Management Strategies for Natural Cider Fermentation: Effects of Sulfite Addition and Acidification in High- and Low-Tannin Cultivars

Isabelle Haser

Thesis submitted to the faculty of the Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of

Master of Science in Life Sciences

In

Food Science and Technology

Amanda C. Stewart, Chair

Jacob Lahne

Elizabeth Chang

April 17, 2023

Blacksburg, Virginia

Keywords: Cider, Pied de Cuve (PDC), Apple Cultivars, Descriptive Analysis, Fermentation Management

Management Strategies for Natural Cider Fermentation: Effects of Sulfite Addition and Acidification in High- and Low-Tannin Cultivars

Isabelle Haser

Academic Abstract

Virginia is the largest apple producing state in the Southeast region of the United States and ranks 10th in most cideries in the US. Natural, or un-inoculated, fermentation methods are of interest to cider producers due to the potential for generating unique and complex aromas and flavors via fermentation with naturally present microbiota. The objective of this study was to determine the effect of common pre-fermentation sulfite addition and pH adjustment on cider chemistry and sensory outcomes for naturally fermented high- and low-tannin apple cultivars. Four treatment conditions were applied to both the high- and the low- tannin cultivars: acidification only, sulfites only, acidification and sulfites, and a control with no pre-fermentation juice chemistry adjustment. The eight experimental ciders were fermented using the Pied de Cuve (PDC) method for natural fermentation. Cider chemistry and sensory parameters were determined, and the treatments imparted key differences in both. Key findings were analyzed for pH, titratable acidity, volatile acidity, malic acid, free/total SO_2, yeast assimilable nitrogen (YAN), total polyphenols, residual sugars, and ethanol. For the acidified condition, the pH was lowered to 3.2 using malic acid. Cider pH ranged from 3.36 ± 0.04 to 3.72 ± 0.07, reflecting a general trend toward rising pH over the course of fermentation. Juice tannins were 0.244 ± 0.003 g/L for Harrison and 0.12 ± 0.01 g/L for GoldRush. Tannins decreased during fermentation; however, Harrison ciders maintained a higher range compared to GoldRush. Sensory characteristics were determined using a Descriptive Analysis (DA) with a trained panel which produced 28 descriptors. Results were examined via analysis of variance (ANOVA) and significant differences for apple cultivar, acid adjustment, and sulfite use were found for both chemistry and sensory parameters. The interaction between high- and low- tannin content and sulfite use had the most impact on the cider chemistry and sensory attributes. This study helps to shed light on the extent to which pre-fermentation pH adjustment and/or sulfite additions can influence the outcomes of natural cider fermentation in both high- and low-tannin cultivars.

Management Strategies for Natural Cider Fermentation: Effects of Sulfite Addition and Acidification in High- and Low-Tannin Cultivars

Isabelle Haser

General Audience Abstract

Cider, also known as "hard cider," is an alcoholic beverage fermented from apples. Virginia is the 6th largest apple producing state in United States and ranks 10th in number of cideries. Natural fermentation uses microorganisms that are present in the environment to ferment cider. This type of fermentation is of interest to the cider industry due to the unique aromas and flavors produced by this method. The objective of this study was to determine the effect of common fermentation management strategies: pre-fermentation sulfite addition and acid adjustment, on cider chemistry and sensory outcomes for naturally fermented high- and low-tannin apple cultivars. Eight experimental ciders were fermented using the Pied de Cuve (PDC) method, which is a type of natural fermentation. Cider chemistry and sensory outcomes were evaluated. The experimental treatments and their interactions imparted key differences in both chemical and sensory outcomes. Cider pH ranged from 3.36 ± 0.04 to 3.72 ± 0.07 reflecting a general trend toward rising pH over the course of fermentation. Juice tannins were 0.244 ± 0.003 g/L for Harrison and 0.12 ± 0.01 g/L for GoldRush. Tannin concentration generally decreased during fermentation; however, Harrison ciders maintained a higher range compared to GoldRush. Sensory characteristics of each cider were determined using a Descriptive Analysis (DA) study, a with a trained panel which produced 28 descriptors, 19 of which were significant. The interaction between high- and low- tannin content and sulfite use had the most impact on the cider chemistry and sensory attributes. This study helps to inform cider producers regarding the impacts of pre-fermentation acid or sulfite additions on natural fermentation, and how those impacts may vary among high- and low-tannin apple cultivars.

Acknowledgements

I would like to thank the following people whose help and support made this project possible. Firstly, I would like to thank my advisor, Dr. Amanda Stewart, who guided and supported me through my research. I would also like to thank the other members of my committee, Dr. Jacob Lahne and Dr. Beth Chang, who provided insights and helpful suggestions through my research. I would also like to thank the Virginia Wine Board for providing funding for this research.

I would also like to thank the faculty and staff of FST. Especially Ann Sandbrook for providing the necessary training to help the chemical analysis run smoothly, and Dr. Ken Hurley for assistance on some tests. Dr. Martha Calvert also deserves thanks for her assistance with data analysis. I would like to thank the rest of the Stewart lab for assisting me both in and out of lab. They have been incredibly supportive through this whole process and were an amazing support system to me. To the other FST graduate students, thank you for providing a friendly community and being there to answer questions. The undergraduate students who helped in both the Stewart and Sensory Evaluation labs also deserve my thanks. Without your hard work this project would not have run as smoothly and efficiently as it did. I would also like to thank my panelists for their time to make the sensory evaluation possible.

Finally, I would like to thank my friends and family outside the FST department. Your support and understanding during these past two years were invaluable to me.

Table of Contents

Chapter 1: Introduction

Hard cider or "cider" is a fermented beverage made from apples. The term "cider" to refer to this beverage is more common outside the United States and will be used as such in this document. The cider industry has grown significantly over the last decade. Between 2013 and 2018, it was estimated that the industry grew by more than 25% each year (Fabien-Ouellet & Conner, 2018). The growth trend is predicted to continue from 2021 to 2026 at a rate of 2.2% annually (Wood, 2021). This is especially important in Virginia, as this was the state with the most prominent growth in the Southeast region of the United States. In 2018, Virginia was listed as the state with the 10th highest number of cideries (Cidermarket, 2018). Virginia is also the sixth largest apple producer in the nation, with over 200 apple varieties being grown throughout the state (Albermale Cider Works, 2021; Virginia Department of Agriculture and Consumer Services, 2021). Apples are considered in the top 20 most valuable crops produced in Virginia (Virginia Department of Agriculture and Consumer Services, 2021). The apples grown in the state are composed of cider and dessert apple varieties. The variety breakdown is roughly 60 cider apple varieties and 160 dessert apple varieties (Albermale Cider Works, 2021).

The rising popularity of cider has increased the demand for quality cider. One way of producing fine cider is through natural fermentation. This method provides unique flavor and aroma characteristics to the cider through the natural microorganisms that are present in the environment. This style creates ciders that are unique and varied depending on location of production. Changes in microbiota present in different orchards or cidery locations has the potential to impart unique cider outcomes, even if the same apples are used. This effect has been observed in wine production from grapes, but has not yet been reported in cider production research (Bouklich et al., 2016). Cider juice amendments can contribute to the quality of cider

produced through both natural fermentation and through inoculation using active dry yeast. Juice amendments can come in the form of pre-, during, or post-fermentation treatments. Some additions, such as acid or sulfites, serve to ensure microbial stability and increase the likelihood that the fermentation progresses as desired. The interactive effects of these additions on the chemistry and sensory properties of cider have not been thoroughly investigated in cider made using natural fermentation.

Although the cider industry is growing steadily, a lack of research-informed technical information for cider production remains. Some research has been done on natural fermentation in wine, and there is interest in extrapolating these results to natural fermentation of cider. While wine and cider are similar, it is important to determine through experimentation whether the findings of wine research hold true for cider. This past research can provide a useful starting point for areas to investigate. Due to the lack of control during some aspects of the natural fermentation process, pre-fermentation additions may be useful tools to ensure a consistent and quality product is produced even with the uncontrolled or natural fermentation approach. An investigation of the effects of pH and sulfite additions prior to natural cider fermentation on cider chemistry and sensory outcomes will provide a starting point for quality cider production using natural fermentation.

1.1 Research Objectives

The long-term objective of this research project is to better understand how pre-fermentation juice chemistry adjustments will affect the chemical and sensory outcomes of ciders fermented using both high- and low-tannin Virginia apples using the pied de cuve (PDC) or other natural cider fermentation methods.

The overall objective of this project is to examine and understand the relationship between tannin level (high- and low-tannin apples) and pre-fermentation management strategies (sulfites and pH) during the natural fermentation process and how these factors affect the chemical and sensory outcomes of the cider.

Specific Objective 1:

Determine the impact of pre-fermentation sulfite addition and pre-fermentation pH adjustment on cider chemistry for high- and low- tannin apple cultivars made using natural cider fermentation.

Working Hypothesis 1:

Fermentations with both a lowered pH and sulfite addition will favor the growth of naturally occurring present sulfite-resistant Saccharomyces yeast strains and thus have more complete fermentation with lower concentrations of residual sugars in ciders compared to ciders with no pH adjustment (acidification) or sulfite addition, where we would expect more growth of bacteria and other yeast strains that may not be sufficiently ethanol tolerant to complete fermentation. In addition, ciders produced using pre-fermentation sulfite and acid addition will have a lower pH and more malic acid remaining at the end of fermentation through the inhibition of lactic acid bacteria growth and metabolism of malic acid to lactic acid. These ciders will also have a higher ethanol content at the end of fermentation, and a lower volatile acidity due to decreased acetic acid bacteria growth. It is also expected that the interaction between these treatments would magnify the results seen in comparison to ciders with only sulfites or only acid. It is also expected that the interaction between these treatments would magnify the results seen in comparison to ciders with only sulfites or only acid. We hypothesize that the difference between

ciders subjected to these treatments will be harder to discern in the high-tannin ciders, due to the natural antioxidant and antimicrobial activity of tannins.

Specific Objective 2:

Determine the impact of pre-fermentation sulfite addition and pre-fermentation pH adjustment on cider sensory outcomes for both high- and low- tannin apple cultivars during natural cider fermentation.

Working Hypothesis 2:

Ciders with sulfite additions and pH adjustment (acidification) will differ from and have fewer undesirable flavors such as acetic acid and acetaldehyde that result from unwanted bacterial growth and metabolism and oxidation compared to cider without both of these additions. Ciders with a lower pH will have more sour, acidic, or tart characteristics than those with no pH adjustment. Ciders with sulfites added will have less sensory faults such as volatile acidity due to their antimicrobial and antioxidant properties. However, sulfites will impart more bitter and drying characteristics on the ciders through their antioxidant action preserving the naturally present bitter and astringent compounds. Furthermore, high-tannin ciders will have less extensive sensory faults due to the antimicrobial and antioxidant effect of the naturally present tannins. High-tannin cultivars will produce a cider that has a richer flavor and increased astringency as compared to low-tannin cultivars. Ciders with pH adjustments and sulfite additions will be dryer and have a more pronounced ethanol flavor.

Chapter 2: Literature Review

2.1 Cider and Cider Production in the United States

Many parts of the world use the term “cider” to refer to a fermented, alcoholic apple beverage. In the United States the term “cider” is often used to describe unfiltered, unpasteurized apple juice, and the term “hard cider” refers to the fermented apple product (Lea & Drilleau, 2003). For the purposes of this document, the term “cider” will refer to the fermented, alcoholic apple beverage.

The cider industry is on an upward trend in the United States. Since 2012, the number of cider makers has increased from 219 to 2,036 in 2021 (Wood, 2021). In addition, revenue from cider made with apples has risen from $121 million in 2012 to $517.6 million in 2021 and makes up 52% of the cider and apple beverage market. The other sector of the market is comprised of cider made from fruit other than apples, such as pears, as well as nonalcoholic or mixed flavor ciders. While there are cider makers in almost every state, the Great Lakes region is responsible for 26.7% of cider establishments followed by the West coast with 25.2% and the Mid-Atlantic region with 17.4% (Wood, 2021). With the industry showing growth in both revenue and number of producers, more research is needed on cider production, quality, and sensory attributes.

2.1.1 Cider Production

Cider can be produced in a multitude of different ways to reach a desired outcome. Like wine, the result is dependent on the apple cultivars, agricultural practices, juice chemistry, yeast strain, fermentation practices, and other conditions. This review will focus on factors that are involved in the cider fermentation process.

To produce cider, some general processing steps are followed. These are: harvesting, milling, pressing, and fermentation. Apple harvest is dependent on apple ripening which can be measured through a variety of indicators such as skin color, firmness, and the starch content (Downing, 1989). Once the apples have reached their desired ripeness, they will be picked from the trees. The harvest can be done either by hand or using machines, the choice typically depends on orchard size. Mechanical harvesting uses methods, such as shaking, to cause the apples to fall from the trees. The machine will then either catch them or let the apples fall to the ground. Preventing apples from falling to the ground is desirable, as damage from falling greatly increases the chance of disease infection within the fruit (Downing, 1989). While this method is commonly used in other countries, it may not be legal to do so in the United States under the new Food Safety and Modernization Act (FSMA). This prevention aims to lessen pathogen related issues that may stem from contact with the ground (Ewing & Rasco, 2018).

Harvested apples can be stored in refrigerated conditions for several weeks to months before processing. If the fruit is kept in a controlled-atmosphere environment (gas-tight or modified atmosphere and refrigerated) the storage can be extended for seven or more months depending on the cultivar, storage facility, and end product being made (Downing, 1989). Storage may positively affect some types of apples used to make cider due to the conversion of the unfermentable starch into fermentable sugars and can also increase flavanol concentrations in cider in certain cultivars (Ewing et al., 2019; Lea & Drilleau, 2003). If apples are stored, they are considered ready to use when they retain an indentation when pressed with a thumb. Both fresh and stored fruit can be used in cider production. Before processing, apples should be sorted and washed to eliminate debris or rotten fruit (Lea & Drilleau, 2003).

Washed apples are milled before being pressed. This step serves to break down the fruit into a mash using a mill so the juice can be extracted more efficiently from the fruit (Downing, 1989; Lea, 2015). The mash is then pressed to extract the juice from the pomace. This can be done in several ways depending on the scale and process type needed. Some common methods include basket presses, hydraulic presses, and belt presses. Pressing aids such as rice hulls may be added to the mash to add firmness and provide channels for the juice to exit. More press aid is needed for stored apples than fresh ones (Downing, 1989). Clarified juice can be achieved through the addition of the pectinase enzyme as well as optional further filtration steps (Lea, 1995). The apple juice can then be fermented using the natural yeast microflora that are present on the apple, or more commonly, through the addition of *Saccharomyces cerevisiae* (*S. cerevisiae*). After fermentation is complete, cider is racked (separated) from the lees (dead yeast). This may occur directly after the fermentation is complete or after an extended maturation period. The cider is then clarified though natural yeast settling, centrifugation, or the use of a fining agent. Fining agents include bentonite, gelatin, isinglass, or chitosan. After clarification, commercial ciders may then be blended for uniformity or with water to achieve the proper alcohol content for sale. (Lea & Drilleau, 2003). Some microbes may cause defects in the cider as they metabolize components of the cider during maturation, storage, and even after bottling. Many of the common cider sensory faults arising due to unwanted microbial growth and metabolism may be prevented using pH adjustments and SO_2 before fermentation. These adjustments can lend competitive advantage to the desirable microorganisms and inhibit unwanted microbial growth. After fermentation, fining agents may be used to remove bacteria before waiting for the organisms to die. The cider may also be pasteurized to ensure shelf life

and microbial safety. Carbonation may be added to some ciders to achieve desired results. This is done through saturating the juice with CO_2 (Downing, 1989).

2.1.2 Natural Yeast Fermentations

The traditional way of making cider involves no external source of yeast, or inoculation. Rather than add yeast, the microbiota naturally present on the apples is relied upon to start the fermentation. Yeast cells are present on the order of 5×10^4 cells/g stored fruit(Lea & Drilleau, 2003). This will result in spontaneous fermentation of the juice within a few hours if the temperature is above 10 °C (Beech, 1993; Lea & Drilleau, 2003). These yeast cells will still be present even after the outside of the apples have been thoroughly washed, due to interior microflora presence, or via adhesion to the waxy exterior of the fruit. The minimum concentration of yeast in juice after pressing has been found to be 10^4 g/mL (Lea & Drilleau, 2003). Letting this natural yeast ferment the cider is one way of conducting a natural fermentation and is the most common method for natural fermentation used in wine and cider making (S. C. Morgan et al., 2019).

Pied de cuve (PDC) is another strategy to produce a natural fermentation. This term is used to describe two different methods of indirect inoculation. One method is to inoculate using a must inoculum that is already fermenting. The other method is to create an inoculum from spontaneously fermenting must in the vineyard or orchard, away from the influence of yeast strains present in the production area. The second method is used to increase the prevalence of non-*Saccharomyces* yeasts (S. C. Morgan et al., 2019). The microbial diversity of fermentations undertaken by commercial yeast do not represent the complexity of the microbial populations that can be found from spontaneous fermentation. (Moschetti et al., 2016). In natural cider fermentation, the first few days are dominated by non-*Saccharomyces* species which multiply

quickly. As the alcohol level rises, the initial fermenters die out and are replaced by *Saccharomyces* spp. (Lea & Drilleau, 2003; Valles et al., 2007). The yeast strains differ based on region or "terroir." In wine fermentations, wine made from identical grape cultivars but grown in different regions have distinct features (Bouklich et al., 2016). While other *Saccharomyces* spp. are present for the beginning and middle of fermentation, *S. cerevisiae* is predominant in the final stages of fermentation (Lea & Drilleau, 2003; Valles et al., 2007). If SO_2 is added to the apple juice prior to fermentation, other *Saccharomyces* spp. are likely to take over and ferment the cider to dryness with more homogenous microflora (Lea & Drilleau, 2003).

PDC can produce flavors that may not be present in cider fermented with a commercial yeast (Moschetti et al., 2016). The resulting product can be more intense and complex with a fuller, rounder palate. These effects may be attributed to the sugars left in the cider due to the fermentation stopping before it reached dryness (Zoecklein et al., 1995). These distinctive flavors are characterized by ethyl acetate, butyrate, and other related esters (Lea & Drilleau, 2003). Terroir of the fermentation will also impact the flavor profile of the cider. "Terroir" refers to regional differences in natural environment such as soil, climate, and topography. In a study of wine and wine grapes, it was found that both the grape microbiota and the wine metabolite profiles can be used to distinguish viticultural areas and individual vineyards. These differences can be found due to changes in sensory and chemical outcome of the wine (Bouklich et al., 2016). Due to the similarity in methods, the importance of terroir for cider characteristics would be the same as wine.

2.2 Apple Juice and Juice Additions

Cider production begins with apple juice. The juice can be fermented fresh or from apple juice concentrate (AJC). In addition to different degrees of juice processing prior to

fermentation, different apple varieties can be used in cider production as well. It is more common in the United States to use dessert apples, apples grown to be consumed fresh, for cider production but cider apple varieties are also used (Cline et al., 2021; Downing, 1989; Lea & Drilleau, 2003). This is likely due to availability, rather than any specific preference for these types in cider production. There are four classifications of cider apple, determined by tannin and acid content: sharp, bittersweet, bittersharp, and sweet (Lea & Drilleau, 2003). Tannic acid is responsible for the "bitter" term and is used for fruit containing >0.2% tannic acid equivalents. Malic acid is used to define the "sharp" term and fruits with >0.45% titratable acidity are considered sharp (Lea & Drilleau, 2003; Thompson-Witrick et al., 2014). There are benefits associated with using cider apples such as their high sugar levels, storage capability, high tannin levels, acidic range, and fibrous structure for ease of pressing (Lea & Drilleau, 2003). Tannins are key to the cider's mouthfeel and body by contributing to bitterness and astringency (Bamforth, 2005; Downing, 1989; Lea & Drilleau, 2003). These compounds are a range of oligomeric procyanidins that can fall into two categories: hydrolyzed and condensed (Lea & Drilleau, 2003; Zoecklein et al., 1995). Larger tannins are more bitter while smaller tannins are more astringent (Lea, 2015). Ciders are typically made through blending juice from different cultivars to ensure a proper balance of sugar, acid, and tannin can be achieved. This method also is more practical for orchard growing and harvesting (Lea & Drilleau, 2003).

2.2.1 Additions and Treatments

Prior to fermentation, the juice is prepared through additions and treatments with effects such as altering the sugar level, yeast nutrient level, pH, and tannin level. These treatments ensure that the fermentation can progress as desired and aim to avoid unwanted off-aromas.

Yeast fermentation converts fermentable sugars into alcohol. This is mainly through the fermentation of glucose and fructose. Sucrose must first be hydrolyzed into its components of glucose and fructose before it can be used (Zoecklein et al., 1995). Many yeast strains express invertase enzymes that can quickly hydrolyze sucrose to glucose and fructose (Zoecklein et al., 1995). Apples have sugar concentrations ranging from 9g/L to 32g/L for glucose, 66 g/L to 96g/L for fructose, 9 g/L to 55g/L for sucrose, and 111g/L to 164g/L for total sugars. These ranges are dependent on cultivar, apple maturity, storage time, growing area, and many other factors (Fuleki et al., 1994; Jackson & Lombard, 1993; Karadeniz & Ekşi, 2002). Sugar accumulates naturally within apples as they mature and are stored (Fuleki et al., 1994; Jackson & Lombard, 1993). To ensure consistency between fermentations or to achieve a specific style, sugars can be added pre-fermentation in the form of beet sugar or cane sugar (Karadeniz & Ekşi, 2002; Lea, 2015) where permitted by local regulations.

Producers may also choose to add yeast nutrients to their juice prior to fermentation. These nutrients may aid in preventing stuck or sluggish fermentations (Ángeles Pozo-Bayón et al., 2009; Bell & Henschke, 2005; *Cider Handbook*, 2018). These additions have been shown to increase the concentration of major nitrogen compounds such as yeast assimilable nitrogen (YAN) in wine. An increase in YAN decreases the likelihood of stuck or sluggish fermentations as well as decreased production of undesirable compounds such as hydrogen sulfide (H_2S). This has to be balanced, however, as excessive YAN remaining in the cider after completion of fermentation also creates the risk for microbial instability and problems with aging. This can be mitigated through the use of diammonium phosphate (DAP). DAP helps develop the yeast population while limiting H_2S and may enhance some of the sensory characteristics of the wine through increased acetate ester concentration (Bell & Henschke, 2005; Gobbi et al., 2013).

Juice pH plays a vital role in ensuring proper fermentation and microbial stability. A pH above 3.8 can lead to microbial contamination and a low-quality final product with color instability and poor taste (Beech, 1993; Jackson & Lombard, 1993; Kodur, 2011; Zoecklein et al., 1995). A high pH also reduces the free sulfur dioxide (SO_2) content in the juice which can impact aging abilities (Jackson & Lombard, 1993). To lower the pH of the juice, exogenous malic acid (the weak organic acid naturally present in apples) can be added to adjust pH within the desirable range of 3.3 to 3.8, then SO_2 can be added (Lea, 2015; Zoecklein et al., 1995). SO_2 is added to inhibit the growth of unwanted bacteria and yeast (Zoecklein et al., 1995). Many selected yeast strains used in cider fermentation are selected for resistance to moderate concentrations of SO_2, which would be inhibitory to most environmental yeast and bacteria (Lea & Drilleau, 2003; Sydney C. Morgan et al., 2019; Zoecklein et al., 1995). While SO_2 is added to the juice, some is also produced naturally by the yeast during the fermentation process. A study examining *S. cerevisiae* found that 20 strains out of 250 produced more than 25 mg/L SO_2 with 5 strains producing 60 to 70 mg/L SO_2 (Zoecklein et al., 1995).

Tannin addition is a widely accepted winemaking practice that has been adopted in the cider industry as well. Commercially available tannins can be selected for addition based on desired outcomes of mouthfeel, structure, color, perception of sweetness, texture, and improved aging ability (*Cider Handbook*, 2018; *Scott Laboratories 2021 Winemaking Handbook*, 2021). These additions can be composed of oak, chestnut, grapes, or other plant material (*Cider Handbook*, 2018; Sanz et al., 2008). Some research has been done on the efficacy of tannins in wine production. It was found that tannins provided an increase of anthocyanin-derived pigments in red wine while another showed no significant difference in color (García-Estévez et al., 2017; Parker et al., 2007). The color of cider is more related to oxidation or degradation, however, and

it is possible to create a high-tannin cider that is extremely light in color if oxidation is completely inhibited (Lea & Drilleau, 2003). Cider apple tannin composition is a topic of current research, and the tannins found in cider apples are of different structures and concentrations compared to those found in white grapes (Ma et al., 2019; Thompson-Witrick et al., 2014). Some tannins such as ferulic acid, when present in sufficient concentration, can even have an anti-fermentative effect on cider fermentation. (Cairns et al. JASBC 2022)(Cairns et al., 2022). The extent to which tannin composition and concentration affect cider fermentation remains a topic of current research.

Sulfites are another pre-fermentation juice addition in cider. This chemical serves as an antimicrobial and antioxidant during the fermentation process. It has been an addition during the winemaking process for hundreds of years (Sydney C. Morgan et al., 2019). While both sulfites and sulfides, in the form of hydrogen sulfide (H_2S), contain sulfur, these compounds play different roles in the fermentation and flavor profile of cider. H_2S is an undesirable odor-active compound with flavors reminiscent of rotten eggs (Zoecklein et al., 1995). Sulfites are an intermediate in the reaction to form methionine and cystine. This reaction can produce some sulfites that contribute to the overall SO_2 concentration as stated above (Zoecklein et al., 1995). If too much SO_2 is added, yeasts may enter the viable but nonculturable (VBNC) state. While this is not common for commercial yeasts as they are selected to withstand SO_2 levels, it may happen for wild yeast strains(Sydney C. Morgan et al., 2019). In a study using two commercial *S. cerevisiae* strains, SO_2 addition was found to impact acetaldehyde and the rate of H_2S production differently in each strain. SO_2 was also found to impact volatile aroma profiles in wine for one of the yeast strains but had no impact on the other. This suggests that SO_2 utilization and resistance is yeast strain dependent (Sydney C. Morgan et al., 2019). In wine PDC

with no SO_2 added, alcoholic and malolactic fermentations occur at the same time (S. C. Morgan et al., 2019).

2.3 Yeast strain and Hydrogen Sulfide

Hydrogen sulfide production is highly affected by yeast strain used for the fermentation process (Manginot et al., 1998; Thomas et al., 1993; Ugliano et al., 2009). Each yeast strain has a different nitrogen requirement, which results in a different amount of H_2S produced as a metabolic intermediate of the sulfide reduction sequence (SRS) pathway (Bell & Henschke, 2005; Manginot et al., 1998). The SRS pathway is initiated when methionine and cystine are depleted during early fermentation. It serves to produce sulfur containing amino acids that are needed during yeast growth, namely cysteine, methionine, and glutathione (Bell & Henschke, 2005).

Both apple and grape juice often lack sufficient concentrations of methionine and cystine to prevent the activation of the SRS pathway, with apple juice being more often deficient than grape. In the conditions these juices present, the SRS pathway will cause the reduction of sulfate to sulfide which will bond to *o*-acetyl homoserine (OHS) and *o*-acetyl serine (OAS) to form methionine and cysteine respectively (Bell & Henschke, 2005; Swiegers & Pretorius, 2005). When there is insufficient nitrogen, H_2S will not have OHS or OAS to bind do and will instead diffuse into the fermenting juice (Bell & Henschke, 2005; Jiranek et al., 1995). H_2S is also released at the end of fermentation when yeast cells experience autolysis, which is more likely to occur in a stuck or sluggish fermentation. H_2S is highly reactive and can generate many compounds that negatively affect flavor, such as mercaptans, dimethyl sulfide, and other volatile sulfur compounds (VSCs) (Swiegers & Pretorius, 2005; Ugliano et al., 2009). It has been found

that methionine pre-fermentation additions can mitigate H_2S and the off flavors associated with its production (Boudreau IV et al., 2017).

H_2S is the most studied VSC due to its association with off flavors of rotten egg and putrefaction. The amount of VSCs produced during fermentation relies on yeast strain, available nutrients, metal ions, and other factors (Ugliano et al., 2009). After fermentation, VSCs can continue to be produced in bottled product with amino acids present. This can result in a higher final concentration of H_2S than was apparent when bottling (Marchand et al., 2000). While H_2S can be beneficial in low concentrations, the easiest way to prevent the formation of off flavors is to stop H_2S from being produced during fermentation, rather than to implement remedial measures after the fact which could themselves negatively impact cider aroma by stripping favorable aromas in addition to H_2S related faults. Some additions such as copper fining agents and post fermentation additions of glutathione that are used to reduce H_2S and SO_2 respectively can contribute to increases in H_2S during storage (Chen et al., 2017; Ugliano et al., 2009). In addition, pre-fermentation Cu^{2+} and SO_2 treatments for wines that were not previously treated with either substance also showed increased VSC production (Bekker et al., 2016). Additional research needs to be conducted to fully understand the methods that occur post-bottling to produce H_2S.

2.3.1 Sensory Attributes of Hydrogen Sulfide

During fermentation, byproducts are produced that impact the flavor of the final product. VSCs are common negative byproducts that produce off-flavor of reduction. Reduction flavor descriptors include cabbage, rotten egg, sulfurous, garlic, onion, and rubber. These attributes can be detected even at low concentrations, which leads to negative sensory experiences and poor quality (Franco-Luesma et al., 2016; Marchand et al., 2000; Swiegers & Pretorius, 2005). While

these descriptors are based on wine studies, similar fermentation methods and yeast strains are used for cider fermentation. There is a gap in knowledge, however, about VSCs in cider, as the difference in chemistry between grape and apple will influence sensory outcomes of fermentation.

Of the negative sensory attributes from VSCs, H_2S is the most commonly recognized. It is also commonly detected in low concentration of 50-80 µg/L (Swiegers & Pretorius, 2005). In addition to producing negative aromas, H_2S can also mask positive aroma compounds. A study showed that concentrations of 0-40 µg/L H_2S significantly decreased citrus, fruits, and floral attributes in wine. At the same time, rotten egg, rotten onion, and other reduction flavor attributes were increased in the wine (Franco-Luesma et al., 2016).

2.3.2 Quantification of Hydrogen Sulfide

There are many methods to measure H_2S. An older method involved cadmium hydroxide traps in a dark room to avoid the photooxidation of cadmium sulfide. Colorimetric analysis had to be conducted immediately on each trap. This method required traps to be replaced every 24 hours and is time consuming, costly, and involves toxic chemicals (Acree et al., 1971; Thomas et al., 1993; Ugliano & Henschke, 2010; Vos & Gray, 1978). Another method is the collection of H_2S on paper tapes containing silver nitrate, lead acetate, mercuric chloride, or dicyanoargentate. These strips change color in the presence of H_2S, and resulting color density can be related to the concentration of H_2S in the sample. This method is less expensive than using glass traps, however, collection can only be performed at one-hour intervals, and is therefore incomplete over the whole fermentation (Natusch et al., 1974; Ugliano & Henschke, 2010).

The current method of sampling H_2S uses transparent tubes packed with a medium that changes color in the presence of H_2S. This method allows for continuous quantitative measurement of fermentation. These tubes are pre-calibrated to a known H_2S concentration with a linear relationship (r^2=0.9997) between the known concentration and the tube reading (Park, 2008). An airtight seal is formed around the fermentation vessel with the H_2S detector tube as the only exit. CO_2 produced during fermentation serves as a carrier for the H_2S to enter the detection tubes. It has been found that there is no interference from mercaptans or SO_2 produced during fermentation. In addition, 90-98% recovery was achieved for varying H_2S concentrations when using detector tubes (Ugliano & Henschke, 2010).

2.4 Yeast Assimilable Nitrogen and Hydrogen Sulfide Production

2.4.1 Yeast Assimilable Nitrogen

Slow or "sluggish" fermentations could be due to a lack of nitrogen. An incomplete use of sugar present in solution, or a "stuck" fermentation, can also result from insufficient nitrogen. Nitrogen is not assimilable to yeast in the gaseous form, rather it must be available to yeast in solution. Low YAN concentration also contributes to H_2S, high alcohol, and lack of favorable compounds produced during fermentation (Boudreau IV et al., 2018; Ingledew & Kunkee, 1985). YAN is composed of ammonium ions and free amino nitrogen (FAN) or primary amino nitrogen (PAN) and is a measure of the nitrogen available for yeast metabolism. Yeast use ammonium and FAN preferentially, however, ammonium can be the only source of YAN for a fermentation (Bell & Henschke, 2005; Boudreau IV et al., 2018). Ammino acids are the predominant FAN source. When ammonium and FAN are used in combination with each other, higher maximum fermentation rates can be achieved. FAN also showed favorable flavor results when used in wine fermentation (Boudreau IV et al., 2018).

Many apple juices have <140 mg N/L YAN. This value is generally considered the minimum recommended concentration for wine production. Currently there is no known minimum YAN for successful cider fermentation, so this value has been adapted by many cidermakers as well (Boudreau IV et al., 2018). In previous wine studies a correlation between soluble solids and YAN was found, such that a higher concentration of soluble solids required a higher YAN concentration (Bisson, 1999). Since cider fermentations contain less soluble solids than wine, it is possible that lower YAN concentrations are required (Boudreau IV et al., 2018). In a PAN and YAN survey of 12 Virginia apple cultivars, it was found that the average YAN and PAN were 59 mg N/L and 53 mg N/L respectively. The highest YAN for these cultivars was found to be 249 mg N/L with 94% of the cultivars having concentrations below 140 mg N/L (Boudreau IV et al., 2018).

2.4.2 Vitamin Deficiencies

Industrial yeast growth uses chemically defined media for cultivation of yeasts (CDMY). Part of this media features vitamins that are required for successful yeast growth. The vitamins in CDMY are riboflavin (B_2), biotin (B_7), thiamine (B_1), pyridoxine (B_6), inositol (B_8), nicotinic acid (B_3), and pantothenic acid (pantothenate or B_5). Para-aminobenzoic acid (B_{10}) is added to promote the growth of yeasts for brewing. The individual amounts of vitamins needed are species and strain dependent, with different *S. cerevisiae* strains requiring different vitamin quantities (Perli et al., 2020). Deficiencies in pantothenic acid and biotin were found to negatively affect fermentation through the excess production of H_2S. Pantothenic acid is required for the synthesis of coenzyme A which makes OAS and OAH. As previously discussed, a lack of OAS and OAH results in increased H_2S production during fermentation. Biotin is a precursor for methionine production (Wainwright, 1970, 1971; Wang et al., 2003). A depletion in methionine

will trigger the SRS pathway which produces H_2S (Bell & Henschke, 2005). Biotin uptake is also essential for yeast growth and metabolic functions (Gorawala, 2012).

H_2S production can be eliminated through sufficient vitamin addition. With an addition of >160 µg/L pantothenic acid, no H_2S was formed during fermentation (Wainwright, 1970). It was found that addition of 200 µg/L pantothenic acid to fermentation conditions that were deficient would cause H_2S production to cease within 24 hours. This addition was added at 48 or 96 hours after yeast inoculation. These results demonstrate that pantothenic acid additions before or during fermentation can be utilized to minimize H_2S production (Edwards & Bohlscheid, 2007). It was also found that fermentations with a high YAN concentration and high pantothenic acid produced less H_2S than fermentations with high YAN and low pantothenic acid. Despite both YAN and pantothenic acid affecting the same metabolic pathways involving H_2S production, it is necessary to ensure there is sufficient pantothenic acid as well as nitrogen (Wang et al., 2003).

2.4.3 Amino Acid Deficiencies

Amino acids such as methionine and cysteine have been found to impact cider. As mentioned previously, these compounds play a role as an inhibitor in the SRS pathway, which will lower H_2S produced during fermentation. However, interactive effects of yeast strain and total YAN have been seen (Bell & Henschke, 2005; Boudreau IV et al., 2017; Wainwright, 1970). When amino acids are the only source of nitrogen, methionine and cystine additions have been shown to increase H_2S liberation (Jiranek et al., 1995). Methionine additions to wine in conjunction to other nitrogen sources was found to decrease H_2S formation. Additions at a rate of 5 mg/L methionine decreased H_2S production when YAN was 53 mg/L, but when YAN was 153 mg/L, 50 mg/L methionine was required to decrease H_2S production. This is still strain

dependent, however, as some yeast strains showed no decrease in H_2S production at any concentration of methionine. This study proposed that higher YAN concentrations increased cell biomass, and therefore more methionine was required to inhibit the SRS pathway (Boudreau IV et al., 2017). These findings demonstrate the importance of understanding the starting juice chemistry of wine and cider.

2.5 Sensory Evaluation of Cider

The accepted sensory evaluation definition according to the Institute of Food Technologists (IFT) and the American Society for Testing and Materials (ASTM) is: a scientific method used to evoke, measure, analyze, and interpret those responses to products as perceived through the senses of sight, smell, touch, taste, and hearing (Lawless & Heymann, 2010; Stone & Sidel, 2004). The field of sensory evaluation of food has grown rapidly in the 20th century. This area of study comprises of a set of techniques for measurement of human responses to foods and beverages. The methods used in this form of testing attempt to isolate the sensory properties of foods and provide information on the sensory characteristics of the food. These methods also serve to provide guidelines for controlled testing conditions to limit biasing factors (Lawless & Heymann, 2010). There are two categories of sensory tests: analytic and affective. Analytic tests focus on collecting information on the properties of the food. Some analytic tests include tetrad tests, triangle tests, and descriptive analysis tests. Affective tests, also known as hedonic tests, focus on collecting information on consumer perceptions of the product. An example of this method is a preference test. The following sections will explore more in-depth applications of sensory evaluation.

2.5.1 Sensory Evaluation Techniques: Difference, Descriptive, and Affective

There are many different sensory evaluation techniques that can be used to determine sensory characteristics of food. One of these methods is descriptive analysis. This method characterizes individual attributes and quantifies their intensities using analytical descriptions, and has been shown to be the most comprehensive and informative sensory evaluation tool (Lawless & Heymann, 2010). The general process for this method is outlined by Lawless and Heymann (2010). This involves training a panel through consensus or ballot training, evaluating panelist consistency, then having panelists evaluate samples (Lawless & Heymann, 2010). The panel should consist of 8-12 trained individuals (Heymann et al., 2012). The small sample size is acceptable due to the high level of calibration of panelists through this process. During calibration, panelists are exposed to all samples and asked to generate descriptors and reference standards. Once these descriptors have been generated, panelists will be asked to rate the intensity of each attribute on a 15-point scale. This will be repeated over two to three sessions to determine the consistency of the panelist. If these results are inconsistent, the panelist will return to the training step. Once the panelists are determined to be consistent, the final evaluation of samples can commence. A randomized set of samples will be presented, and attributes of each sample are evaluated before moving to the next sample. This data is then collected and analyzed (Lawless & Heymann, 2010). The descriptive analysis method has been adapted and variations have been made to suit different studies (Lawless & Heymann, 2010). There are multiple ways to conduct this type of study based on comparing different descriptive analyses (Lestringant et al., 2018).

Difference testing is a simpler method of sensory testing that can be achieved with untrained panelists. This involves testing to see if there is a difference between two types of products. Panelists are asked to sample usually two or three products and determine if there is a

difference, and if so which one is different (Lawless & Heymann, 2010). This was classically seen in the Carlsberg breweries and Seagram's distilleries in the 1940s. These tests involved two products picked from the same batch and one product picked from a different batch. The objective for the panelists was to find the sample from the different batch. This test served to screen beer tasters to ensure they could distinguish between different flavor types. Later, this test was used to ensure consistent quality between batches of product (Helm & Trolle, 1946; Peryam & Swartz, 1950). Data analysis for this method can be performed based on frequency and proportion of correct answers (Lawless & Heymann, 2010).

The third major type of sensory testing is affective testing. This method, also known as hedonic testing, focuses on the degree of liking or disliking a product. This is commonly measured with a 9-point hedonic scale which was developed in the 1940s. This scale usually ranges from dislike extremely to like extremely with a neutral position at the center. A larger panel is necessary for this method in order to achieve statistically significant data. This method allows for more probing into what people specifically like or dislike about the product, such as color or flavor aspects (Lawless & Heymann, 2010).

2.5.2 Development of a Cider Sensory Lexicon

A lexicon is a tool that can be used to help panelists describe the sensory characteristics of cider. A lexicon is defined as a standardized vocabulary that aids in the communication of perceived sensory attributes and can assist in product development, quality control, and in understanding consumer perceptions through the use of consistent language (Drake & Civille, 2003; Lawless & Civille, 2013). The lexicons can be used by panels to consistently describe products. A more well-defined and well-documented lexicon can lead to higher calibration of a trained sensory panel. Panel selection is important for lexicon development as there are many

qualities, such as abstract thinking and flexibility, that aid in the success of forming an accurate lexicon (Lawless & Civille, 2013).

There are many factors that go into making an effective lexicon. First and foremost, it must be descriptive and discriminating. This can be done by using a large and varied sample set when developing the lexicon. Another important quality is a lack of redundancy. Each term should describe its own flavor or aspects of multiple flavors (Drake & Civille, 2003). This cannot be resolved by the sensory panel, however, as the panelists might insist that terms are different when they are actually the same (Lawless & Heymann, 2010). The terms must also be precise and have a clear meaning in order to achieve consistent results. This may be done by grouping terms together to demonstrate which aspect of a particular thing is being referenced, such as burnt/charcoal. To achieve this consistency, references may be used. Reference standards also help lexicons be accurately communicated between research groups (Drake & Civille, 2003). Once terms are made, references can be used to help reach a standardized consensus for the food item in question (Qin et al., 2018).

Only a few lexicon developing studies have been performed on cider. While there are extensive lexicons for beer and wine, the more recent increase in popularity of cider, and a wide range of cider styles at that, has created more of a need for the development of a cider lexicon. A 2018 study by Qin et al. established a lexicon for UK and Scandinavian ciders. This had 23 attributes with 17 for odor, 4 for taste, and 2 for mouthfeel (Qin et al., 2018). A separate study was conducted for Virginia ciders. This was done using a Check-All-That-Apply (CATA) method (Phetxumphou et al., 2020). CATA is a method for rapid sensory profiling which consists of a list of sensory attributes. Subjects only have to indicate presence or absence of descriptors in a product (Varela & Ares, 2012). This study gathered initial terms from cider

descriptors and condensed terms to a smaller set by removing synonyms and non-actionable words. The smaller list was then used in the CATA study. This resulted in a lexicon with 26 attributes with 14 for flavor and aroma, 3 for taste, and 5 for mouthfeel. The flavor and aroma terms are alcohol, beer-like, berries, candies, earthy/musty, fermented apples, floral, fresh apples, fruity, metallic, mild, overripe apples, putrid, synthetic, tart, vegetal/grassy, wine-like, and woody. The taste attributes are bitter, sour, and sweet. The mouthfeel attributes are carbonated, crisp, dry, sharp, and smooth (Phetxumphou et al., 2020).

2.6 Conclusions and research needs identified

The current literature that is available demonstrates that further research in cider fermentation and the effects of fermentation management strategies on the sensory and chemical characteristics of cider is necessary. Many of the sources of information currently available on cider production are limited in scope of apple varieties and conditions employed, and/or borrow substantial information from the research done on wine, which is similar but not identical to cider. There is especially little research on natural fermentations as a method of cider production. The research that has previously been done on both cider and natural fermentation will provide a useful starting point to build upon further through the future research that will be conducted. The impacts of pre-fermentation juice treatments (pH adjustment and SO_2 addition), apple cultivar, and natural fermentation will be assessed to better understand their effects on the chemical and sensory outcomes of the finished cider.

Chapter 3: Pre-Fermentation Management Strategies for Natural Cider Fermentation: Effects of sulfite addition and acidification in high- and low-tannin cultivars

3.1 Introduction

Many parts of the world use the term "cider" to refer to a fermented, alcoholic apple beverage. In the United States the term "cider" is often used to describe unfiltered, unpasteurized apple juice, and the term "hard cider" refers to the fermented apple product (Lea & Drilleau, 2003). Herein, "cider" will refer to the fermented, alcoholic apple beverage.

The cider industry is on an upward trend in the United States. Since 2012, the number of cider makers has increased from 219 to 2,036 in 2021 (Wood, 2021). In addition, revenue from cider made with apples has risen and currently makes up 52% of the cider and apple beverage market. The other sector of the market is comprised of cider made from fruit other than apples, such as pears, as well as nonalcoholic or mixed flavor ciders. With the industry showing growth in both revenue and number of producers, more research is needed on cider production, quality, and sensory attributes. Currently, there is still a lack of research-based information on cider production, specifically for pre-fermentation treatment on natural fermentation methods.

Cider can be produced in a multitude of different ways to reach a desired outcome. Like wine, the result is dependent on the apple cultivars, agricultural practices, juice chemistry, yeast strain, fermentation practices, and other conditions. Past research has shown that apple cultivar is believed to be the main factor that contributes to cider quality and sensory properties (Rosend et al., 2019). Apples fall into three main categories: dessert, culinary, and cider. Dessert apples are typically the best eaten fresh, culinary apples are the best to cook with, and cider apples are typically the best to ferment into cider (Jacobsen, 2014). Cider apples have higher levels of

tannins and acids, and can be further broken down into four classifications determined by tannin and acid content: sharp, bittersweet, bittersharp, and sweet (Jacobsen, 2014; Lea & Drilleau, 2003). Tannic acid is responsible for the "bitter" term and is used for fruit containing >0.2% tannic acid equivalents. Malic acid is used to define the "sharp" term and fruits with >0.45% titratable acidity are considered sharp (Lea & Drilleau, 2003; Thompson-Witrick et al., 2014). Compared to cider apples, dessert apples have a lower tannin and acid concentration, but a higher nitrogen concentration (Lea, 2015; Prolux & Nichols, 2003; Thornton, 2014; Valois et al., 2006; Watson, 2013). Despite the category names, in the United States it is also common to use dessert apples for cider production (Cline et al., 2021; Downing, 1989; Lea & Drilleau, 2003).

Tannins have been shown to play an important role in the mouthfeel and body outcomes of cider. This is done through contributing to the bitterness and astringency of the cider (Bamforth, 2005; Downing, 1989; Lea & Drilleau, 2003). These compounds are a range of oligomeric procyanidins that can fall into two categories: hydrolyzed and condensed (Lea & Drilleau, 2003; Zoecklein et al., 1995). Larger tannins are more bitter while smaller tannins are more astringent (Lea, 2015). Tannins are water soluble polyphenols which exhibit antimicrobial properties (Scalbert, 1991). Past research on polyphenol concentrations in apples show that there is a wide range of polyphenols depending on apple cultivar (Thompson-Witrick et al., 2014). The two cultivars selected for this study, Harrison (high-tannin fruit or HTF) and GoldRush (low-tannin fruit or LTF), have been characterized to have different polyphenol levels. GoldRush was shown to contain less than half the total polyphenols as compared to Harrison, 359(74.5) mg/L galic acid equivalents compared to 926(9.1) mg/L galic acid equivalents (Thompson-Witrick et al., 2014). Differing polyphenol levels have been shown to impact both the fermentation kinetics and cider aroma (Cairns et al., 2022).

Fermentation method is also a key factor in the sensory outcome of cider (Cairns et al., 2022). This study focuses on natural cider fermentation using the Pied de Cuve (PdC) method. This method creates an inoculum from spontaneously fermenting must in an orchard, without the involvement of commercial yeast. This method is used to increase the prevalence of non-*Saccharomyces* yeasts (S. C. Morgan et al., 2019). The yeast strains differ based on region or "terroir." In wine fermentations, wine made from identical grape cultivars but grown in different regions have distinct features (Bouklich et al., 2016). Typically, compared to fermentation inoculated with commercial yeast strains, natural fermentation takes longer to start, and are more prone to becoming stuck or sluggish (Boulton et al., 1999). As a result, ciders made using PdC are less likely to ferment to dry, and will have higher residual sugars as compared to ciders fermented with commercial yeasts (Littleson et al., 2023). PDC can produce interesting and desirable flavors that may not be present in cider fermented with a commercial yeast (Littleson et al., 2023; Moschetti et al., 2016; Zoecklein et al., 1995).

Prior research on PdC cider fermentation of Harrison and GoldRush apples has shown the differences in these apples based on yeast strain, and polyphenol levels (Cairns et al., 2022; Littleson et al., 2023). There is a lack of research, however, on the effects of common fermentation management strategies, pH adjustment and sulfite addition, on natural fermentation. Past research has not, however, examined the effect of sulfites or pH adjustment as pre-fermentation treatments (Littleson et al., 2023).

Apple juice pH plays a vital role in ensuring proper fermentation and microbial stability of cider. Acid content varies depending on the apple cultivar selected (Lea & Drilleau, 2003). A pre-fermentation pH above 3.8 can lead to microbial contamination and a low-quality final product with color instability and poor taste (Beech, 1993; Jackson & Lombard, 1993; Kodur,

2011; Zoecklein et al., 1995). To lower the pH of the juice, exogenous malic acid (the weak organic acid naturally present in apples) can be added to adjust pH within the desirable range of 3.3 to 3.8, then sulfites (SO_2) can be added if desired (Lea, 2015; Zoecklein et al., 1995). Over the course of cider fermentation, the pH will rise, so pH adjustment to the juice can ensure that cider stays within a safe pH range throughout fermentation.

Sulfites are another common pre-fermentation juice addition in cider, serving as an antimicrobial and antioxidant during the fermentation and aging process (Sydney C. Morgan et al., 2019). If SO_2 is added to the apple juice prior to fermentation *Saccharomyces* spp. are more likely to take over the PdC and ferment the cider to dryness with more homogenous microflora (Lea & Drilleau, 2003). Many commercial yeast strains used in cider fermentation are selected for resistance to moderate concentrations of SO_2, which would be inhibitory to most environmental yeast and bacteria (Lea & Drilleau, 2003; Sydney C. Morgan et al., 2019; Zoecklein et al., 1995). While SO_2 is added to the juice, some is also produced naturally by the yeast during the fermentation process. A study examining *S. cerevisiae* found that 20 strains out of 250 produced more than 25 mg/L SO_2 with 5 strains producing 60 to 70 mg/L SO_2 (Zoecklein et al., 1995).

While there have been more sensory studies on cider in recent years, there is less information on cider compared to beer and wine (Heymann & Ebeler, 2017). In recent years, more studies have focused on sensory evaluations of US ciders, especially from Virginia and the Northeast (Calvert et al., 2023; Cole et al., 2022; Kessinger et al., 2021; Littleson et al., 2023). There are many different methods of collecting sensory data, however, this study will use a descriptive analysis (DA) study. This method characterizes individual attributes and quantifies their intensities using analytical descriptions and has been shown to be the most comprehensive

and informative sensory evaluation tool. The general process of this method is panelist training followed by product evaluation (Lawless & Heymann, 2010). The attributes generated will form a lexicon for the ciders, and individual attributes can be analyzed in correlation to chemical analysis and fermentation treatment (Cole et al., 2022; Littleson et al., 2023).

The objective of this study was to determine the effect of apple cultivar and pre-fermentation treatment on the chemistry and sensory properties of naturally fermented cider. These factors were chosen to fill in previously mentioned gaps in knowledge. Ciders were produced using two different apple cultivars and four different pre-fermentation treatments. A DA was conducted on the experimental ciders once fermentation was complete. Standard chemical analysis was performed on both the juice and cider. Sensory and chemical properties of the ciders were analyzed to determine the relationship between the two.

3.2 Materials and Methods

3.2.1 Apples and Apple Juice

Harrison and GoldRush apple cultivars were selected, as they are commonly used for cider production in the Commonwealth of Virginia and represent examples of a relatively high- and low-tannin cultivar, respectively. Harrison, a heritage cider apple cultivar, and GoldRush, a relatively new dessert apple cultivar that is increasingly used for cider production, were purchased from Silver Creek Orchards (Tyro, VA, U.S.A) (Janick, 2001; Watson, 2013). Apples were harvested at commercial maturity and placed in cold storage until being transported to the Virginia Tech Department of Food Science & Technology (Blacksburg, VA, U.S.A) pilot plant 1-3 weeks following the 2022 harvest. The apples were stored at 4 °C in bushel-sized cardboard boxes for three days before being pressed. Each cultivar was pressed separately using a Goodnature X-1 Industrial Cold Press Juicer (Goodnature, Buffalo, NY, U.S.A). A sufficient

amount of apples were pressed until about 75 L of juice was obtained from each cultivar. Juice was collected in cleaned and sanitized 20 L food-grade polypropylene containers. Solids that were present in the juice were not racked off. After pressing, potassium metabisulfite (KMBS) (Presque Isle Wine Cellars, North East, PA, U.S.A) was added to 30 L of juice from each cultivar to a target value of 50 mg/L free SO_2. Any processing or fermentation equipment that could not be autoclaved was sanitized prior to use with Ster-Bac Quat sanitizer (Ecolabs, St. Paul, MN, U.S.A) diluted to a no-rinse concentration per the manufacturer's instructions. Juice was stored at 4 °C for 24 hours until the fermentation protocols described in the Fermentation Methods section (3.2.3) began.

3.2.2 Juice Chemistry

The following methods were employed to measure the chemical parameters of apple juice before fermentation: pH (probe, Thermo Scientific ROSS Ultra Triode Electrode Model 107BNUMD, Thermo Fisher Scientific, Waltham, MA,USA), soluble solids (Extech RF15 Portable Sucrose Brix Refractometer, Extech Instruments, Nashua, NH, USA), total YAN in the form of primary amino nitrogen (PAN) (Megazyme PANOPA Enzyme Kit, Megazyme International, Wicklow, Ireland) and ammonia (Megazyme Ammonia ion (rapid) Enzyme Kit, Megazyme International, Wicklow, Ireland), malic acid (Megazyme L-Malic Acid Assay Kit, Megazyme International, Wicklow, Ireland), initial sugars (Megazyme Sucrose/D-Glucose/D-Fructose Assay Kit, Megazyme international, Wicklow, Ireland), free SO_2 and total SO_2 (AOAC procedure 990.28), total polyphenols (Folin-Ciocalteau assay (Spanos & Wrolstad, 1990)), and titratable acidity (AOAC procedure 962.12 and 936.16).

3.2.3 Fermentation

Prior to fermentation, all equipment was cleaned and sanitized or sterilized using an Autoclave. Sanitizer was Ster-Bac Quat sanitizer (Ecolabs, St. Paul, MN, U.S.A) diluted to a no rinse concentration. The 1-gallon (3.875 L) glass carboys used as fermentation vessels were autoclaved. Stoppers and airlocks were submerged in sanitizer for 25 min. Sanitizer was drained from airlocks prior to use.

This experiment had a full-factorial design with two factors: apple cultivar and pre-fermentation treatment. Juice pressed from the two cultivars, Harrison and GoldRush, were each divided into four parts. Each part received a different pre-fermentation treatment: acidification only, sulfites only, acidification and sulfites, and a control with no pre-fermentation treatment. This resulted in eight treatments overall, each carried out in quadruplicate. This design allowed us to determine if chemical or sensory parameters varied due to the experimental factors (cultivar and pre-fermentation treatments), and/or due to the interaction between factors.

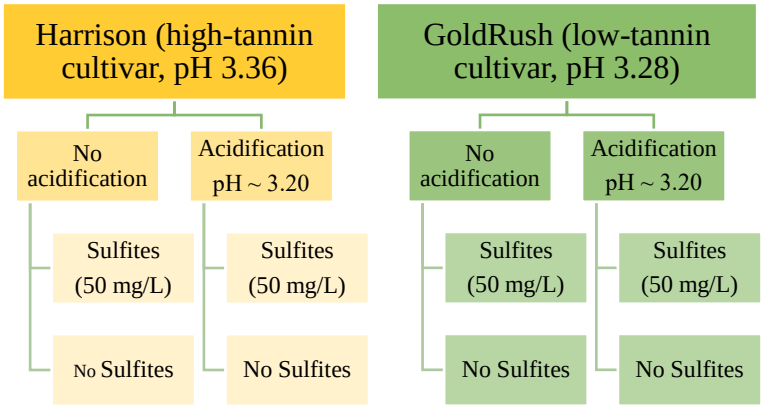

Figure 1: Experimental cider production scheme detailing treatment conditions for all ciders.

Potassium metabisulfite (KMBS) was added to the sulfite addition juice treatments approximately 30 min after pressing. This was added in a sufficient amount to obtain 50 mg/L free SO_2. All juice was stored at 4 °C for four days prior to inoculation. Approximately eight

hours prior to inoculation, juice was removed and allowed to come to room temperature (20 °C). It is possible that microbiota in the juice may have been active in the time prior to inoculation due to the presence of native yeasts in the juice. However, since all vessels were treated the same, we can expect that any early fermentation during this time would be similar among treatments. This was not actively monitored as a part of the experiment.

Prior to inoculation, malic acid (Presque Isle Wine Cellars, North East, PA, U.S.A) was added to juice for acidification pre-fermentation treatment. The pH of the juice was measured, and acid was added to adjust the pH to 3.20. Once added, the juice was mixed, and the pH was re-measured to confirm the pH change.

All fermentations were started using the pied du cuve (PdC) method to obtain a culture of ambient environmental microbiota in a small amount of juice. The PdC inoculum was added to the juice in a 1:3 ratio of inoculum culture: juice. To generate the inoculum, 19 L of juice from each cultivar was placed in a separate, sanitized, food-safe 5-gallon (18.9 L) buckets under a tree

Figure 2. Example of pied de cuve strategy set up at Kentland Orchard.

in an established apple orchard (Kentland Farms Orchard, Blacksburg, VA, U.S.A). A cheese cloth was loosely secured around the top of each bucket to prevent insects and physical debris from entering. Buckets of juice were placed inside a large dog crate which was tied to the trunk of a tree, to prevent animal tampering. The dog crate had ample airflow to ensure microorganisms could still interact with the juice. Juice was allowed to remain in the orchard for 72 h (Figure 2). During this time, temperatures ranged from -1.7 to 21 °C. Time was chosen based on previous research on time sufficient to allow a natural fermentation to begin (Littleson et al., 2023).

Both the juice and inoculum were allowed to come to room temperature before combining. For each treatment type, 3.785 L of inoculum was added to 11.35 L of juice, consistent with the 1:3 inoculation ratio. Inoculum and juice were mixed thoroughly and samples for future analysis were taken at this time. After inoculation, fermentations were conducted in an 18 °C environmental chamber. Each 1-gallon (3.785 L) carboy was filled approximately two-thirds (2.52 L) full. One-third of the volume was left empty to provide adequate head space and room for mixing. A one hole, size 6 rubber stopper and a twin-bubble airlock (The Vintage Shop, Delta, BC, Canada) filled with water were used to close the carboy. During the first two weeks of fermentation, each carboy was mixed vigorously until no sediment could be seen on the bottom three times a day. Mixing was performed by shaking and swirling the carboy to re-suspend the yeast in solution. Care was taken to avoid contaminating the stopper and airlock during this process. Until there were visible signs of fermentation, the stopper and airlock were also removed once per mixing session to allow oxygen to enter. After two weeks, carboys were mixed twice a day, and after three to four weeks, carboys were only mixed once each day.

Fermentation progress was monitored through measurement of the concentration of residual sugars (glucose, fructose, and sucrose). Carboys were mixed then brought into a laminar flow hood. The stopper and airlock were briefly removed. A 2 mL sterile serological pipette (Falcon Serological Pipette 2 mL, Corning, Corning, NY, U.S.A) was used to transfer 1 mL of cider into a labeled 1.5 mL centrifuge tube. Tubes were placed in a -20 °C freezer until the time of sugar concentration analysis. Every three to four days, an additional sample was taken at this time for microbial community analysis (to be completed outside the scope of this thesis). The same procedure was followed as for sugar concentration sampling; however, the centrifuge tubes were autoclaved prior to use. Microbial community analysis samples were also stored in a -20 °C freezer until the time of analysis.

Ciders were bottled once visual signs (e.g., lack of bubbling, separation of layers) and residual sugars indicated that fermentation was complete. Residual sugar testing confirmed the end of fermentation once values did not change for three or more days. Ciders were promptly bottled once fermentation was complete. All four carboys of each treatment were combined in a sanitized food-grade container. A sample of this cider was tested for free sulfites. KMBS was added to each treatment to achieve 25 mg/L free SO_2 regardless of pre-fermentation treatment. Cider was then placed in autoclaved 357 mL bottles and stored at 4 °C until the time of sensory evaluation.

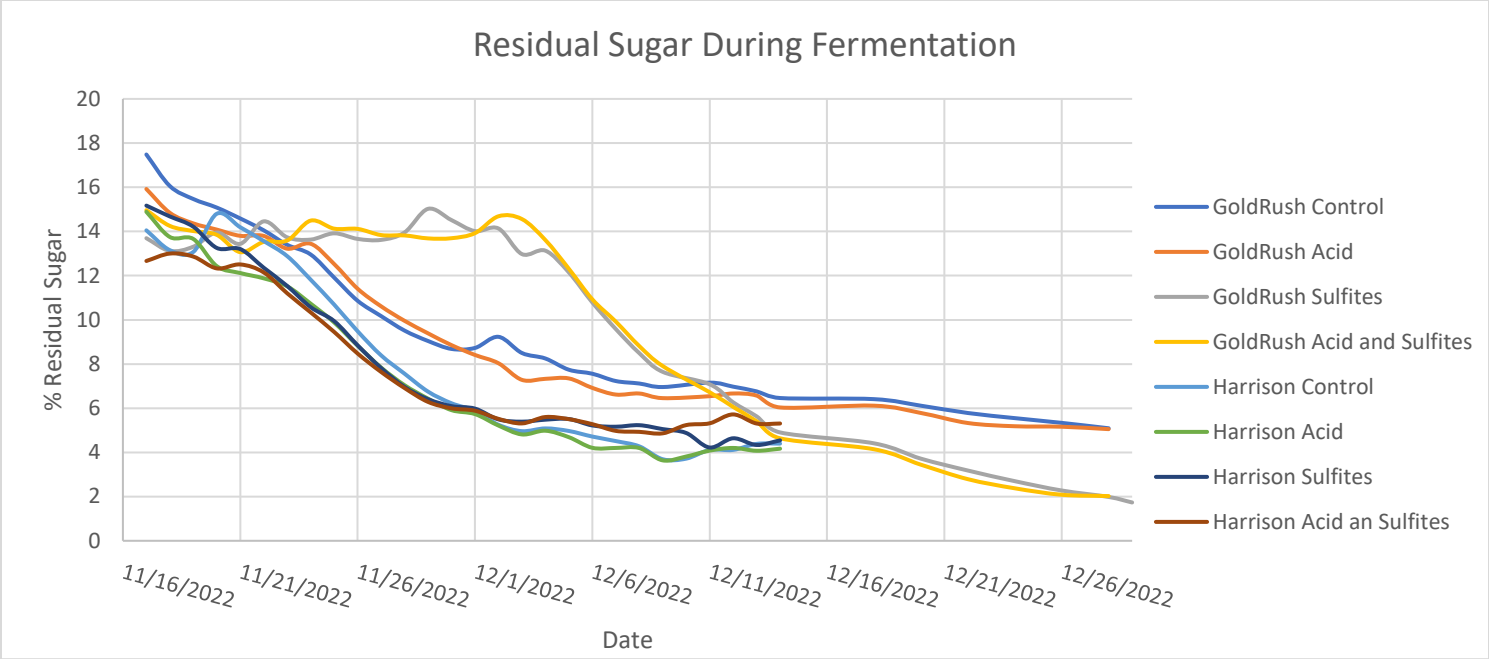

Figure 3. Residual sugars for each treatment type during fermentation.

Chemistry parameters, as outlined in the following sections, were determined for each of the experimental replicates after fermentation had finished.

3.2.4 Cider Chemistry

The following methods were used to measure chemical parameters of interest in the cider after fermentation: pH (probe, Thermo Scientific ROSS Ultra Triode Electrode Model 107BNUMD, Thermo Fisher Scientific, Waltham, MA,USA), total YAN in the form of primary amino nitrogen (PAN) (Megazyme PANOPA Enzyme Kit, Megazyme International, Wicklow, Ireland) and ammonia rapid test (Megazyme Ammonia ion (rapid) Enzyme Kit, Megazyme International, Wicklow, Ireland), residual sugar (Megazyme Sucrose/D-Glucose/D-Fructose Assay Kit, Megazyme International, Wicklow, Ireland), volatile acidity (AOAC procedure 964.08), titratable acidity (AOAC procedure 962.12 and 936.16), free SO_2 and total SO_2 (AOAC procedure 990.28), and ethanol content (AOAC procedure 984.14).

3.2.5 Sensory Training and Evaluation

A descriptive analysis (DA) sensory study was chosen to evaluate the sensory attributes of the cider samples after fermentation. This method allows for comparison between samples and provides the most complete set of descriptors (Heymann et al., 2014). This study was approved by the Virginia Tech Human Research Protection Program (IRB #19-939). Six panelists were recruited (4 females and 2 males, ages 21-60+) from Virginia Tech and the surrounding community. Prior to starting the study, panelists confirmed that they were at least 21 years of age and had consumed alcoholic beverages before. Panelists were provided with snacks and soft drinks as incentives, but no monetary incentives.

The consensus style DA sensory study was carried out as outlined in Heyman et al. (Heymann et al., 2014). A lexicon was created through consensus in a series of eight 60-minute training sessions over three weeks. All eight cider samples were presented to panelists multiple times in a random order to generate sensory descriptions (aroma, taste, and mouthfeel). Each sample was served in 44 mL pours in black wine glasses covered with plastic watch glasses at around 40 °C. Between sessions, nitrogen was added to bottles to maintain sample freshness and consistency. Panelists were asked to first smell samples, then taste and expectorate. During training sessions, panelists worked to come to a consensus on descriptive terms. Reference standards were provided for each term to achieve uniformity and consistency between panelists. A total of 28 terms were generated with recipes. After the creation of a lexicon, panelists were trained on a 15-point line scale to evaluate the intensity of each term. Group training was then used to minimize variation in scale usage.

Ciders were then evaluated using the lexicon created through consensus training. These evaluations were conducted in standard sensory booths. Each panelist took part in four 60-minute

evaluations with six ciders per session (for a total of three sensory replicated per cider sample). Samples were randomized for each evaluation session using a Latin Square design (see Appendix B for sample presentation order) Prior to each evaluation session, panelists were provided with reference standards to refresh themselves on terms. At the request of panelists, paper and a pencil were provided to take notes during evaluation. Samples were randomized with three-digit codes. Data collection was through Compusense Cloud (Guelph, ON, Canada) on an iPad.

Table 1. Sensory attributes generated during consensus training and their corresponding recipes.

Attribute	Recipe
Aroma (orthonasal)	
Citrus	1 strip of orange peel, and two strips each of lemon, lime, and grapefruit peel, gently squeezed to express oils
Red Fruit	25g each of raspberries, blueberries, blackberries, and cherries (with pits removed) mashed
Banana	2 1 cm sliced of banana
Floral	1 bouquet of fresh flowers (rose, daisies, snapdragon, thistle, dianthus)
Grassy	4 sprigs fresh cut grass clippings (Virginia Tech, Blacksburg, VA, USA)
Musty	40 g wet leaf litter and debris (Virginia Tech, Blacksburg, VA, USA)
Woody	20 g Feline Pine™ litter (Arm & Hammer, Trenton, NJ, USA), 40 mL distilled water
Yeasty	½ tsp yeast (The Kroger Co., Cincinnati, OH, USA), 250 mL warm distilled water, ¼ tsp sugar (The Kroger Co., Cincinnati, OH, USA)
Spiced	1 dash apple pie spice (The Kroger Co., Cincinnati, OH, USA)
Chemical	1 Sharpie® marker (Sharpie, ©Newell Brands, Atlanta, GA, USA)
Nail Polish Remover	10 mL 0.5% ethyl acetate solution
Sulfur	1.5 tsp hard-boiled egg (The Kroger Co., Cincinnati, OH, USA) mashed
Rotten Fruit	15 g expired GoldRush apple with 2 x 1 in banana peel
Metallic	1 penny rubbed between fingers
Flavor (retronasal)	
Vinegar	20 mL solution: 50 mL apple cider vinegar (The Kroger Co., Cincinnati, OH, USA), 200 mL distilled water
Apple	5 g Gala apple diced
Honey	5 g Private Selection™ Raw & Unfiltered Clover Honey (The Kroger Co., Cincinnati, OH, USA)
Caramel	½ Werther's Original® Chewy Caramel (Storck U.S.A, Chicago, IL, USA)
Pear	20 mL RW Knudsen Family® pear juice (©Knudsen & Sons, Inc., The J.M. Smucker Company, Orrville, OH, USA)

Grape	20 mL Welch's Concord Grape Juice (
Alcohol	7 mL of solution: 20 mL Luksusowa vodka (),
Basic Taste	
Sweet	20 mL solution: 15g pure cane granulated sugar (The Kroger Co., Cincinnati, OH, USA), 250 mL distilled water
Salty	20 mL solution: 1.5 g salt (The Kroger Co., Cincinnati, OH, USA), 250 mL distilled water
Bitter	15 mL Old Nation Boss Tweed Double IPA (Old Nation Brewing Co., Williamston, MI, USA)
Sour	20 mL solution: 1.5 g malic acid (LD Carlson Co, Kent, OH, USA), 200 mL distilled water
Mouthfeel	
Fizzy	10 mL club soda (The Kroger Co., Cincinnati, OH, USA)
Drying	7 mL of solution: 10 mL Simple Truth® (The Kroger Co., Cincinnati, OH, USA), 40 mL distilled water
Thick	15 mL Guinness™ Draught Stout (nitrogenated) (Guinness & Co., Dublin, Ireland)

3.2.6 Data Analysis

Juice chemistry data obtained from each cultivar, prior to fermentation, was analyzed using a two-sample t-test assuming equal variances, with each apple cultivar as the sample. This was done to evaluate differences between cultivars for each chemical parameter. Juice chemistry analysis was also performed on samples collected after the addition of pre-fermentation treatments. For juice with acidification treatments, pH was measured, while free and total sulfites were measured for juice with sulfite additions. Chemistry results from pre-treatment and post-treatment (after pH adjustment and sulfite addition to relevant treatment conditions) juice analyses were compared using a two-sample t-test assuming equal variances. This was done to ensure significant differences were achieved as intended in the pre-fermentation juice treatments. All juice analyses were run in analytical triplicate.

Cider chemistry data was analyzed using three-way analysis of variance (ANOVA) with apple cultivar, acid adjustment, and sulfite use as the parameters. For all cider tests, Tukey's Honestly Significant Difference (HSD) was performed to evaluate significant values, adjusted p-values $p < 0.05$. Cider chemistry tests were run in analytical triplicate on each fermentation

replicate. The exception to this is residual sugar, which was tested for consecutive days throughout the fermentation, and was run with only the biological (fermentation) replicates, no analytical replicates. Values for each fermentation replicate were used to calculate the mean for each treatment.

Results from the Sensory DA study were analyzed to determine if there were significant differences between sensory attributes overall and to determine the impact of apple cultivar and per-fermentation treatment on the significance of terms. This was analyzed using MANOVA with apple cultivar, acid adjustment, and sulfite use as factors. Panelist and panelist replicate were included in the MANOVA to account for unwanted variations (Rencher & Christensen, 2012). A pseudo-mixed ANOVA was then conducted with each of the 28 attributes as the independent variable (Heymann et al., 2014). This was done to determine if there were significant differences for each term based on apple cultivar, acid adjustment or sulfite use. Radar plots were then created to visualize the significant descriptors for each fermentation condition. Principal component analysis (PCA) plots were also created for sensory attributes and samples. This was done to better understand the relationships between attributes and samples and to see the differences in sensory profile between samples.

All statistical analyses were performed using RStudio, version 4.2.3 (R Core Team, 2023). Code and data are available from the corresponding author upon request.

3.3 Results and Discussion

3.3.1 Juice and Cider Chemistry

Juice chemistry was analyzed using a standard pre-fermentation juice panel: pH, titratable acidity (TA), malic acid (MAL), Free Sulfur Dioxide (FSO_2, mg/L), Total Sulfur Dioxide (TSO_2), yeast assimilable nitrogen (YAN), total polyphenols, soluble solids (°Brix), and initial sugars.

Significant differences among treatments (Table 2) were found in all attributes except MAL, initial sugars, FSO_2, and TSO_2. The lack of a significant difference in MAL may be due in part to analytical error, leading to a higher-than-expected standard deviation. GoldRush and Harrison apples from the same orchard have been used in previous studies with similar MAL results of 8.14(0.06) g/L and 8.61(0.06) g/L respectively (Littleson et al., 2023). The initial sugar values for both cultivars also did not show significant difference, despite significant differences in soluble solids (°Brix). This could potentially be due to the presence of other soluble solids like sorbitol in the juice that were not sucrose, fructose, or glucose, and therefore not measured with the sugar kit which targeted glucose, fructose, and sucrose (Belitz & Grosch, 2009). While past research on GoldRush and Harrison apples from the same orchard did not report initial sugar content as glucose, fructose, and sucrose, it did report soluble solids, which were consistent with the current findings that Harrison was higher in soluble solids than GoldRush. However, the past study reported higher soluble solids overall with Harrison at 16.75(0.05) °Brix and GoldRush at 14.2(0.00) °Brix (Littleson et al., 2023). Variation in soluble solids from year to year is normal, and to be expected. This can result from many factors including the heat accumulation during growing season, harvest maturity, and crop load (Peck et al., 2016). In both sulfite analyses there was no significant difference based on apple cultivar, and confirms that there was no appreciable sulfites present in the apple juice prior to our additions (Kim et al., 2000).

The other juice chemistry parameters varied significantly between the Harrison and GoldRush cultivars, which is not surprising since the rationale for selecting these cultivars for the experiment was due to their expected differences in initial juice chemistry. Some of the chemistry parameters that differed between Harrison and GoldRush have the potential to impact the fermentation kinetics, sensory outcomes, or both. GoldRush juice had a higher TA, while

Harrison had higher pH, YAN, total polyphenols, and soluble solids compared to GoldRush. Higher concentrations of soluble solids and total polyphenols in Harrison juice is consistent with previous reports for this cultivar (Littleson et al., 2023; Thompson-Witrick et al., 2014). A higher TA in GoldRush juice is also consistent with some previous studies (Thompson-Witrick et al., 2014), however, it conflicts with other past studies (Littleson et al., 2023). This indicates that the TA of GoldRush and Harrison apples may vary from year to year, due to growing season and/or orchard management factors.

Table 2. Initial juice chemistry results before implementation of pre-fermentation treatments.

Test	Harrison	GoldRush	p-value
pH	3.36(0.01)	3.28(<0.001)	0.0002*
Titratable Acidity (TA, g/L)	5.7(0.3)	6.3(0.2)	0.04*
Malic Acid (g/L)	0.97(0.07)	0.7(0.2)	0.08
Free Sulfur Dioxide (FSO_2, mg/L)	0.3(0.2)	0.1(0.2)	0.5
Total Sulfur Dioxide (TSO_2, mg/L)	0.7(0.2)	0.7(0.2)	1
Yeast Assimilable Nitrogen (YAN, mg/L N)	60(3)	48(5)	0.02*
Total Polyphenols (g/L)	0.245(0.004)	0.13(0.01)	0.0001*
Soluble Solids (°Brix)	14.4(0.2)	13.8(0.1)	0.006*
Initial Sugars (g/L)	81(10)	120(20)	0.05
Glucose (g/L)	9(2)	8(2)	0.4
Fructose (g/L)	41(4)	60(10)	0.06
Sucrose (g/L)	30(5)	60(10)	0.03*

Values are given as mean (SD) for analytical triplicates.
* Indicates a significant difference ($p < 0.05$) in the given juice chemistry parameter between apple cultivars

Chemical parameters for the juice after pre-fermentation treatments were applied are reported in Table 3. This was done to ensure there was a significant difference in pH and sulfites between the control and each treatment type prior to initiating the experiment. It was found that there was a significant pH difference between the control and the treated sample for all treatments with acid adjustment conditions.

Table 3. Pre-fermentation treatment chemistry results for adjusted values.

Pre-fermentation Treatment	pH adjustment	Free Sulfites	Total Sulfites
Harrison with Acid	3.21(<0.001) *	–	–
Harrison with Sulfites	3.36(0.01)		
Harrison with Acid and Sulfites	3.20(0.01) *		
GoldRush with Acid	3.21(0.006) *	–	–
GoldRush with Sulfites	3.28(<0.001)		
GoldRush with Acid and Sulfites	3.21(0.006) *		

Values are given as mean (SD) for analytical triplicates.
***** Indicates a significant difference between the given chemistry parameter in the treated v. untreated juice

After fermentation was complete, relevant chemical parameters were determined for all ciders to examine the effects of each pre-fermentation treatment on cider chemistry outcomes (Table 4). This involved a standard cider chemistry panel of pH, titratable acidity (TA), volatile acidity (VA), malic acid, free sulfur dioxide (FSO_2), total sulfur dioxide (TSO_2), yeast assimilable nitrogen (YAN), total polyphenols, alcohol by volume (ABV) and residual sugar (RS). Significant differences among treatments were found for pH, malic acid, total polyphenols, and ABV. There were significant differences based on apple cultivar for total polyphenols and ABV. This was expected since the pre-fermentation soluble solids concentrations determine potential alcohol content and differed between the cultivars. Similarly, the cultivars were selected based on their high- and low-tannin content, thus differences in polyphenol concentration were to be expected in the juice and cider. Acid adjustment prior to fermentation also resulted in significant differences in pH of the ciders, as would be expected.

More interestingly, sulfite addition pre-fermentation resulted in significant differences in pH, malic acid, total polyphenols, and ABV in the cider. There was no significant difference found for YAN for any treatment condition. This is likely due to the yeast nutrients being consumed during the fermentation process. The initial YAN present in juice was lower than the recommended amount of 140 mg N/L YAN. This value is generally considered the minimum

recommended concentration for wine production. Currently there is no known minimum YAN for successful cider fermentation, so this value has been adapted by many cidermakers as well (Boudreau IV et al., 2018). There was also no significant difference in residual sugar for any treatment condition. This could be due to the fermentations not going to completion, which is in agreement to past research and is common for natural fermentations (Littleson et al., 2023; Zoecklein et al., 1995).

There were no significant interactions found between apple cultivar and acid adjustment. This means that based on apple cultivar selected (high v. low tannin), pH adjustment from 3.28 to 3.20 for Harrison and 3.36 to 3.20 for GoldRush will not have a significant effect on the cider chemistry outcomes. There were also no significant interactions found between acid adjustment and sulfite use (Appendix C). Similarly, there were no significant differences in cider chemistry outcomes based on the effect of acid adjustment and sulfite use.

There were significant differences for some interactions between apple cultivar and sulfite use (Appendix C). This shows that depending on the apple cultivar used, the sulfite treatment will have a different effect on chemistry values. These effects were not significantly different for all interactions. Every interaction between apple cultivar and sulfite use was significant for MAL except those between Harrison with and without sulfites. This is likely due to the amount of malic acid being consistent in the starting juice, and not having any further additions being considered in this interaction. This interaction also resulted in a significant difference in YAN for Harrison and GoldRush without sulfites. This is most likely due to the high YAN for GoldRush with only pH adjustment. This value may be high due to analytical error, as there is a high standard deviation. There is also a significant difference for this interaction in RS between GoldRush with and without sulfites. GoldRush ciders without sulfites

have significantly higher RS than those with sulfites. This is most likely due to the long lag phase of the fermentation which allowed sucrose to be converted into glucose and fructose, which are more readily useable by some strains of yeast.

Table 4. Cider chemistry results based on fermentation quadruplicates and analytical triplicates.

	Harrison				GoldRush			
Chemical Analysis	Control	Acid	Sulfites	Acid and Sulfites	Control	Acid	Sulfites	Acid and Sulfites
pH[b,c]	3.52(0.008)	3.43(0.03)	3.6(0.2)	3.42(0.005)	3.72(0.07)	3.5(0.2)	3.47(0.09)	3.36(0.04)
Titratable Acidity (TA, g/L)[a,b,c]	4.0(0.8)	4(1)	4.1(0.9)	5.8(6)	4(1)	6(2)	6(1)	7(1)
Volatile Acidity (VA, g/L)								
Malic Acid (g/L)[c]	5(1)	4(2)	3(2)	4.6(0.7)	3(5)	2(2)	6(2)	6(1)
Free Sulfur Dioxide (FSO_2, mg/L)								
Total Sulfur Dioxide (TSO_2, mg/L)								
Yeast Assimilable Nitrogen (YAN, mg/L N)	9(4)	9(2)	10(4)	9(3)	8(2)	12(6)	12(5)	14(7)
Total Polyphenols (g/L)[a,c]	0.17(0.03)	0.15(0.03)	0.19(0.04)	0.20(0.03)	0.064(0.001)	0.07(0.05)	0.158(0.002)	0.16(0.01)
Alcohol by Volume (ABV, %v/v)[a,c]	4(1)	3.6(0.8)	3.8(0.6)	3.7(0.9)	4.1(0.9)	5(1)	7(1)	6(1)
Residual Sugar (RS, g/L)	36(10)	28(13)	38(7)	39(4)	35(9)	62(50)	13(8)	13(12)
Glucose (g/L)[a,c]	1.5(0.8)	1.6(0.3)	0.4(0.3)	0.6(0.3)	6(3)	5(3)	1(1)	2(2)
Fructose (g/L)	6.2(0.6)	6(3)	6.6(0.6)	6(2)	12(8)	10(5)	6(3)	6(6)
Sucrose (g/L)	29(10)	22(9)	31(7)	32(5)	17(16)	46(51)	6(6)	5(5)

Values are given as mean (SD)
Superscripts indicate significant ($p < 0.05$) analysis of variance (ANOVA) results.
[a] Significantly different for the apple cultivar
[b] Significantly different for acid adjustment
[c] Significantly different for sulfite use

3.3.1 Sensory Evaluation

During the descriptive analysis study, 28 terms were generated. Of these terms, 22 were found to be significant without considering sample: panelist interaction. After further analysis, 19 terms were found to be significant after accounting for sample and panelist interaction.

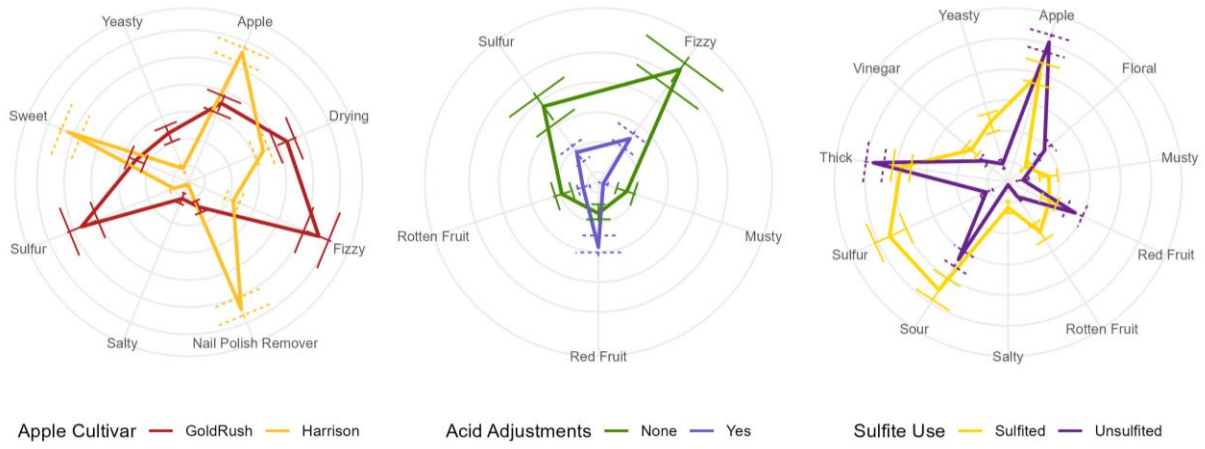

Figure 4. Radar plots for statistically significant sensory attributes based on apple cultivar, acid adjustment, and sulfite use. Significance was found using analysis of variance (ANOVA).

Attributes that were significantly different by apple cultivar, acid adjustment, or sulfite use can be seen in the radar plots in Figure 4. Descriptors that differ based on apple cultivar were "apple," "drying," "fizzy," "nail polish remover," "salty," "sulfur," "sweet," and "yeasty." GoldRush had higher mean values for all attributes except "nail polish remover," "apple," and "sweet." Ciders made from Harrison apples had a higher RS at the end of fermentation, which agrees with having a higher mean sweetness compared to GoldRush. (Nail polish and VA data, sulfur and SO_2 data)

Attributes that were significantly different by acid adjustment were "fizzy," "sulfur," "musty," "red fruit," and "rotten fruit" (Table 5). Ciders without a pH adjustment had a higher mean for all terms except "red fruit." This suggests that by adjusting the starting pH from 3.36 or 3.28 to around 3.20 may prevent the formation of off flavors such as "fizzy," "sulfur," "musty," and "rotten fruit."

The interaction between apple cultivar and acid adjustment was also examined to find significant attributes. These terms were "sulfur," "grape," and "fizzy" (Table 5). This shows that based on apple cultivar chosen, acid adjustment will make a difference in these three terms.

Attributes that were significantly different for sulfite use were “apple,” “floral,” “musty,” “red fruit,” “rotten fruit,” “salty,” “sour,” “sulfur,” “thick,” “vinegar,” and “yeasty.” Ciders with sulfites had higher means for all attributes except “apple,” “floral,” “red fruit,” and “thick.” This was contrary to our expectation. We had expected that sulfite use would lead to a decrease in negative or off-flavor descriptors, but these results show that sulfite addition increased negatively associated descriptors like “musty,” “rotten fruit,” “vinegar,” and “yeasty.” This could be due to sulfites inhibiting yeasts that could have fermented the juice but were not as resistant to sulfur dioxide as selected strains for cidermaking would be. This could have suppressed natural yeasts and allowed growth of spoilage organisms.

The interaction between apple cultivar and sulfite use was also examined. There were 17 terms that were found to be significantly different for sulfite use depending on apple cultivar chosen. These terms can be found in Table 5. There were more terms that were significant for this interaction than the other two interactions examined. This agrees with the chemistry data which also showed the most significant data was in the apple cultivar and sulfite use (Appendix C). This finding is useful to cidermakers because it demonstrates that sulfite recommendations are likely not generalizable across different apple cultivars with different starting chemistry, and that differences beyond pH should be considered in determination of a sulfite addition strategy to achieve stylistic goals.

The interaction between acid adjustment and sulfite use was also examined for significant terms. This resulted in the fewest number of terms, being “sulfur” and “fizzy” (Table 5). This may indicate that the interaction between these two treatments has the least effect on the sensory quality of the cider.

Some terms were common across more than one set of interactions. The “grape” attribute was present in both the apple cultivar interactions. This is of note, as “grape” was not significant to any of the individual treatment conditions. This suggests that there may be some interaction between tannin levels and the pre-fermentation treatment that results in the frequency of sensory attributes that would not be otherwise present. The “sulfur” attribute was significant in all three interactions. This was also a significant term on all three of the individual treatments.

Table 5. Statistically significant attributes for treatment interactions

Apple Cultivar and Acid Adjustment	Apple Cultivar and Sulfite Use	Acid Adjustment and Sulfite Use
Sulfur	Sulfur	Sulfur
Grape	Sweet	Fizzy
Fizzy	Salty	
	Bitter	
	Sour	
	Vinegar	
	Apple	
	Honey	
	Red fruit	
	Pear	
	Grape	
	Banana	
	Musty	
	Yeasty	
	Rotten fruit	
	Drying	
	Thick	

PCA plots were generated to explore differences between the ciders regarding their overall sensory profile (Figures 5 and 6). Figure 5 shows all attributes and their relationship and intensities relative to each other. This can be further examined by looking at the correlation between samples and terms (Figure 6). This shows that the two GoldRush ciders with sulfite treatments are separated from the rest of the ciders. These two fermentations took the longest to

start fermenting, had the lowest residual sugars and the highest ABV compared to the other ciders. The GoldRush cider with no pre-fermentation sulfite additions are both separated from the other GoldRush ciders and from each other. The GoldRush cider with no pre-fermentation treatments is on its own, while the cider with pre-fermentation acidification is grouped with the Harrison ciders. This suggests that the GoldRush cider with acidification has similar sensory attributes to Harrison ciders. All the Harrison ciders were more clustered together than the GoldRush ciders. This is in contrast to previous PdC fermentations with these cultivars, which showed more variability with the Harrison ciders (Littleson et al., 2023). This may be due to the use of a higher starting concentration of sulfites in this study than the previous study. Sulfites have antimicrobial properties and could therefore have impacted the fermentation kinetics in these ciders (S. C. Morgan et al., 2019). In addition, the PdC inoculum will naturally vary from year to year and the inoculum community itself may well have interacted differently with the two cultivars than it had in past years, despite the fact that it was generated in the same orchard at a similar time of year, and that the fruit for both cultivars was also obtained from the same orchard in our study as in that of Littleson et al.. This finding emphasizes the complexity of managing natural fermentations, and the variation that can arise even when management decisions are fairly consistent.

Based on sugar levels seen during fermentation (Figure 3), GoldRush and Harrison ciders were expected to show differences in sensory attributes. GoldRush ciders with pre-fermentation sulfite addition displayed similar sugar levels during the fermentation process. Both treatments took longer to start fermentation than all other treatments. GoldRush ciders without pre-fermentation sulfite addition also displayed similar sugar levels during fermentation. While these ciders are not closely related on the second dimension of the PCA plot (PC2), they are closer

together on the first dimension (PC1) and are both on the left side of the plot (Figure 6). Harrison ciders were closely grouped together in sugar levels during the entire fermentation process for all treatment types. This is reflected in their much closer grouping on the PCA plot compared to GoldRush ciders. This suggests that the similarities between fermentation kinetics and residual sugar levels will result in similar sensory attributes in the resulting ciders. The closer grouping of Harrison ciders as compared to GoldRush are not in agreement with previous studies which illustrated the opposite findings (Littleson et al., 2023). This difference in finding is likely due to the use of different pre-fermentation treatments and conditions.

Multivariate analysis with PCA allows for the examination of the correlations between sensory attributes which would otherwise be non-significant (Rencher & Christensen, 2012). PC1 separates attributes related to fruitiness, sweetness, and chemicals (e.g., “honey,” “apple,” “nail polish remover”) from those related to earthiness and sourness (e.g., “salty,” “grassy,” “vinegar”). PC2 shows much less separation than PC1, with 12.84% variance compared to 71.99% variance. There is some separation on PC2 with terms like “grassy” and “fizzy” being separated from terms like “vinegar” and “chemical.” These two dimensions account for approximately 85% of all the variation in the dataset, indicating a very good dimensional reduction fit.

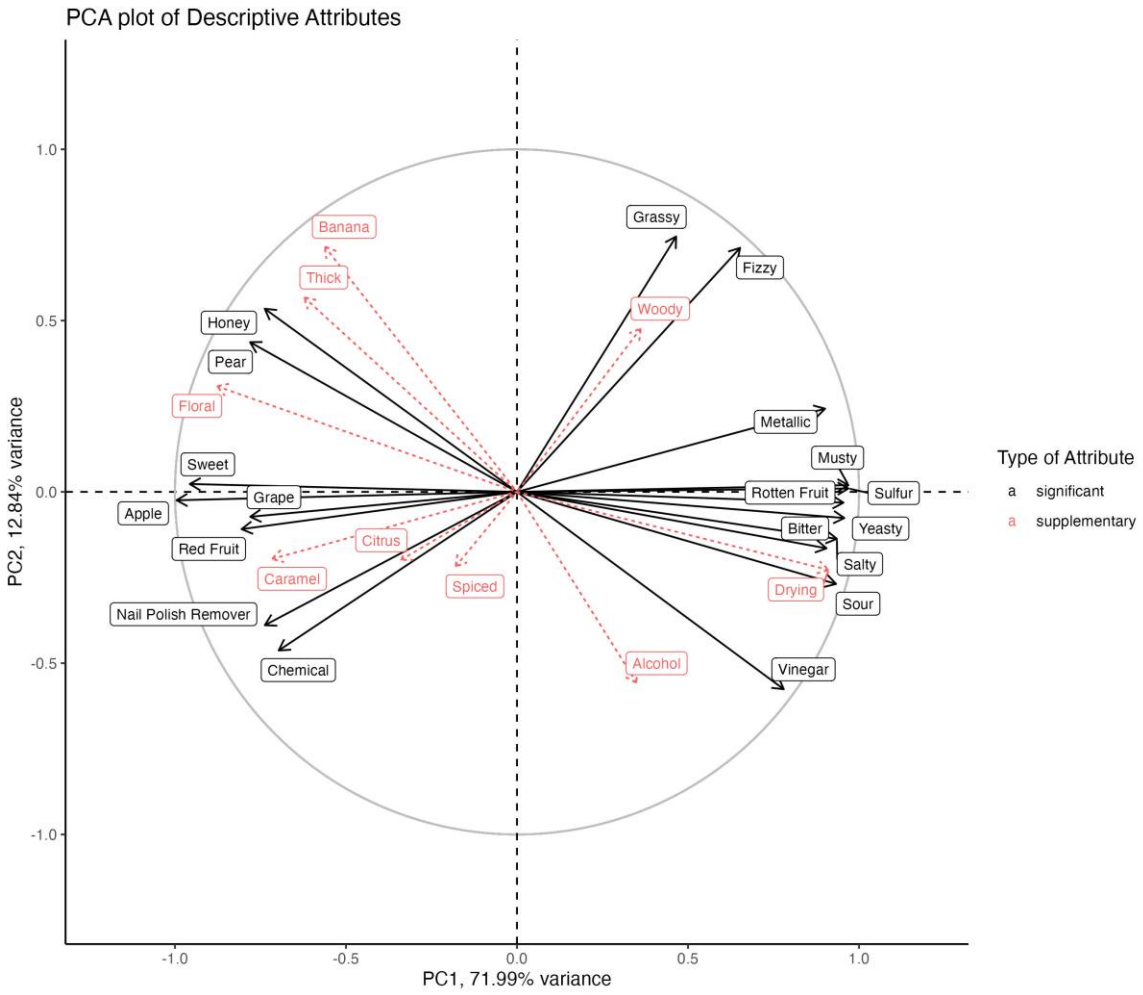

Figure 5. Principle component analysis (PCA) plot showing significant and nonsignificant sensory attributes("loadings"). Attributes are visualized as correlations, therefore terms with longer arrows indicate higher loading in relevant dimensions.

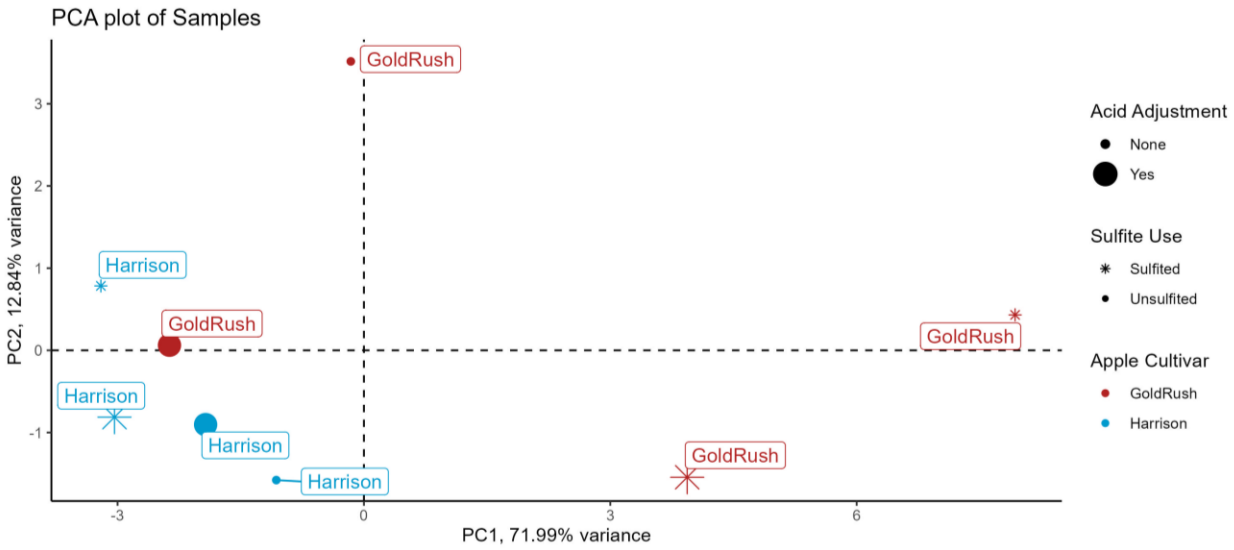

Figure 6. Principal component analysis (PCA) plot for samples ("factor") on all sensory descriptors. The dimensions of the plot can be understood though interpretation combined with Figure 5.

With the samples plotted in the PCA space (Figure 6), PC1 separates GoldRush ciders with sulfites from Harrison ciders. PC2 separates the GoldRush cider with no pre-fermentation treatments from the other ciders. GoldRush ciders fermented with sulfites are associated with terms like "sulfur," "rotten fruit," and "vinegar," while the GoldRush cider with no pre-fermentation treatment is associated with "honey" and "pear." Harrison ciders are more associated with terms like "chemical," "sweet," and "red fruit." This is reflected in the fermentation kinetics of the different ciders (Figure 3).

These findings illustrate the importance of tannin levels in apples when fermenting with a natural fermentation style. While the individual groupings contrast with pervious literature findings, having differences between sensory attributes based on apple cultivar is consistent. This study used 50 mg/L KMBS as a pre fermentation treatment, which is higher than previous literature (Littleson et al., 2023). The high tannin levels in cider apples provide more targets for sulfite binding, preserving some flavor compounds and taking sulfites out of solution. This

interaction decreases tannin activity and may decrease astringency (Ma et al., 2018). Decreased tannin activity may increase the fermentation rate in PdC, as it may mitigate the antimicrobial effects of tannins. This interaction has also been shown to impact the overall sensory characteristics of the ciders (Cairns et al., 2022). Higher tannin concentrations may allow Harrison ciders to ferment consistently regardless of sulfite use. This finding is useful for cidermakers in that it helps to shed light on the reasons why sulfite use may not be deemed important or helpful by cidermakers who ferment mainly high-tannin apples, like those in the UK. For lower tannin fruit, the use of sulfites may provide a more impactful and useful strategy to meet stylistic goals for natural cider fermentation.

3.4 Conclusion

The objective of this study was to determine the effects of apple cultivar and pre-fermentation juice treatments (pH adjustment and sulfite addition) on cider chemistry and sensory values and to determine the relationship between them. Ciders were fermented using juice from two apple cultivars with four different pre-fermentation treatments. Chemical analysis was performed on the juice and cider to examine the chemical properties of cider. A DA sensory analysis study was conducted to examine the sensory properties of the ciders.

Juice and cider chemistry revealed statistically significant differences between apple cultivars. Chemical analysis of cider also revealed significant differences between acid adjustment and sulfite use. Sensory evaluation of the ciders showed significant differences in the attributes of the ciders. The interactions between treatments were also analyzed and showed significant differences for chemistry and sensory data. These results confirm that pre-fermentation treatments have an impact on the chemistry and sensory attributes of cider. This study shows that results of natural fermentation differ based on apple cultivar, and more research

needs to be done to establish fermentation management strategies based on apple cultivar. This research also shows that juice chemistry has an impact on cider outcomes and sensory attributes, however, the chemistry varies between years and should be regularly assessed prior to fermentation.

Chapter 4: Conclusions and Future Work

Cider has a long and rich history in the United States, at one point being the most popular beverage (Watson, 2013). Currently, the industry is once again experiencing growth, and is expected to do so in the coming years (Wood, 2021, 2023). Since 2012, the number of cider makers has increased from 219 to 2,036 in 2021 (Wood, 2021). This is especially important in Virginia, as this was the state with the most prominent growth in the Southeast region of the United States. In 2018, Virginia was listed as the state with the 10th highest number of cideries (Cidermarket, 2018). Virginia is also the sixth largest apple producer in the nation, with over 200 apple varieties being grown throughout the state (Albermale Cider Works, 2021; Virginia Department of Agriculture and Consumer Services, 2021). Looking at the relationships between apple cultivar and pre-fermentation treatment and how these factors effect chemistry and sensory attributes of the cider is important information to relay to local industry.

The objective of this research was to determine the effects of apple cultivar and pre-fermentation treatment on cider chemistry and sensory values and to determine the relationship between them. Ciders were fermented using juice from two apple cultivars with four different pre-fermentation treatments, for a total of eight ciders produced. Chemical analysis was performed on the juice and cider to examine the chemical properties of cider. A DA sensory analysis study was then conducted to examine the sensory properties of the ciders.

There is still room for future work in many areas of this study. Work could be continued both from this study as well as past research on similar topics. As cider grows in popularity in the United States, there is room for more research on apple cultivars that are native to different regions of the country. This could possibly extend to research on the effects of apple cultivar and terroir on both ciders inoculated with commercial yeast and PdC.

Further research could also be done with PdC fermentations on Harrison and GoldRush apples. Based on the effects of sulfites on GoldRush fermentation kinetics and sensory properties, a concentration dependent sulfite addition study would be an interesting avenue to explore. Dosing GoldRush and Harrison PdC with different amounts of sulfites as a per-fermentation treatment could lead to the discovery of the optimal concentration for minimal sensory faults.

Research could also be done on the inoculation ratio used in PDC. A larger microbial load could possibly decrease the lag phase in the cider, speeding up fermentation. In addition, an impact on fermentation kinetics could impact the sensory attributes of the ciders. Higher microbial loads may also contribute to different attributes in the ciders. This could potentially decrease the likelihood of stuck or sluggish fermentations.

One area of research that was not explored in this study was the use of yeast nutrients. While there are other studies being conducted on the effects of yeast nutrients on the sensory properties of cider, the combination of yeast nutrients, PdC, and sulfites would be a logical next step to this thesis work. As some fermentations with sulfites appeared to become stuck or sluggish, resulting in opportunities for off-flavor development, adding yeast nutrients may allow PdC ciders to ferment to dry.

Another logical next step from this thesis work would be to conduct further sensory testing on naturally fermented ciders. Consumer studies on acceptability and/or willingness to pay could provide useful information on the effect of pre-fermentation treatments on market liking. This information could help those in industry make decisions on implementing the treatment strategies mentioned in this thesis work into their production.

Bibliography

Acree, T. E., Sonoff, E. P., & Splittstoesser, D. F. (1971). Determination of hydrogen sulfide in fermentation broths containing SO 2. *Applied microbiology*, *22*(1), 110-112. https://doi.org/10.1128/am.22.1.110-112.1971

Albermale Cider Works. (2021). https://www.albemarleciderworks.com/orchard/apple-varieties

Ángeles Pozo-Bayón, M., Andújar-Ortiz, I., & Moreno-Arribas, M. V. (2009). Scientific evidences beyond the application of inactive dry yeast preparations in winemaking. *Food Research International*, *42*(7), 754-761. https://doi.org/https://doi.org/10.1016/j.foodres.2009.03.004

Aubin, A. J., Hynes, M., & Shocktor, J. P. (2007). ACQUITY UPLC for the Rapid Analysis of Amino Acids in Wine. https://www.waters.com/nextgen/lt/en/library/application-notes/2007/acquity-uplc-for-the-rapid-analysis-of-amino-acids-in-wine.html

Bamforth, C. W. (2005). *Food, fermentation, and micro-organisms*. Blackwell Science. http://site.ebrary.com/id/10240504

Beech, F. W. (1993). Yeasts in cider-making. In *The Yeasts* (pp. 169-213). Elsevier. https://doi.org/https://doi.org/10.1016/B978-0-08-092543-1.50015-1

Bekker, M. Z., Smith, M. E., Smith, P. A., & Wilkes, E. N. (2016). Formation of Hydrogen Sulfide in Wine: Interactions between Copper and Sulfur Dioxide. *Molecules*, *21*(9), 1214. https://www.mdpi.com/1420-3049/21/9/1214

Belitz, H. D., & Grosch, W. (2009). *Food Chemistry Berlin Allemange*. Springer.

Bell, S.-J., & Henschke, P. A. (2005). Implications of nitrogen nutrition for grapes, fermentation and wine. *Australian Journal of Grape and Wine Research*, *11*(3), 242-295. https://doi.org/https://doi.org/10.1111/j.1755-0238.2005.tb00028.x

Bisson, L. F. (1999). Stuck and Sluggish Fermentations. *American Journal of Enology and Viticulture*, *50*(1), 107. http://www.ajevonline.org/content/50/1/107.abstract

Boudreau IV, T. F., Peck, G. M., Ma, S., Patrick, N., Duncan, S., O'Keefe, S. F., & Stewart, A. C. (2017). Hydrogen sulphide production during cider fermentation is moderated by pre-fermentation methionine addition. *Journal of the Institute of Brewing*, *123*(4), 553-561. https://doi.org/https://doi.org/10.1002/jib.449

Boudreau IV, T. F., Peck, G. M., O'Keefe, S. F., & Stewart, A. C. (2018). Free amino nitrogen concentration correlates to total yeast assimilable nitrogen concentration in apple juice. *Food Science & Nutrition*, *6*(1), 119-123. https://doi.org/https://doi.org/10.1002/fsn3.536

Bouklich, N. A., Collins, T. S., Masarweh, C., Allen, G., Heymann, H., Ebeler, S. E., & Mills, D. A. (2016). Associations among Wine Grape Micropbiome, Metabolome, and Fermentation Behavior Suggest Microbial Contribution to Regional Wine Characteristics. *mBio*, *7*(3). https://doi.org/10.1128/mBio.00631-16

Boulton, R. B., Singleton, V. L., Bisson, L. F., & Kunkee, R. E. (1999). *Principles and Practices of Winemaking*. Springer. https://doi.org/https://doi.org/10.1007/978-1-4757-6255-6

Cairns, P., Hamilton, L., Racine, K., Phetxumphou, K., Ma, S., Lahne, J., Gallagher, D., Huang, H., Moore, A. N., & Stewart, A. C. (2022). Effects of Hydroxycinnamates and Exogenous Yeast Assimilable Nitrogen on Cider Aroma and Fermentation Performance. *Journal of the American Society of Brewing Chemists*, 1-12. https://doi.org/10.1080/03610470.2021.1968171

Calvert, M. D., Neill, C. L., Stewart, A. C., & Lahne, J. (2023). Sensory descriptive analysis of hard ciders from the Northeast and Mid-Atlantic United States. *Journal of Food Science*. https://doi.org/https://doi.org/10.1111/1750-3841.16507

Chen, Y., Jastrzembski, J. A., & Sacks, G. L. (2017). Copper-Complexed Hydrogen Sulfide in Wine: Measurement by Gas Detection Tubes and Comparison of Release Approaches. *American Journal of Enology and Viticulture*, *68*(1), 91. https://doi.org/10.5344/ajev.2016.16024

Cider Handbook. (2018). Scott Laboratories.

Cidermarket. (2018). *Cider Maker Survey*. http://cydermarket.com/cider-maker-survey.html

Cline, J. A., Plotkowski, D., Beneff, A., & Beres, B. (2021). Juice attributes of Ontario-grown culinary (dessert) apples for cider. *Canadian Journal of Plant Science*, *101*(4), 536-545. https://doi.org/10.1139/cjps-2020-0223

Cole, E., Stewart, A. C., Chang, E. A. B., & Lahne, J. (2022). Exploring the Sensory Characteristics of Virginia Ciders through Descriptive Analysis and External Preference Mapping. *Journal of the American Society of Brewing Chemists*, 1-13. https://doi.org/10.1080/03610470.2022.2119535

Downing, D. L. (1989). *Apple Cider*. Springer US. https://doi.org/10.1007/978-1-4684-8225-6_8

Drake, M. A., & Civille, G. V. (2003). Flavor Lexicons. *Comprehensive Reviews in Food Science and Food Safety*, *2*(1), 33-40. https://doi.org/https://doi.org/10.1111/j.1541-4337.2003.tb00013.x

Edwards, C. G., & Bohlscheid, J. C. (2007). Impact of pantothenic acid addition on H2S production by Saccharomyces under fermentative conditions. *Enzyme and Microbial Technology*, *41*(1), 1-4. https://doi.org/https://doi.org/10.1016/j.enzmictec.2007.03.002

Ewing, B. L., Peck, G. M., Ma, S., Neilson, A. P., & Stewart, A. C. (2019). Management of Apple Maturity and Postharvest Storage Conditions to Increase Polyphenols in Cider. *HortScience horts*, *54*(1), 143-148. https://doi.org/10.21273/hortsci13473-18

Ewing, B. L., & Rasco, B. A. (2018). Food Safety Modernization Act Produce Safety Rule Compliance for United States Hard Cider Producers Using Ground-harvested Apples. *HortTechnology hortte*, *28*(6), 698-705. https://doi.org/10.21273/horttech04096-18

Fabien-Ouellet, N., & Conner, D. (2018). The Identity Crisis of Cider. *Journal of Food Research*, *7*(2). https://doi.org/10.5539/jfr.v7n2p54

Franco-Luesma, E., Sáenz-Navajas, M.-P., Valentin, D., Ballester, J., Rodrigues, H., & Ferreira, V. (2016). Study of the effect of H2S, MeSH and DMS on the sensory profile of wine model solutions by Rate-All-That-Apply (RATA). *Food Research International*, *87*, 152-160. https://doi.org/https://doi.org/10.1016/j.foodres.2016.07.004

Fuleki, T., Pelayo, E., & Palabay, R. B. (1994). Sugar Composition of Varietal Juices Produced from Fresh and Stored Apples. *Journal of Agricultural and Food Chemistry*, *42*, 1266-1275.

García-Estévez, I., Alcalde-Eon, C., Puente, V., & Escribano-Bailón, M. T. (2017). Enological Tannin Effect on Red Wine Color and Pigment Composition and Relevance of the Yeast Fermentation Products. *Molecules*, *22*(12), 2046. https://www.mdpi.com/1420-3049/22/12/2046

Gobbi, M., Comitini, F., D'Ignazi, G., & Ciani, M. (2013). Effects of nutrient supplementation on fermentation kinetics, H2S evolution, and aroma profile in Verdicchio DOC wine production. *European Food Research and Technology*, *236*(1), 145-154. https://doi.org/10.1007/s00217-012-1870-0

Gorawala, P. (2012). *Agricultural Research Updates* (S. Mandhatri, Ed. Vol. 4). Nova Publishers.
Helm, E., & Trolle, B. (1946). Selection of a taste panel. *Wallerstein Lab. Commun*, *9*(28), 181-194.
Heymann, H., & Ebeler, S. E. (2017). *Sensory and Instrumental Evaluation of Alcoholic Beverages*. Elsevier.
Heymann, H., King, E. S., & Hopfer, H. (2014). Classical Descriptive Analysis. In P. Varela & G. Ares (Eds.), *Novel Techniques in Sensory Characteriation and Consumer Profiling* (pp. 9-40). Taylor and Francis Group, LLC.
Heymann, H., Machado, B., Torri, L., & Robinson, A. L. (2012). HOW MANY JUDGES SHOULD ONE USE FOR SENSORY DESCRIPTIVE ANALYSIS? *Journal of Sensory Studies*, *27*(2), 111-122. https://doi.org/https://doi.org/10.1111/j.1745-459X.2012.00373.x
Ingledew, W., & Kunkee, R. E. (1985). Factors influencing sluggish fermentations of grape juice. *American Journal of Enology and Viticulture*, *36*(1), 65-76.
Jackson, D. I., & Lombard, P. B. (1993). Environmental and management practices affecting grape composition and wine quality--a review. *American Journal of Enology and Viticulture*, *44*, 409-430.
Jacobsen, R. (2014). *Apples of Uncommon Character: Heirlooms, Modern Classics, and Little-Known Wonders*. Bloomsbury Publishing USA.
Janick, J. (2001). 'Goldrush' Apple. *Journal of the American Pomological Society*, *55*, 194-196. https://www.pubhort.org/aps/55/v55_n4_a31.htm
Jiranek, V., Langridge, P., & Henschke, P. A. (1995). Regulation of hydrogen sulfide liberation in wine-producing Saccharomyces cerevisiae strains by assimilable nitrogen. *Applied and environmental microbiology*, *61*(2), 461-467. https://doi.org/10.1128/aem.61.2.461-467.1995
Karadeniz, F., & Ekşi, A. (2002). Sugar composition of apple juices. *European Food Research and Technology*, *215*(2), 145-148. https://doi.org/10.1007/s00217-002-0505-2
Kessinger, J. N., Earnhart, G., Hamilton, L., Phetxumphou, K., Neill, C., Stewart, A. C., & Lahne, J. (2021). Exploring Perceptions and Categorization of Virginia Hard Ciders through the Application of Sorting Tasks. *Journal of the American Society of Brewing Chemists*, *79*(2), 187-200. https://doi.org/10.1080/03610470.2020.1843927
Kim, H.-Y., Lee, Y.-J., Hong, K.-H., Kwon, Y.-K., Ko, H.-S., Lee, Y.-K., & Lee, C.-W. (2000). Studies on the Contents of Naturally Occuring Sulfite in Foods. *Korean Journal of Food Science and Technology*, *32*(3), 544-549.
Kodur, S. (2011). Effects of juice pH and potassium on juice and wine quality, and regulation of potassuim in grapevines through rootstocks (*Vitis*): a short review. *Vitis - Journal of Grapevine Research*, *50*(1), 1-6. https://doi.org/https://doi.org/10.5073/vitis.2011.50.1-6
Lawless, H. T., & Heymann, H. (2010). *Sensory Evaluation of Food* (2 ed.). Springer. https://doi.org/https://doi.org/10.1007/978-1-4419-6488-5
Lawless, L. J. R., & Civille, G. V. (2013). Developing Lexicons: A Review. *Journal of Sensory Studies*, *28*(4), 270-281. https://doi.org/https://doi.org/10.1111/joss.12050
Lea, A. (2015). *Craft Cider Making: Third Edition*. The Crowood Press Ltd.
Lea, A., & Drilleau, J.-F. (2003). Cidermaking. In J. R. P. Andrew G. H. Lea (Ed.), *Fermented Beverage Production* (2 ed., pp. 59-87). Springer Science + Business Media. https://doi.org/10.1007/978-1-4615-0187-9

Lea, A. G. H. (1995). Apple juice. In P. R. Ashurst (Ed.), *Production and Packaging of Non-Carbonated Fruit Juices and Fruit Beverages* (pp. 153-196). Springer US. https://doi.org/10.1007/978-1-4615-0949-3_6

Lestringant, P., Delarue, J., & Heymann, H. (2018). Do panelists memorize products when performing descriptive analysis on few products? *Journal of Sensory Studies*, *33*(1), e12305. https://doi.org/https://doi.org/10.1111/joss.12305

Littleson, B., Chang, E., Neill, C., Phetxumphou, K., Sandbrook, A., Stewart, A. C., & Lahne, J. (2023). Sensory and chemical properties of Virginia hard cider: Effects of Apple Cultivar Selection and Fermentation Strategy. *Journal of the American Society of Brewing Chemists*, *81*(1), 141-154. https://doi.org/https://doi.org/10.1080/03610470.2022.2057780

Ma, L., Watrelot, A. A., Addison, B., & Waterhouse, A. L. (2018). Condensed Tannin Reacts with SO2 during Wine Aging, Yielding Flavan-3-ol Sulfonates. *Journal of Agricultural and Food Chemistry*, *66*(35), 9259-9268. https://doi.org/10.1021/acs.jafc.8b01996

Ma, S., Kim, C., Neilson, A. P., Griffin, L. E., Peck, G. M., O'Keefe, S. F., & Stewart, A. C. (2019). Comparison of Common Analytical Methods for the Quantification of Total Polyphenols and Flavanols in Fruit Juices and Ciders. *Journal of Food Science*, *84*(8), 2147-2158. https://doi.org/https://doi.org/10.1111/1750-3841.14713

Manginot, C., Roustan, J. L., & Sablayrolles, J. M. (1998). Nitrogen demand of different yeast strains during alcoholic fermentation. Importance of the stationary phase. *Enzyme and Microbial Technology*, *23*(7), 511-517. https://doi.org/https://doi.org/10.1016/S0141-0229(98)00080-5

Marchand, S., de Revel, G., & Bertrand, A. (2000). Approaches to Wine Aroma: Release of Aroma Compounds from Reactions between Cysteine and Carbonyl Compounds in Wine. *Journal of Agricultural and Food Chemistry*, *48*(10), 4890-4895. https://doi.org/10.1021/jf000149u

Morgan, S. C., Haggerty, J. J., Johnston, B., Jiranek, V., & Durall, D. M. (2019). Response to Sulfur Dioxide Addition by Two Commercial Saccharomyces cerevisiae Strains. *Fermentation*, *5*(3), 69. https://www.mdpi.com/2311-5637/5/3/69

Morgan, S. C., McCarthy, G. C., Watters, B. S., Tantikachornkiat, M., Zigg, I., Cliff, M. A., & Durall, D. M. (2019). Effect of sulfite addition and pied de cuve inoculation on the microbial communities and sensory profiles of Chardonnay wines: dominance of indigenous Saccharomyces uvarum at a commercial winery. *FEMS Yeast Res*, *19*(5). https://doi.org/10.1093/femsyr/foz049

Moschetti, G., Corona, O., Gaglio, R., Squadrito, M., Parrinello, A., Settanni, L., Barone, E., & Francesca, N. (2016). Use of fortified pied de cuve as an innovative method to start spontaneous alcoholic fermentation for red winemaking. *Australian Journal of Grape and Wine Research*, *22*(1), 36-45. https://doi.org/https://doi.org/10.1111/ajgw.12166

Natusch, D. F. S., Sewell, J. R., & Tanner, R. L. (1974). Determination of hydrogen sulfide in air. Assessment of impregnated paper tape methods. *Analytical Chemistry*, *46*(3), 410-415. https://doi.org/10.1021/ac60339a001

Park, S.-K. (2008). Development of a method to measure hydrogen sulfide in wine fermentation. *Journal of Microbiology and Biotechnology*, *18*(9), 1550-1554.

Parker, M., Smith, P. A., Birse, M., Francis, I. L., Kwiatkowski, M. J., Lattey, K. A., Liebich, B., & Herderich, M. J. (2007). The effect of pre- and post-ferment additions of grape derived tannin on Shiraz wine sensory properties and phenolic composition. *Australian Journal of*

Grape and Wine Research, *13*(1), 30-37. https://doi.org/https://doi.org/10.1111/j.1755-0238.2007.tb00069.x

Peck, G., McGuire, M., Boudreau, T., & Stewart, A. (2016). Crop Load Density Affects 'York' Apple Juice and Hard Cider Quality. *HortScience horts*, *51*(9), 1098. https://doi.org/10.21273/hortsci10962-16

Perli, T., Wronska, A. K., Ortiz-Merino, R. A., Pronk, J. T., & Daran, J.-M. (2020). Vitamin requirements and biosynthesis in Saccharomyces cerevisiae. *Yeast*, *37*(4), 283-304. https://doi.org/https://doi.org/10.1002/yea.3461

Peryam, D. R., & Swartz, V. W. (1950). Measurement of sensory differences. *Food Technol*, *4*(1), 390-395.

Phetxumphou, K., Cox, A. N., & Lahne, J. (2020). Development and Characterization of a Check-All-That-Apply (CATA) Lexicon for Virginia Hard (Alcoholic) Ciders. *Journal of the American Society of Brewing Chemists*, *78*(4), 299-307. https://doi.org/10.1080/03610470.2020.1768784

Prolux, A., & Nichols, L. (2003). *Cider: Making, Using, and Enjoying Sweet & Hard Cider*. Storey Publishing.

Qin, Z., Petersen, M. A., & Bredie, W. L. P. (2018). Flavor profiling of apple ciders from the UK and Scandinavian region. *Food Research International*, *105*, 713-723. https://doi.org/https://doi.org/10.1016/j.foodres.2017.12.003

R Core Team. (2023). *R: A language and environment for statisitcal computing*. In

Rencher, A. C., & Christensen, W. F. (2012). Methods of Multivariate Analysis. In (3rd ed.). Wiley.

Rosend, J., Kuldjärv, R., Rosenvald, S., & Paalme, T. (2019). The effects of apple variety, ripening stage, and yeast strain on the volatile composition of apple cider. *Heliyon*, *5*(6), e01953. https://doi.org/https://doi.org/10.1016/j.heliyon.2019.e01953

Sanz, M. L., Martínez-Castro, I., & Moreno-Arribas, M. V. (2008). Identification of the origin of commercial enological tannins by the analysis of monosaccharides and polyalcohols. *Food Chemistry*, *111*(3), 778-783. https://doi.org/https://doi.org/10.1016/j.foodchem.2008.04.050

Scalbert, A. (1991). Antimicrobial properties of tannins. *Phytochemistry*, *30*(12), 3875-3883. https://doi.org/https://doi.org/10.1016/0031-9422(91)83426-L

Scott Laboratories 2021 Winemaking Handbook. (2021). Scott Laboratories.

Spanos, G. A., & Wrolstad, R. E. (1990). Influence of Processing and Storage on the Phenolic Composition of Thompson Seedless Grape Juice. *Journal of Agricultural and Food Chemistry*, *38*, 1565-1571. https://doi.org/https://doi.org/10.1021/jf00097a030

Stone, H., & Sidel, J. L. (2004). *Sensory Evaluation Practices* (3 ed.). Academic Press. https://doi.org/https://doi.org/10.1016/B978-012672690-9/50005-6

Swiegers, J. H., & Pretorius, I. S. (2005). Yeast Modulation of Wine Flavor. In *Advances in Applied Microbiology* (Vol. 57, pp. 131-175). Academic Press. https://doi.org/https://doi.org/10.1016/S0065-2164(05)57005-9

Thomas, C. S., Boulton, R. B., Silacci, M. W., & Gubler, W. D. (1993). The Effect of Elemental Sulphur, Yeast Strain, and Fermentation Medium on Hydrogen Sulfide Production during Fermentation. *American Journal of Enology and Viticulture*, *44*(2). https://www.ajevonline.org/content/ajev/44/2/211.full.pdf?casa_token=iBOC-45E2JIAAAAA:8pM_gsWOtFZzZyCU5CE_PCOgTSGQtAG23WW6_0kCWTSs-HNIywSvomiih7cIwdy9J7exk-4YIK8

Thompson-Witrick, K. A., Goodrich, K. M., Neilson, A. P., Hurley, E. K., Peck, G. M., & Stewart, A. C. (2014). Characterization of the Polyphenol Composition of 20 Cultivars of Cider, Processing, and Dessert Apples (Malus × domestica Borkh.) Grown in Virginia. *Journal of Agricultural and Food Chemistry*, *62*(41), 10181-10191. https://doi.org/10.1021/jf503379t

Thornton, C. (2014). *Cider Apples*. Leaves of Gold Press.

Ugliano, M., Fedrizzi, B., Siebert, T., Travis, B., Magno, F., Versini, G., & Henschke, P. A. (2009). Effect of Nitrogen Supplementation and Saccharomyces Species on Hydrogen Sulfide and Other Volatile Sulfur Compounds in Shiraz Fermentation and Wine. *Journal of Agricultural and Food Chemistry*, *57*(11), 4948-4955. https://doi.org/10.1021/jf8037693

Ugliano, M., & Henschke, P. A. (2010). Comparison of three methods for accurate quantification of hydrogen sulfide during fermentation. *Analytica Chimica Acta*, *660*(1), 87-91. https://doi.org/https://doi.org/10.1016/j.aca.2009.09.049

Valles, B. S., Bedriñana, R. P., Tascón, N. F., Simón, A. Q., & Madrera, R. R. (2007). Yeast species associated with the spontaneous fermentation of cider. *Food Microbiology*, *24*(1), 25-31. https://doi.org/https://doi.org/10.1016/j.fm.2006.04.001

Valois, S., Merwin, I. A., & Padilla-Zakour, O. I. (2006). Characterization of Fermented Cider Apple Varieties Grown in Upstate New York. *J. Am. Pomol. Soc.*, *60*, 113-128.

Varela, P., & Ares, G. (2012). Sensory profiling, the blurred line between sensory and consumer science. A review of novel methods for product characterization. *Food Research International*, *48*(2), 893-908. https://doi.org/https://doi.org/10.1016/j.foodres.2012.06.037

Virginia Department of Agriculture and Consumer Services. (2021). *Virginia's Top 20 Farm Commodities*. Retrieved May 31, 2022 from https://www.vdacs.virginia.gov/agriculture-top20.shtml

Vos, P. J. A., & Gray, R. S. (1978). The Origin and Control of Hydrogen Sulfide During Fermentation of Grape Must. *American Journal of Enology and Viticulture*, *30*(3), 187-197. https://www.ajevonline.org/content/ajev/30/3/187.full.pdf

Wainwright, T. (1970). Hydrogen Sulphide Production by Yeast under Conditions of Methionine, Pantothenate or Vitamin B6 Deficiency. *Microbiology*, *61*(1), 107-119. https://doi.org/https://doi.org/10.1099/00221287-61-1-107

Wainwright, T. (1971). Production of H2S by Yeasts: Role of Nutrients. *Journal of Applied Bacteriology*, *34*(1), 161-171. https://doi.org/https://doi.org/10.1111/j.1365-2672.1971.tb02275.x

Wang, X. D., Bohlscheid, J. C., & Edwards, C. G. (2003). Fermentative activity and production of volatile compounds by Saccharomyces grown in synthetic grape juice media deficient in assimilable nitrogen and/or pantothenic acid. *Journal of Applied Microbiology*, *94*(3), 349-359. https://doi.org/https://doi.org/10.1046/j.1365-2672.2003.01827.x

Watson, B. (2013). *Hard and Sweet*. The Countryman Press.

Wood, G. (2021). *Cider Production* (OD5335). I. World.

Wood, G. (2023). *Good harvest: The industry will likely benefit from an influx of new entrants* [Industry Report](OD5335).

Zoecklein, B. W., Fugelsang, K. C., Gump, B. H., & Nury, F. S. (1995). *Wine Analysis and Production* (B. W. Zoecklein, Ed. 1 ed.). Springer. https://doi.org/https://doi.org/10.1007/978-1-4757-6978-4

Appendix A

Pied de Cuve Inoculation and Fermentation Protocol

Materials

Good Nature press, two Good Nature press bags, 32 one-gallon carboys, 32 stoppers, 32 airlocks, Harrison apples (Silver Creek Farms), GoldRush apples (Silver Creek Farms), 50 mL centrifuge tubes, 12 20 L food-grade containers with lids, large spoon, Sanitizer, tape, marker, potassium metabisulfite (KMBS), rope, large dog crate, cheese cloth, blanket, malic acid, funnel, five-gallon bucket, auto siphon with bottling wand

Day 1:

1. Place a new Good Nature press bag into the washed and sanitized Good Nature press and press and juice apples until 20 gallons of juice has been obtained. Reserve 5 50 mL tubes of juice per cultivar.
2. Pour the juice into sanitized and labeled five-gallon food-grade buckets. For each cultivar, four food-grade containers had three gallons of juice and one had four gallons of juice. Discard pomace and press bag after each cultivar has been pressed.
3. Add sulfites in the form of potassium metabisulfite (KMBS) to two treatments per cultivar, or four treatments total. KMBS is added at a rate of 50 mg/L to the three-gallon treatments. Approximately 0.96 g KMBS was added to the juice and mixed.
4. Juice was placed into a 4 °C refrigerator for storage.

Day 2: Pied de Cuve (PDC)

5. Remove the two containers with four gallons of juice from the refrigerator. Transport them to Kentland Farms Virginia Tech orchard. Place the bottom of the dog crate next to an apple tree and line the bottom with a blanket.
6. Place the two containers in the dog crate and remove their lids. Tie cheese cloth onto the open tops of the containers and finish assembling the dog crates. Use a rope to tie the dog crate shut as well as to secure it to the tree.

Day 5: Juice Additions and Inoculation

7. Untie and disassemble the dog crate. Remove cheese cloth from containers and replace lids. Transport back to lab.
8. Measure pH of Harrison and GoldRush Juice. Use malic acid to lower the pH to 3.20 in treatments where acid adjustment is required. Mix well.
9. Prepare sanitizer to a no rinse condition and sanitize 32 stoppers and airlocks.
10. Add one gallon of Harrison inoculum to each Harrison juice sample. Mix well. Reserve 5 50 mL centrifuge tubes per treatment type.
11. Use a funnel to add ~2.5 L of inoculated juice to each 1-gallon carboy for 4 carboys per treatment type. Cap with stopper and airlock filled to max line with water. Label carboys with treatment type and number 1 through 4.
12. Repeat steps 9 and 10 with GoldRush juice.
13. Place carboys in an 18 °C room for fermentation

Day 6 to End: Fermentation Management and Sampling

14. Remove stopper on one carboy and gently shake. Replace stopper and continue shaking until little to no sediment remains on bottom. Repeat for each carboy.

15. Shake three times a day every day for the first two weeks. Once fermentations start rapidly bubbling, omit the step to open the carboy. After two weeks shake twice a day, and after three to four weeks shake only once a day.
16. After one shaking time each day, sample each carboy for sugar and every 3-5 days for microbial content.
17. Move shaken carboy into sterile hood and remove stopper. Use a sterile 2 mL serological pipette to move 1 mL into a labeled 1.5 mL microcentrifuge tube.
18. If also sampling for microbial ecology, use the same pipette to move 1 mL sample into a labeled autoclaved 1.5 mL microcentrifuge tube.
19. Discard pipette and replace the stopper. Close microcentrifuge tube(s) and repeat steps 17 and 18 for all carboys. Replace carboys into the temperature-controlled room and place 1 mL samples in -20 °C freezer for storage until tests are run.
20. Once results from Megazyme fructose, glucose, sucrose test show consistent readings for multiple days, cider is ready to be bottled.

Bottling

21. Using aluminum foil and autoclave tape, prepare washed 375 mL bottles for the autoclave. Each treatment should have ~24 bottles prepared. Autoclave bottles on the GRAV15 setting.
22. Prepare sanitizer to a no rinse concentration and sanitize a 5-gal container. Pour sanitizer into a 5-gal bucket. Place bottle caps in sanitizer as well as a clean auto siphon with a bottling wand attachment.
23. Prepare and label 5 50 mL centrifuge tubes per carboy as well as one extra per treatment type.

24. Use sanitized auto siphon to move cider from each carboy of a single treatment type into the sanitized 5-gal food-grade container. Try to get as little sediment as possible while maximizing the amount of cider recovered. Fill the appropriate 5 50 mL centrifuge tubes while transferring cider. Repeat this process for the remaining 3 biological reps of this treatment.
25. Use the auto siphon to mix the combined samples and take 10 mL in a 15 mL centrifuge tube for sulfite testing. Test free SO_2 of combined cider using SOP methods. Calculate how much free SO_2 is present, if any, and calculate the amount of KMBS needed to adjust total mixed volume to 25 ppm free SO_2.
26. Add KMBS to cider in the 20 L container and mix to dissolve. Fill the last 50 mL centrifuge tube with the sulfited cider.
27. Use the bottling wand to transfer cider into labeled 375 mL bottles. Fill one to two inches below the top. Cap bottles and place in labeled box. Place boxes in refrigerator until use. Place centrifuge tubes into the -20 °C freezer until they can be tested.
28. Repeat bottling process for the remaining 7 ciders, rinsing and sanitizing the siphon and container between each cider.

Appendix B

Sensory evaluation cider presentation order

Samples & Blinding Codes

Sample Number	Blinding Code for Rep 1	Blinding Code for Rep 2	Blinding Code for Rep 3	Sample Name
1	732	726	833	GoldRush, no acid adjustment, no sulfites
2	752	119	841	GoldRush, acid adjustment, no sulfites
3	668	742	335	GoldRush, no acid adjustment, sulfites
4	193	575	341	GoldRush, acid adjustment, sulfites
5	653	287	302	Harrison, no acid adjustment, no sulfites
6	744	141	633	Harrison, acid adjustment, no sulfites
7	442	451	895	Harrison, no acid adjustment, sulfites
8	319	993	769	Harrison, acid adjustment, sulfites

Serving Layout for Partial Present Block Evaluation 1

	Rep 1					
Panelist	**1**	**2**	**3**	**4**	**5**	**6**
1	8 - 319	7 - 442	1 - 732	6 - 744	2 - 752	5 - 653
2	5 - 653	4 - 193	6 - 744	3 - 668	7 - 442	2 - 752
3	1 - 732	8 - 319	2 - 752	7 - 442	3 - 668	6 - 744
4	6 - 744	5 - 653	7 - 442	4 - 193	8 - 319	3 - 668
5	7 - 442	6 - 744	8 - 319	5 - 653	1 - 732	4 - 193
6	4 - 193	3 - 668	5 - 653	2 - 752	6 - 744	1 - 732

Serving Layout for Partial Present Block Evaluation 2

	Rep 1		**Rep 2**			
Panelist	**7**	**8**	**1**	**2**	**3**	**4**
1	3 - 668	4 - 193	3 - 742	2 - 119	4 - 575	1 - 726
2	8 - 319	1 - 732	7 - 451	6 - 141	8 - 993	5 - 287
3	4 - 193	5 - 653	3 - 742	2 - 119	4 - 575	1 - 726
4	1 - 732	2 - 752	4 - 575	3 - 742	5 - 287	2 - 119
5	2 - 752	3 - 668	6 - 141	5 - 287	7 - 451	4 - 575
6	7 - 442	8 - 319	8 - 993	7 - 451	1 - 726	6 - 141

Serving Layout for Partial Present Block Evaluation 3

	Rep 2				Rep 3	
Panelist	**5**	**6**	**7**	**8**	**1**	**2**
1	5 - 287	8 - 993	6 - 141	7 - 451	2 - 841	1 - 833
2	1 - 726	4 - 575	2 - 119	3 - 742	1 - 833	8 - 769
3	5 - 287	8 - 993	6 - 141	7 - 451	8 - 769	7 - 895
4	6 - 141	1 - 726	7 - 451	8 - 993	5 - 302	4 - 341
5	8 - 993	3 - 742	1 - 726	2 - 119	2 - 841	1 - 833
6	2 - 119	5 - 287	3 - 742	4 - 575	3 - 335	2 - 841

Serving Layout for Partial Present Block Evaluation 4

	Rep 3					
Panelist	**3**	**4**	**5**	**6**	**7**	**8**
1	3 - 335	8 - 769	4 - 341	7 - 895	5 - 302	6 - 633
2	2 - 841	7 - 895	3 - 335	6 - 633	4 - 341	5 - 302
3	1 - 833	6 - 633	2 - 841	5 - 302	3 - 335	4 - 341
4	6 - 633	3 - 335	7 - 895	2 - 841	8 - 769	1 - 833
5	3 - 335	8 - 769	4 - 341	7 - 895	5 - 302	6 - 633
6	4 - 341	1 - 833	5 - 302	8 - 769	6 - 633	7 - 895

Appendix C

Significant difference p-values for interactions between apple cultivar, acid adjustment, and sulfite use in cider chemistry

Interaction	Treatment Difference	pH	Titratable Acidity (TA, g/L)	Volatile Acidity (VA, g/L)	Malic Acid (MAL, g/L)	Yeast Assimilable Nitrogen (YAN, mg/L N)	Free Sulfur Dioxide (FSO_2, mg/L)	Total Sulfur Dioxide (TSO_2, mg/L)	Total Polyphenols (g/L)	Alcohol by Volume (ABV)	Residual Sugars (RS, g/L)	Glucose (g/L)	Fructose (g/L)	Sucrose (g/L)
Apple Cultivar: Acid Adjustment	Harrison:none-GoldRush:none	–	–		–	–			–	–	–	–	–	–
	GoldRush:yes-GoldRush:none	–	–		–	–			–	–	–	–	–	–
	Harrison:yes-GoldRush:none	–	–		–	–			–	–	–	–	–	–
	GoldRush:yes-Harrison:none	–	–		–	–			–	–	–	–	–	–
	Harrison:yes-Harrison:none	–	–		–	–			–	–	–	–	–	–
	Harrison:yes-GoldRush:yes	–	–		–	–			–	–	–	–	–	–
Apple Cultivar: Sulfite Use	Harrison:sulfites-GoldRush:sulfites	0.03*	–		0.00*	0.84			0.02*	0.00*	0.05	0.58	–	–
	GoldRush:unsulfited-GoldRush:sulfites	0.00*	–		0.00*	0.26			0.00*	0.00*	0.007*	0.00*	–	–
	Harrison:unsulfited-GoldRush:sulfites	0.17	–		0.00*	0.45			1.00	0.00*	0.34	1.00	–	–
	GoldRush:unsulfited-Harrison:sulfites	0.00*	–		0.00*	0.70			0.00*	0.34	0.77	0.00*	–	–
	Harrison:unsulfited-Harrison:sulfites	0.92	–		0.85	0.10			0.03*	0.79	0.82	0.70	–	–
	Harrison:unsulfited-GoldRush:unsulfited	0.00*	–		0.00*	0.008*			0.00*	0.90	0.32	0.00*	–	–
Acid Adjustment : Sulfite use	yes:sulfites-none:sulfites	–	–		–	–			–	–	–	–	–	–
	none:unsulfited-none:sulfites	–	–		–	–			–	–	–	–	–	–
	yes:unsulfited-none:sulfites	–	–		–	–			–	–	–	–	–	–
	none:unsulfited-yes:sulfites	–	–		–	–			–	–	–	–	–	–
	yes:unsulfited-yes:sulfites	–	–		–	–			–	–	–	–	–	–
	yes:unsulfited-none:unsulfites	–	–		–	–			–	–	–	–	–	–

*Indicates a significant ($p<0.05$) difference based on treatment interactions analyzed with HSD

−Indicates not significant according to ANOVA results