a growing Eastern wine market (Bettini, 2013). Although Massachusetts ranks 24th in the nation for wine production by volume, there is a growing number of craft wineries and high quality producers (2013 Statistical Report – Wine). The Southeastern New England AVA is home to 23 wineries (American Winery Guide 2014). This geographic area is located at the same latitude as some of the world's best wine regions and enjoys a moderating oceanic effect (AVA §9.72 2013).

Recent National Economic Trends

Production and consumption trends have increasingly flavored United States wine producers as European vineyard surface area fell by 13% from 2000 to 2011, while United States vineyard surface area grew by a modest 2% (Bettini 2013). This trend coincides with an increase in consumption by the US wine market by 34% over the same (OIV Statistical Report 2012). This combined with the US Supreme Court Granholm decision increasing interstate competition in the wine market has led to a “perfect storm” where producers are increasingly driven to produce better quality wines at an accessible price point (Hinman, 2005). This makes winemaking a particularly promising professional field for young engineering students.

3. Introduction

3.1 Project Sponsor

Zoll Cellars is a winery located in Shrewsbury, MA owned and operated by Frank Zoll since 2008. Zoll Cellars is a micro-winery with production at approximately 600 cases of high to premium quality of wine per year. Wines are sold at local wine boutiques and restaurants, such as the Wine Vine on West Street and the Sole Proprietor on Highland St in Worcester, Massachusetts. Frank and Justin also sell the wine at about a dozen farmer’s markets every week across the state during the sales season. The wine can also be purchased directly through the website, zollwine.com.

3.2 Existing Wine Products

Zoll currently offers a variety of wine products to consumers through local wine boutiques, direct sale, and farmers markets. The wines are priced between \$10 and \$25 per bottle. Not pictured are two of Zoll Cellars perennial best sellers, the medium bodied spicy Cabernet Franc and the full bodied luscious Sandcastle Blend.



Figure 4: Zoll Cellars current vintages. Form left to right: Hard Cider, Wildflower Mead, Vidal Blanc, Riesling, Lighthouse Blend, Pinot Noir. (Zoll 2014)

4. Areas of Interest

Three principal areas of interest were identified by the author during interviews with the project sponsor, professor Kmiotek and professor Timko.

4.1 Research Process Variables

Wines that offer higher quality will sell better and will increase profits for the winemaker (Hinman, 2005). Consumers will also benefit from access to a higher quality product and a more pleasurable experience. Many factors are involved in making quality wines that are perceived as having high quality and creating these desirable factors is the job of a winemaker (Cordente 2012). Choices by the

winemaker can include yeast selection, added supplements, grape skin contact time, oak aging, sulfite addition, filtration and a host of additional techniques. By using scientific and engineering principals to identify the methods, materials, and processes to craft better wines, the product can be optimized to meet consumer needs.

4.2 Scalable Process Development

Developing new wine styles can be an expensive proposition for a commercial winery. Uncertainty in process variables in addition to market instability can inhibit the introduction of new products. However as markets shift the winery must be able to capitalize on emerging trends and introduce new products to the market (Hinman 2005). The number of new recipes or methods that can be evaluated is limited by the volume of grapes from the harvest that can be spared and time required to prepare and evaluate research projects. Creating a research and development program to identify new winemaking techniques at a minimum capital cost with quick turnaround and a small fruit investment will greatly benefit the winemaker in making informed choices for each vintage.

4.3 Yeast Selection

Wine yeasts have been studied extensively with grapes from other wine regions and have been characterized well. However Eastern US grapes vary considerably from those produced in other regions in tartaric acid content and several other factors, thus the characteristics of fermentation and finish quality will also be affected (Rodriguez-Nogales, Fernandez, & Vila-Crespo 2009). Studying these effects on yeast performance can give winemakers a better sense of which yeasts will produce favorable characteristics in their wines.

5. Engineering Study Proposal

The conclusion of Section I is a one-to-one proposal to Engineering objectives in Section II.

5.1 Research

This will be an engineering study of the process variables of interest to Zoll Cellars.

5.2 Scale

Develop a sustainable winemaking research program to evaluate scalable processes.

5.3 Yeast Selection

Yeast selection in winemaking is a principal interest of the engineering study.

Section II



7. Engineering Objectives

7.1 Research

Develop methods for testing process variables

7.2 Scalability

Evaluate scalability of research methods to commercial processes

7.3 Yeast

Study yeasts strain as a process variable in winemaking process

8. Rationale

8.1 Research

This engineering study is valuable because it offers a high information to cost ratio when evaluating wine making process variables. The number of process variable that are possible to evaluate effectively per liter of invested wine is a measure of information. The median price per bottle of Zoll Cellars wine is \$15 (Zoll 2014). Maximizing the return in information from invested research wine is the soft metric for success for the research program.

8.2 Scalability

Better wine products that can be produced in a commercial scale is the final goal of the research program. Honing product variables in a development program is the offers a more consistent product upon launch to consumer market. This research program will be considered successful if recommendations are implemented and commercial scale processes reflect learned knowledge in the lab.

8.3 Yeast

Knowing which yeast strains produce good wine from the fruit that Zoll Cellars is using is an important process variable. This engineering study will better equip Zoll cellars to produce improved wine and will inform the author's winemaking style. The metric for this process variable will be reflected in notes each strain that will serve as reference material during winemaking season in subsequent years.

9. State of the Art

9.1 Lab Bench Methods

Bench scale studies of using the micro fermentation method were developed in the late 90's and reported in the literature. Romano was the first to report a procedure where grape musts were sterilized and fermented in 250 mL Erlenmeyer flasks under a layer of mineral oil (Romano, et al. 1998). The effects of grape varietal and growing region were studied using micro fermentation in a study published by Sarmiento, et al. (2001). Similar research has also been reported by a Portugal group with a clearer focus on the analytic chemistry and grape growing processes (Coelho, et al. 2006).

Modern analytical chemistry techniques offer a means to evaluate wine in greater detail than ever before. Because wine is a complex solution of many compounds, separation by gas chromatography is the most common technique used for analysis (López 2002). Analysis by direct injection of wine has been reported but has not gained widespread use (Villen, et al. 1995). The "fast" methodology that has been reported and adopted by several groups requires a liquid extraction of analyte with dichloromethane (Ortega 2001). Most recently a number of groups report using SPME extraction to prepare analytes for injection into their chromatography column (López 2002), (Coelho 2006), (Gonzalez 2011), (Torrens 2004).

9.2 Scaling Research

The research published of micro fermentation also notes the importance of scaling effects, where Romano followed there 1998 publication with a scale studies in 2003, concluding that differences between commercial and micro reactors were not significant. Torrens, et al. examined semi industrial fermentation and these performed relative to commercially available major producers (Torrens 2008).

Vilanova in 2012 utilized 16 L intermediate size fermenters are concluded that the wine produced was not significantly different than comparable commercial fermentations carried out in the same winery.

9.3 Yeast selection studies

The assessment of commercially available yeast in winemaking by various groups has been a very active area of research. Some groups focus on the assessment of commercial strains in unique varieties (Vilanova 2012) (Torrens 2008). Others have isolated and grown cultures of wild yeast strains for characterization and possible commercialization (Romano 2003) (Ortiz-Muñiz 2010). These studies most often use sensory analysis by expert palates combined with analytical chemistry techniques to characterize the wines and produce a recommendation (Torrens 2008) (Vilanova 2012) (Rodriguez 2009) and (González 2011). These methods will be employed to make yeast selection recommendations for future winemaking projects.

10. Approach

Wine was studied by setting up micro batches and manipulating variables independently to identify targets for commercialization. The primary variable of interest was selection of commercially available yeast strain, while nutrient addition and blending properties of small batches were also explored. The resulting wines and ciders were analyzed using standard vintner's tests and by gas chromatography with mass spectrometer detection. Finally a procedure for rapid prototyping of hard cider was developed.

11. Methods

The methods utilized in this engineering study were chosen after consulting the literature and were improved throughout the study by iteration of the method. The designs were evaluated using axiomatic design to inform the process.

11.1 Micro Fermentation

Method development

The micro-process research approach utilized in this project was made to mirror the process variables found in production of the commercial wine at the host winery. This included using similar timeframes or reference points in the production schedule and environmental conditions for the crush, fermentation and press. These micro studies were used over several experiments to evaluate 1) scalability and feasibility of micro-processes 2) yeast selection for Eastern US grapes 3) kinetics during fermentation and 4) cider fermentation. The micro-fermenter design was the workhorse of the study and was used to produce upwards of two dozen unique fermentations.



Figure 5: Micro fermentation process flow diagram including images of the micro wines produced

The micro fermentation process was conducted in quart sized Mason Jars fitted with an air lock. White wines were fermented as juice while red wines were fermented with about 100 mL of skins. The jars were weighed at the beginning of the fermentation and periodically throughout the fermentation to determine the mass of carbon dioxide evolved from the system. This gave an indication as to the overall progress of the fermentation and the total amount of ethanol present in the wine.

The micro fermenters were constructed from mason jars in quart, pint and 8 oz mason jars at different points in the study. Different lid designs were tried were tried as well eventually producing a design that was easier to make, sealed the contents better and cost less. The lid serves two main functions; to seal the wine from contaminants present in the atmosphere and to allow the release of carbon dioxide produced during fermentation.



Figure 6: Three design iterations for the micro fermentation experimental setup.

The first design solution was to fit a #12 holed stopper directly to the mason jar with a bird cage airlock fitted into the center hole to allow gas to escape. This design was costly (\$7/unit) and relies entirely on friction grip from compression of the bung to maintain a good seal. The next iteration was to drill a 3/8" hole in the jar lids to allow a serpentine airlock to be inserted and sealed with a gasket, wood glue or silicone caulk, in chronological order. This design was cheaper and allowed for a good positive mechanical seal to be formed by the jar lid and utilized commonly available materials already present at the winery. The latest iteration was to pour vegetable oil onto the surface of the must within a beer bottle. This design is the simplest and cheapest, however additional effort is required to extract the wine from under the vegetable oil and this design has yet to be fully optimized for lab use.

Wine Micro Fermentation

The wine micro fermentations were carried out in the fall of 2013 as fruit arrived at the winery for commercial production. The active fermentations had finished by January and the wines were sealed to prevent volatile decay and oxidation.

Material sourcing

The four fruit harvests that were made available for micro-fermentation were Westport Massachusetts Chardonnay (MACH), Cutchogue Long Island New York Cabernet Franc (NYCF), Lake Cayuga New York Cabernet Sauvignon (NYCS), and Portsmouth Rhode Island Cabernet Franc (NYCF). Yeasts available for use in this study was limited to commercial yeasts from sources identified by the sponsor. From this list of available yeasts, three were chosen for each varietal. At the end of the press, the leftover juices from the NYCS series and the RICF series were blended to form the single varietal 123 blends.

Fermentation

The fermentation schedule was determined by the time of arrival of fruit at the winery. Once fruit arrived and were crushed as commercially sized batches, samples of must were taken for micro fermentation. By completing the crush with the commercial batches the micro process was as close to the commercial process as possible. Dried yeast (0.5 g) was rehydrated in 10mL of warm water for 15 minutes. An addition of 15mL of juice for 5 minutes was then performed to acclimatize the yeast before pitching in the micro fermenters and stirring to homogeneity. This procedure is given in the yeast manufacturer’s instructions, scaled proportionally to the volume of juice in the micro fermenters. Fermentation was monitored and was judged to end by the movement of gas bubbles through the airlock.

Table 1: Micro fermentation varieties and yeast choice

Micro Fermentation	Date	1	2	3	4	123
MB	October 5	D 254				
MACH	October 10		D 47	D 254	K1-V1116	
NYCF	October 22	RC 212	D 80	BM 4x4		NYCF(1+2+3)
RICF	October 26	RC 212	D 80	BM 4x4		RICF(1+2+3)
NYCS	November 8	RC 212	D 80	D 254	D254	
VB	November 5	D 47				

Racking, Pressing and Aging

The red wines were pressed to separate skins from finished wine 5-10 days after the end of fermentation. The press was a basket screen strainer placed in a funnel with a collection jar at the bottom. Due to the reduction in volume (~250 mL) from the removal of the skins, the wines were stored in smaller pint (500 mL) mason jars. This resulted in a surplus of 250 mL of finished for each fermentation. To complete an exploratory study, these remains were mixed in equal parts from for each series and the resulting blends were stored in 500 mL jars. The chardonnay and vidal were not racked and were allowed to age on the lees, which is common in white wines to increase body and mouth feel.

Culture media

A series of kinetics studies were conducted in a culture media inoculated with sugar. Sucrose was added to distilled water up to 22 brix and the solution was buffered with three salts; KH₂PO₄ [8.0 g/L], (NH₄)₂SO₄ 5.0 [g/L], and MgSO₄*7 H₂O [1.0 g/L]. Yeast process variables were studied in round one with four yeast strains were used being studied at the 0.2 g inoculation level (RC212, D80, VIN13, and EC-1118) and one micro fermenter inoculated with 1 g of EC-1118 to examine effect of yeast mass on the rate. Round two examined sucrose concentration, doubling the salt and a low yeast inoculation. These fermentations were weighed daily to measure fermentation progress by emission of CO₂.

Cider Studies

The micro fermentation method was applied to hard apple ciders in the spring of 2014. Three iterations of the method were exercised with the second attempt being most successful

Apple sourcing

Gala and Macintosh apples were sourced from Ricker Hill Orchards in Turner Maine. Golden Delicious apples were purchased at Price Chopper on park Ave Worcester Massachusetts.



Figure 7: Cider micro fermentations 1-12. The first four from left are Macintosh ciders, the right four are Gala on four different yeasts, the middle four are blends of Golden Delicious, Gala and Macintosh.

Micro process adapted

The micro fermentation procedure developed for the hard ciders followed the same principal steps as the wine making for white grapes, however a micro crush and press procedure was also developed to enable the process to be commercial batch independent. This means that micro fermentations can be conducted year round from store bought apples. The crush was completed using a food processor to blend whole apples into pulp. The pulp was then sandwiched between paper towel sheets and pressed with a rolling pin to extract juice. From there the fermentation followed the white wine procedure. The fermenters were massed daily to measure fermentation progress until airlocks settled.

Micro process execution

The cider studies that were most intriguing were started on April 4 2014. A series of 12 micro fermenters were prepared using 8 oz mason jars and the most advanced lid design to date. Two process variables were identified for study; yeast selection and blending properties of apple varieties.

Yeast selection matrix

Table 2: The cider fermentations followed the following matrix setup for yeast/apple combinations. The yeasts are listed across the top and the two apple varieties were listed on the side.

	71B	QA23	KV-1116	EC-1118
Macintosh	1	2	3	4
Gala	9	10	11	12

Blending table

Table 3: The same series included an apple blending study. The same yeast (EC-1118) was to examine the effects of blends on flavor profile.

	Golden Delicious	Golden Delicious Macintosh	Golden Delicious Gala	Golden Delicious Macintosh Gala
EC-1118	5	6	7	8

11.2 Analytical Chemistry

The analytical chemistry was conducted to keep records and explain variation in results. The vintner's standard tests refer to tests that are routinely performed at the winery and are performed on every wine produced at Zoll Cellars.

Vintner's Standard Tests

Standard tests currently used by the winery fall into three categories; 1) sugar content and concentration, 2) Acid chemistry and buffer capacity 3) Sulfite concentration.

Sugar content is measured with two instruments to verify results. A hydrometer is used to measure density, which is linearly dependent on the sugar content. The Brix scale is traditionally used in winemaking, which is defined as a weight percent of sucrose in water solution.

$$\left[1^{\circ}Bx = \frac{1g\text{Sucrose}}{100g\text{Solution}} \right]$$

Refractometry is used with fresh juice (unfermented) to measure sugar concentration. A refractometer is a small device with a sample plate and an eyepiece that measures the diffraction of light through the analyte. The reading is taken by looking through the eyepiece and reading the measurement off of a scale with units in brix. Any discrepancies between the refractometer and hydrometer readings are noted in the notes for sugar content.

Acid chemistry of wines is tracked by pH and by the titratable acidity of analyte. Measurements of pH were taken by a Milwaukee MW 102 pH/temperature probe. A sodium hydroxide titration with 0.1 molarity NaOH and several drops of phenolphthalein in 10 mL of wine to determine the titratable acidity. The calculation works out to 10 times the volume (in mL) of base used is the titratable acidity (in g/L). These two factors are related but can vary, especially if the acidity is adjusted by bicarbonate addition.

Gas Chromatography

Gas chromatography was performed on the wine and cider studies using the same procedure. Wine analytes were extracted using 3 mL of wine, 7 mL of water, 2.25 g NaCl, 15 μ l of the internal standard and 0.4 mL dichloromethane in a 15 mL test tube. The analyte was shaken for 15 minutes by hand, spun in the centrifuge for 5 min at 3000 rpm, and extracted by pipet from the bottom of the tube.



Figure 8: Pipet tip immersed in the dichloromethane extract at the bottom of the centrifuge tube. To the left is a GC sample vial and lid. Notice the solids collected at the phase interface

The internal standard for the GC was prepared as an ethanol solution with 140 μ g/ml of each compound: 2-butanol (2B), 4-methyl-2-pentanol (4M), 4-hydroxy-4-methyl-2-pentanone (4O) and 2-octanol (2O).

Target compounds for each internal standard

Table 4: Target compounds for GC/MS analysis and the internal standards that they would be compared against to get concentration data.

2-butanol	4-methyl-2-pentanol	4-hydroxy-4-methyl-2-pentanone	2-octanol
2B	4M	4O	2O
Acetaldehyde	Ethyl acetate	Propanoic acid	Ethyl hexanoate
Diacetyl	Isobutyl acetate	Butyric acid	Ethyl octanoate
1-Butanol	Isoamyl acetate	Isobutyric acid	Ethyl decanoate
Isobutanol	Hexyl acetate	Isovaleric acid	Phenylethyl acetate
Isoamyl alcohol	Ethyl propanoate	Ethyl lactate	Diethyl succinate
	Ethyl butyrate	Ethyl 3-hydroxybutyrate	Hexanoic acid
	Ethyl isobutyrate	g-Butyrolactone	Octanoic acid
	Ethyl 3-methylbutyrate	Methionol	Decanoic acid
	1-Hexanol	Benzyl alcohol	b-Phenylethanol
	cis-3-Hexenol		Acetoin
			Furfural

The cider samples were extracted by a similar method, but no internal standard was used after that failed to bear results in the wine GC runs that it was used for. The following amounts were utilized to complete the extraction: 5 mL of cider, 5 mL water, 2.25 g NaCl, 1.0 mL dichloromethane.

The gas chromatography method was determined by a careful tuning of the method presented by Ortega et al (2001). The important parameters are as follows. Injection was done by the AOC-20i auto sampler injecting 0.5 µl of analyte in splitless mode with the injection port at 230°C. The carrier gas was controlled at constant pressure of 80 kPa. Column oven temperature profile: hold at 50°C (2 min), ramp 10°C/min (20 min) to 250°C, hold for 3 minutes. The mass spectrometer settings were as follows; interface temp 230 °C and ion source 200 °C, with the detection window starting at 3 minutes to the end at 25 min.

11.3 Sensory Descriptions

An unexpected skill that was required to complete this project was the ability to discern between subtle flavor and texture differences in the wine and convey that with descriptive vocabulary. This is perhaps the most important test in a winemaker's arsenal is their own sensory descriptors of the wine from tasting and smelling samples taken along the way. Wines were evaluated at the end of the study to measure the flavor profiles. Notes on aroma, flavor, body and acid (cider only) were recorded and recommendations for yeast selection in the next winemaking style were made and accepted by the project sponsor. The recommended yeasts are not published to protect the proprietary advantage gained by the sponsor, but tasting notes are presented.

12. Results

The results will be given in four sections to reflect an increasingly complex picture of the wine.

12.1 Analytical chemistry

12.2 Mass balances

12.3 Gas Chromatography

12.4 Sensory descriptors

12.1 Analytic Chemistry

The standard tests are summarized in table 4. None of the wines for micro fermentation fell outside the envelope for normal values so no corrective action was necessary. Nutrient and sulfite addition was 0.2 grams of each for every micro fermenter, except NYCS4. The exceptional result of the season was the titratable acidity of the NY Baco Noir that was not part of the micro fermentation study. With an acid level of 13.5 g/L, the flavor profile of the wine was extremely strong at the front of the palate and needed 2 lbs of sodium bicarbonate to balance the acid.

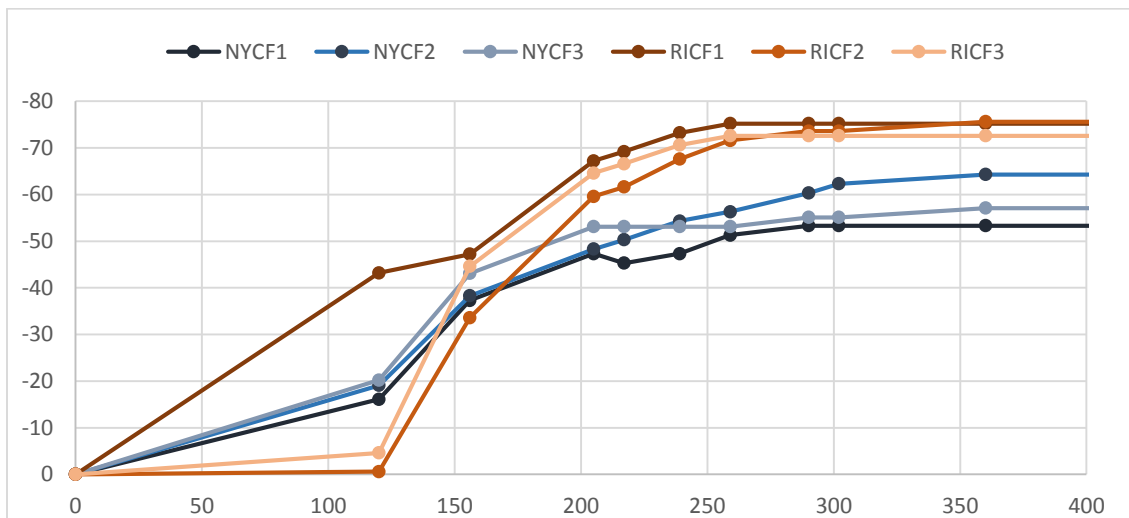
Table 5: The standard test results for the wines of the 2013 winemaking season.

	Date	Temp (F)	Sugar (°Bx)	pH	Tartaric acid (g/L)
Westport MA Chardonnay	11-Oct	55	21	3.8	7.9
Cutchogue NY Cab Franc	22-Oct	55	22.5	3.8	7.5
Portsmouth RI Cab Franc	23-Oct	60	21.5	4.1	6.7
Portsmouth RI Vidal Blanc	3-Nov	60	21.5		8.25
Lake Cayuga NY Cab. Sauv.	8-Nov	50	23	3.5	5

12.2 Mass Balance

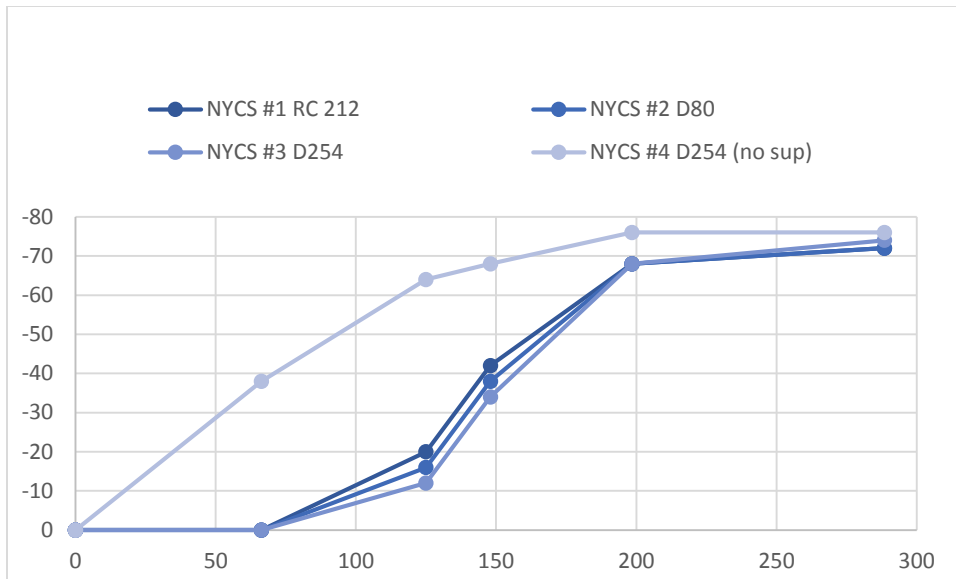
The New York and Rhode Island Cabernet Franc micro fermentation mass tables are shown below. The progress of the fermentations appear to follow a first order rate law, with rate of evolution of carbon dioxide dropping off to zero after 10-14 days for all micro fermentations. There is an interesting period at the beginning of each fermentation where the yeast take a period of up to four days to begin fermenting. It is possible that this apparent shock is due to the rehydration methodology, and it could be a potential future project to examine this in greater detail.

Table 6: Mass loss for NY and RI Cabernet Franc wines



The cabernet sauvignon micro fermentations (table 4) followed a similar pattern and evaporated a similar mass of carbon dioxide. An interesting discrepancy occurred where one of the micro fermentations did not display the characteristic lag in fermentation. This micro fermenter, NYCS4 was the only one conducted without supplemental nutrient added to the must so the role of yeast nutrient in inhibiting the kinetics is another question that came from this study of one.

Table 7: Mass loss rates for NY Cabernet Franc



12.3 Gas Chromatography

The results of the gas chromatography runs were chromatographs showing peaks for each compound as it was eluted from the column. The mass spectrometer analyzed each peak and reported an ion fragment spectrum with a probable chemical species and the relative percent abundance. This analysis showed that gas chromatography can be used to evaluate wine in this lab, however more work is needed before chromatography results can be used by the winemaker to inform decision making in the process.

The wine samples were run on March 27, 2014 and the results of the session are given here. The chromatograms detailed beautiful results when the analysis was completed for a few good runs. Early chromatography attempts had a high rate of failure to obtain results. After three months of trying, these bore out the first results. These analysis were also characterized by frequent failures to obtain even one distinguishable peak.

NYCS 4 was a lucky one. The chromatograph here shows 48 unique peaks with a large array of flavor active compounds.

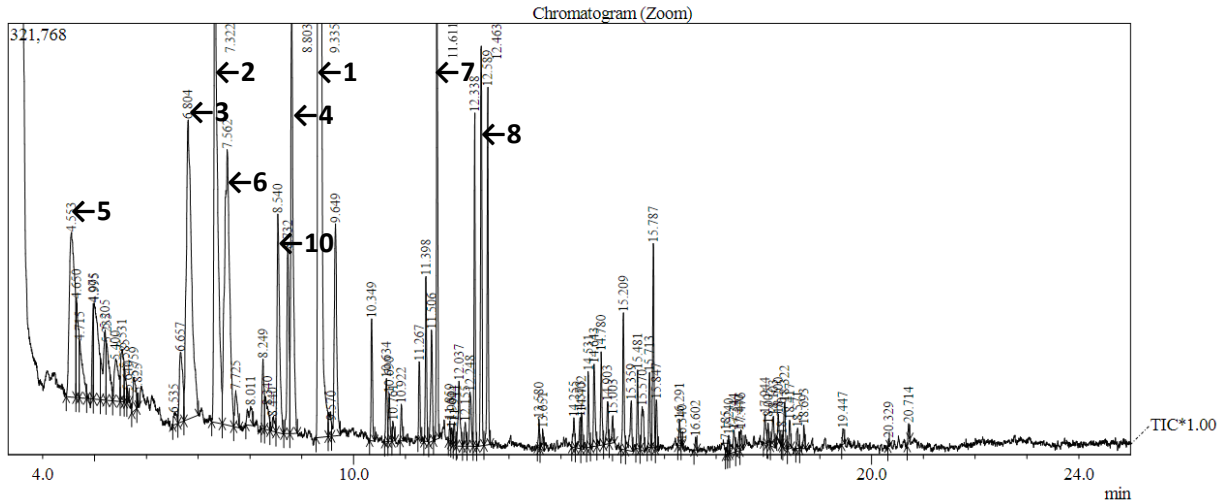


Figure 9: NYCS4 the best chromatograph and fermentation profile, the peaks are labeled in order of abundance and the compound names and data are listed below.

The 10 most concentrated species are listed. The presence of phenylethyl alcohol in such high proportions is still unexplained but must be the result of contamination.

Table 8: 10 most concentrated compounds

Rank	% Area	Time	Relative height	Compound
1	45.69	9.34	48.58	Phenylethyl Alcohol
2	7.07	7.32	4.12	2-Octanol
3	6.28	6.80	2.49	Pentanoic acid, 2,4-dimethyl-3-oxo-, methyl ester
4	3.97	8.80	3.48	m-Toluic acid, 6-ethyl-3-octyl ester
5	3.25	4.55	1.19	3-Acetoxydodecane
6	3.04	7.56	2.23	Heptane, 2,5,5-trimethyl-
7	2.5	11.61	3.96	Benzene, 1,3-bis(1,1-dimethylethyl)-
8	2.35	12.46	3.34	2-Isopropyl-5-methyl-1-heptanol
9	2.14	7.53	1.83	Pentane, 1-butoxy-
10	2.12	8.54	1.79	Acetophenone

The full list of compounds is shown on the next page.

Table 9: Compound table for a typical wine run. The compounds are ranked by their order of elution

Order	Time	% Area	Relative height	Compound
1	4.553	3.25	1.19	3-Acetoxydodecane
2	4.995	1.74	0.73	Formic acid, hexyl ester
3	5.4	0.39	0.25	Styrene
4	6.65	0.96	0.52	3-(Hydroxy-phenyl-methyl)-2,3-dimethyl-octan-4-one
5	6.804	6.28	2.49	Pentanoic acid, 2,4-dimethyl-3-oxo-, methyl ester
6	7.322	7.07	4.12	2-Octanol
7	7.525	2.14	1.83	Pentane, 1-butoxy-
8	7.562	3.04	2.23	Heptane, 2,5,5-trimethyl-
9	8.249	0.36	0.47	Thiophene, tetrahydro-2-methyl-
10	8.54	2.12	1.79	Acetophenone
11	8.732	1.39	1.44	1-Dodecanol, 3,7,11-trimethyl-
12	8.803	3.97	3.48	m-Toluic acid, 6-ethyl-3-octyl ester
13	9.335	45.69	48.58	Phenylethyl Alcohol
14	9.649	2	1.73	3-Methylbenzyl alcohol
15	10.349	0.7	1.02	Butanedioic acid, diethyl ester
16	10.634	0.42	0.53	Octanoic acid, ethyl ester
17	10.69	0.29	0.4	Dodecane
18	10.755	0.11	0.15	Undecane, 4,6-dimethyl-
19	10.922	0.2	0.31	Dodecane, 4-methyl-
20	11.267	0.43	0.66	Dodecane, 4,6-dimethyl-
21	11.398	0.85	1.38	Dodecane, 4,6-dimethyl-
22	11.506	0.75	0.93	Dodecane, 2,6,11-trimethyl-
23	11.611	2.5	3.96	Benzene, 1,3-bis(1,1-dimethylethyl)-
24	11.944	0.11	0.19	Dodecane, 4,6-dimethyl-
25	12.037	0.48	0.52	Cetene
26	12.248	0.41	0.44	5-Oxotetrahydrofuran-2-carboxylic acid, ethyl ester
27	12.338	1.61	2.78	11-Methyldodecanol
28	12.463	2.35	3.34	2-Isopropyl-5-methyl-1-heptanol
29	12.589	1.79	2.99	2-Isopropyl-5-methyl-1-heptanol
30	13.58	0.12	0.22	Decanoic acid, ethyl ester
31	13.651	0.11	0.18	Tetradecane
32	14.531	0.37	0.63	Eicosane
33	14.643	0.52	0.69	Hexadecane, 2,6,11,15-tetramethyl-
34	14.78	0.53	0.78	10-Methylnonadecane
35	14.903	0.25	0.35	Eicosane
36	15.209	0.73	1.12	Phenol, 2,4-bis(1,1-dimethylethyl)-
37	15.359	0.21	0.35	1-Hexadecanesulfonyl chloride
38	15.481	0.39	0.66	1-Dodecanol, 2-hexyl-
39	15.57	0.4	0.37	1-Dodecanol, 2-hexyl-
40	15.713	0.41	0.62	1-Dodecanol, 2-hexyl-
41	15.787	1.09	1.71	Benzene, 1,1'-(1-methylethylidene)bis-
42	15.847	0.24	0.41	1-Hexadecanesulfonyl chloride
43	16.291	0.15	0.21	Triethylidene mannitol
44	17.935	0.2	0.2	(2,3-Diphenylcyclopropyl)methyl phenyl sulfoxide, trans-
45	18.103	0.34	0.26	1-Hexadecanesulfonyl chloride
46	18.2	0.13	0.21	Octacosyl trifluoroacetate
47	18.322	0.24	0.35	1-Dodecanol, 2-hexyl-
48	18.417	0.17	0.23	Triacetyl heptafluorobutyrate

The analysis revealed an tremendous result of 48 compounds where before only the one was thought to exist. The general functional groups found were esters, ethers, higher alcohols, alkanes and sulfonyls. One compound was found far in excess of every other peak; phenylethyl alcohol accounted for 45% of the peak area. This made all of the gas chromatography runs look as though there was one peak until the baseline was sufficiently magnified. This has been encountered on every run since and needs to be addressed as a study refinement for next year. The results of the wine gas chromatography runs was that practice improves results and that the first runs rarely work the best. Practice, especially perfect practice, makes perfect.

Cider chromatography runs

The chromatography runs for the cider were a great follow up study. Below is the result of 10 chromatographs obtained from ciders 1-12, excluding 1 and 3 due to issues with those extractions. Subtle variations can be seen in the chromatographs, especially near the 20.5 minute mark.

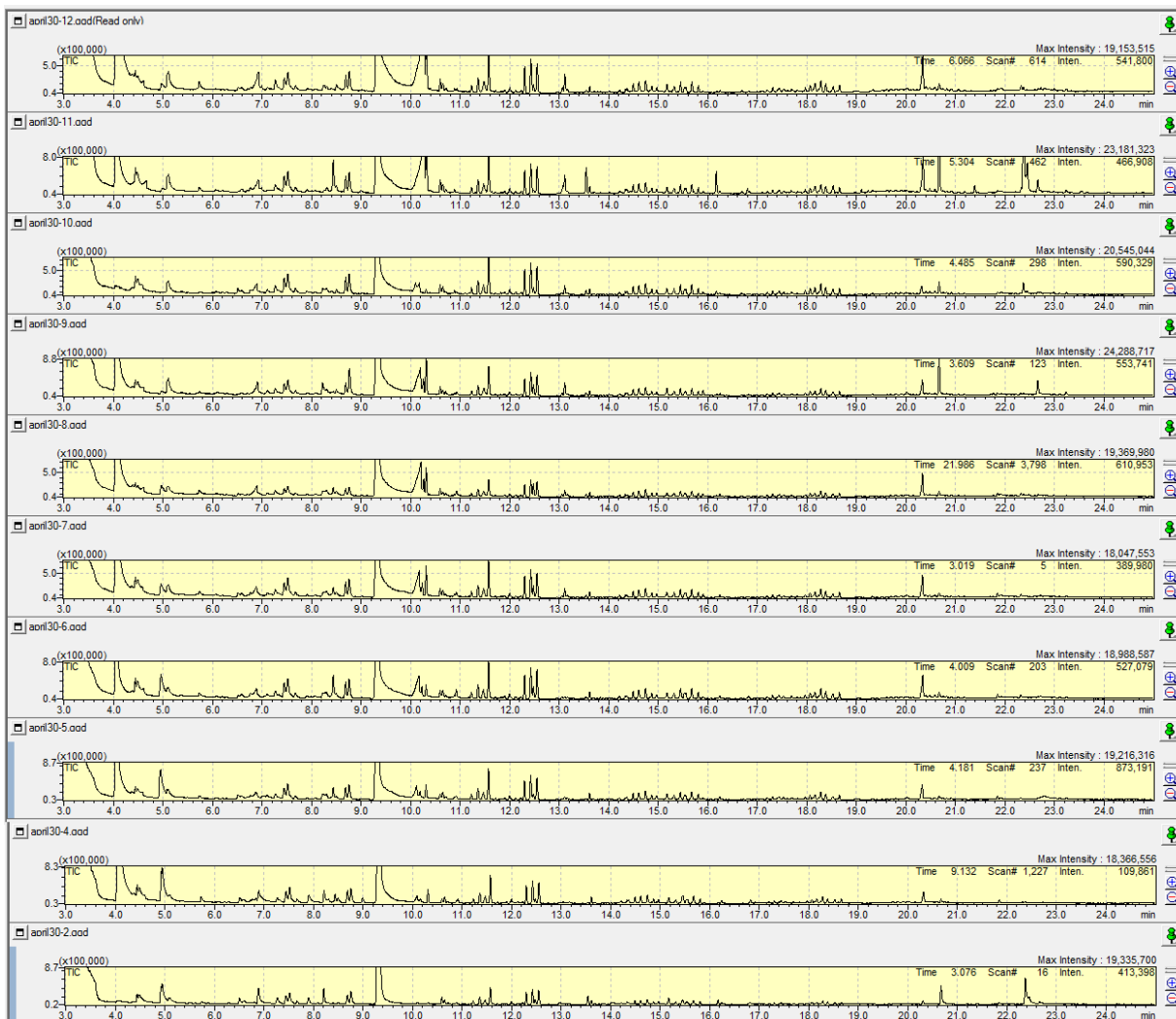


Figure 10: Cider fermentation chromatographs, not pictured are ciders 1&3 because the auto injector failed to pick up sufficient sample to be detected by the column

The 20 highest concentrated compounds eluted from the column for a typical run are shown in a compound table on the next page. A very large number of peaks were detected in these runs, with greater than 150 peaks being common. This chromatograph yielded 178 peaks with the 20 most abundant compounds being displayed in the table for clarity.

Table 10: The 20 most concentrated species in a typical cider run

Relative Abundance	Area %	Order	Time	Height %	Compound
1	3.81	66	11.577	6.07	Benzene, 1,3-bis(1,1-dimethylethyl)-
2	3.50	28	7.524	2.58	Nonane, 2,6-dimethyl-
3	3.23	76	12.429	4.38	2-Isopropyl-5-methyl-1-heptanol
4	2.72	11	5.111	1.62	1-Butanol, 3-methyl-, acetate
5	2.70	78	12.556	3.91	2-Isopropyl-5-methyl-1-heptanol
6	2.44	39	8.762	2.66	1-Decene, 2,4-dimethyl-
7	2.33	8	4.489	1.61	2,4-Dimethyl-1-heptene
8	2.26	22	6.893	1.20	Hexanoic acid
9	2.23	75	12.304	3.39	2-Isopropyl-5-methyl-1-heptanol
10	2.21	27	7.452	2.00	Heptane, 2,5,5-trimethyl-
11	1.67	38	8.691	2.28	1-Decene, 2,4-dimethyl-
12	1.65	51	10.103	1.30	Ethyl hydrogen succinate
13	1.63	154	20.671	1.74	Hexadecanoic acid, ethyl ester
14	1.52	7	4.448	2.01	2,4-Dimethyl-1-heptene
15	1.50	52	10.178	1.26	Octanoic acid
16	1.40	34	8.302	0.65	Nonane, 4,5-dimethyl-
17	1.39	65	11.473	1.25	Dodecane, 2,6,11-trimethyl-
18	1.39	98	14.747	1.46	Hexadecane, 2,6,11,15-tetramethyl-
19	1.38	130	18.285	1.46	1-Dodecanol, 2-hexyl-

12.4 Sensory Descriptors

Wine tasting

The concluding test for these wines was a sensory analysis to determine flavor profile and character. This information was used to make a recommendation for yeast choice for a commercial batch in the coming year.

Table 11: This table contains tasting notes taken with the project sponsor, Frank Zoll

ID	Aroma	flavor	body	Finish
MACH 2	honey, melon, citrus, VA	quints, tart, grassy, VA	medium 3	Perceptible acid
MACH 3	citrus, melon, floral	off sweet, lemon grass, under ripe pineapple	medium 3	Short and crisp
MACH 4	pungent, banana, woody	vanilla custard	medium 3+	Long velvety
VB 1	ethene, rotting apples, acetone	acetone, rancid almond, oxidized	medium 4	Creamy rich
RICF 1	tart raspberry, plum	Acidic, strawberry, raspberry, herbaceous, salty plum wine	Light 2+	short clean
RICF 2	tart raspberry, plum, menthol	Acidic, strawberry, raspberry, herbaceous, tartness	Light 2+	short clean
RICF 3	Fruit forward, earthy	Balanced acid, fruit forward, black berry	Medium 3	complex chocolate
RICF 123	jammy, earthy	Smokey, earthy, balanced acid	Medium 3	balanced
NYCF 1	ripe fruit, boyson berry	Menthol, robotussin, VA	Medium 3+	longer finish
NYCF 2	plum, cherry, H2S	Menthol, red berry, earthy	Medium 3	fresh mint
NYCF 3	jammy, earthy	Balanced acid, fruit forward, black berry	Medium 3	Clean light
NYCF 4	dried fruit, fruit forward, caramel	cranberry, fruit, cherry, robotussin	Medium 3	Long complex
NYCS 1	plum, floral, sulfur	cranberry, ripe acid,	Medium 4	Acidic
NYCS 2	plum, sour cherry	cranberry, tobacco, earth	Medium 3+	Acidic
NYCS 3	raspberry, acetone, currant, tart acid	light acid, raspberry, strawberry	Medium 3-	Short mineral
NYCS 4	light raspberry, earth, floral geranium	watermelon jolly rancher, raspberry	Medium 3	Fruity

The micro fermentations varied significantly within each series, showing that yeast choice did have a significant impact on aroma, flavor, body, and finish. By arranging the frequency of tasting terms that were used for each yeast a rudimentary understanding of how each yeast acts.

Flavor descriptors for each yeast, with the frequency noted beside each descriptor. To improve the study a better more focused survey could be developed with a narrower defined set of flavors to better capture the set of flavors expressed in tasting notes. A double blind experiment would also improve the results. Repeatability was not tested in this experiment and would be an interesting to see the same study completed for each sample in triplicate to determine the variance within each setup. This experience was tremendously enjoyable and proved the value of the method for evaluate

Yeast strain	Aroma	Flavor
D254 (3)	Floral (2), citrus, melon, raspberry (2), acetone, currant, geranium, acetone, tart acid	raspberry (2), off sweet, lemon grass, under ripe pineapple, watermelon Jolly Rancher, light acid
D 47 (2)	ethene, rotting apples, acetone, citrus, melon, floral	acetone, rancid almond, oxidized, off sweet, lemon grass, under ripe pineapple
K1-V1116	pungent, banana, woody	vanilla custard
RC 212 (3)	plum (2), ripe fruit, boyson berry, tart raspberry, sour cherry	Robotussin, VA, Acidic, strawberry, raspberry, herbaceous, salty plum, cranberry, tobacco, earth
D 80 (3)	plum (3), sour cherry, raspberry, cherry, H ₂ S, menthol,	Earthy (2)Menthol, red berry, cranberry, strawberry, raspberry, tobacco,
BM 4x4 (2)	fruit forward (2), dried fruit, earthy, caramel	fruit (2), cherry, black berry, cranberry, robotussin

Cider Tasting

The cider micro fermentations sensory descriptors. Again some flavors jumped out as particularly appealing less than appealing. The cider results, when organized by process variable did not show evidence strong evidence of a yeast dominated flavor profile. Results also did not seem to be a strong function of apple strain. The results showed that each sample was differentiable, but there seems to be a complex interaction occurring that is beyond the grasp of this author. Recommendations for future research would definitely include performing all experiments in duplicate so that random variation could be ruled out of the analysis.

Table 12: Cider tasting notes, ranked by process viable of interest

ID	PV	aroma	flavor	Acidity	Body	Finish
1	71 B	apple seed, floral	Bitter, acidic, straw,	acid+	medium +	chalky
9	71 B	buttery, chardonnay	tart grape, copper, balanced sour	medium	medium	lingering
2	QA 23	Bright acid, apple sauce, sweet	Sweet maple, sour apple, green wood	balanced	medium	sweet
10	QA 23	nail polish, honey suckle, melon	Grape, tart, apple sauce	light	medium	light but smooth
3	K1-V1116	Floral, apple seed	apple skin, petroleum jelly, chemical	light	medium	chalky, sour
11	K1-V1116	yeast, honey	floral	medium	medium +	light
4	EC-1118	barnyard, yeast,	Yeast, straw, apple blossom	light	light +	none
12	EC-1118	yeast, roasted nuts, fruit forward	balanced, straw, yeast, mineral water	light	medium	light clean
5	GD	peach, pear, honeysuckle,	apple, petroleum, balanced	light	medium	soft yeast
6	GD, Mac	mead, honey suckle, melon	sweet apple pie, dough, straw,	light	medium	soft rich
7	GD, Gala	bread, pastry	light, white grape, flour	light	light +	soft yeast
8	GD, Mac, Gala	apple seed, green grass, lemon	tart apple pie, flour, menthol	medium	medium	soft yeast

13. Conclusions

13.1 Research Method

Micro fermentation is a useful method for creating and evaluating process variables.

Better temperature and process control could yield better results.

Gas chromatography is a viable method for determining the concentration of compounds in the wine.

48 compounds were characterized in wine, and 178 compounds were found in a hard cider.

Improvements in chromatography could come from installing a better column or better analytics.

13.2 Scalability

The scalability of the process was confirmed by tasting the commercial, test and micro fermentations.

This could be further refined by controlling for additional environmental variables, such as light, temperature and filtration in the future.

13.3 Yeast selection

Recommendations for yeast were made based upon the results of the micro fermentations.

The yeast strains D-47, K1-V1116 and BM 4x4 were associated with more ripe fruit flavors and creamier textures.

The yeast strains RC 212 and D80 brought more acidic and fruity flavors to mind

Section III

14. Recommendations

14.1 Beverage Engineering Lab Proposal

While this project was able to deliver results to the sponsor and much was learned about micro process systems in winemaking, a far greater set of variables remain to be studied in further detail. High interest in this area of research has been expressed by WPI students and at this time 14 students in 5 project groups have signed on for wine MQP projects in the 2014-2015 academic year. Several project proposals are outlined here as well as general recommendations for what could become WPI's newest research lab. This research lab is tentatively proposed as the WPI Beverage Engineering Lab.

One possible benefit of this lab would be to increase appreciation for the craft of producing fine alcohol and in encouraging responsible alcohol consumption. A possible opportunity for an off campus wine appreciation club could also serve to promote the recreational tasting and critical evaluation of wines. Students in this lab should show a desire to improve the state of responsible alcohol consumption among their peers. Students should show leadership on and off campus in reducing reckless alcohol consumption and changing the attitudes of their peers. The social mission of this lab should not be understated, and the reckless behavior of any student in this lab would seriously endanger the important and stimulating research that should be conducted here. This may be the most important of the missions within the WPI community.

WPI's envisioned beverage engineering laboratory could be a tremendous asset to the department and an active area of research. Students, faculty, general public and potential employers have all expressed interest in the research conducted and a general enthusiasm for applying engineering to alcohol production was common. Of the three students working on alcohol projects this year, Danielle Dechaine and myself accepted job offers from Gallo Winery and Ricker Hill Cidery, respectively. Research opportunities in alcohol are abundant and the benefit of such research is usually a tangible benefit to consumers.

Several facility upgrades would benefit such a lab tremendously, but research could be successful so long as there are dedicated and passionate people working together. A dedicated laboratory space for food safe micro fermentation and wine handling would be a great step in upgrading the on campus research facilities. Better ambient temperature controls for micro fermentation temperature would improve the quality of the studies and reduce uncertainty in results. This space could be shared between several groups and might allow better exchange of ideas between groups. Members of the beverage engineering lab, as an integral part of their education, should meet off campus periodically for wine tasting and palate training.

14.2 Project Topics

Gas Chromatography and Analytic Chemistry

A group should be dedicated to analytic chemistry and the analysis of the data produced by it. A new column for the GC, tentatively identified as the carbowax type, should be purchased to improve the sensitivity of the GC to the flavor compounds of interest. Analytical tests described by OIV methods could also be used to study wines

High Performance Liquid Chromatography

A group could explore the possibility of using HPLC to measure sugar profiles of wine must and finished wines to measure sugar content. One barrier for this project is obtaining HPLC time and the training required to use the technique.

Micro Process Wine

The list of process variables of interest in micro vinification are of no end. Zoll cellars has grape vines, so harvest date and sun exposure on the vine could be a micro study. Fermentation factors that could yield informative results include changing the pH, temperature, nutrient levels, addition of oak, and skin contact time. The blending properties, container variation and post fermentation processing are elements of winemaking finished wine that is an interesting field.

Process Engineering Cider

The author of this project is going to work for Ricker Hill Farms in Turner, Maine to design and run a hard cider process. The first year of operation at the cidery is bound reveal problems in production that students may be interested in solving as a project. Challenges to this project are physical distance to the project site and the undetermined nature of the project. The candidate sponsor has expressed interest in hosting a project. This project may be considered more suitable for students with an interest in plant startup or process troubleshooting, skills that are highly valued by employers in an increasingly competitive labor market

Sensory and Analytical Testing Survey

One of the more interesting aspects of this project was the tasting evaluation. For students with a strong interest in winemaking as a profession a trained palate is critical. Developing methods to evaluate wine character using the literature and analytical chemistry methods would benefit other groups in the beverage engineering lab by giving them a tool to evaluate their creations.

Home Brewing Design

Students may wish to broaden the audience of brewers by designing home brewing setups and popularizing the craft among the general campus community. Outreach and popularization of home brewing could be an important goal of this project. Recipe design, cost analysis and marketing strategies would be primary design goals.

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