Virginia-grown Cider: How do Cultivar and Fermentation Strategies affect Cider Chemistry and Flavor?

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Virginia-grown Cider: How do Cultivar and Fermentation Strategies affect Cider Chemistry, Flavor and Consumer Valuation?

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Technical Abstract

The US cider market has expanded in recent years, but limited research-based information is available on fermentation management. This study investigates how apple cultivar and yeast inoculation affect the chemical and sensory properties of cider. Four ciders were produced in triplicate using combinations of two different apple cultivars - Harrison, a cider cultivar and GoldRush, a dessert cultivar - and two fermentation strategies - inoculated with dry active yeast EC1118 or Pied de Cuvé ambient fermentation. Ciders were analyzed for alcohol content, free/total SO₂, titratable acidity, volatile acidity, malic acid, pH, and residual sugar. Sensory evaluation was conducted using Descriptive Analysis with trained panelists. Results were analyzed via ANOVA and Principal Component Analysis. Apple cultivar and fermentation method resulted in significant differences for chemistry and sensory parameters. Malic acid concentration was greater in the control ciders while concentrations of both residual sugar and volatile acidity were higher in the PDC ciders. The interactions effect of cultivar*fermentation method influenced both malic acid and residual sugar concentrations, where concentration differences between control and ambient ciders is smaller for GoldRush than for Harrison, showing that fermentation style produces different results across cultivars. Volatile acidity produced opposite interaction effects as differences between fermentation styles was larger for GoldRush. For sensory attributes, Harrison ciders
produced high intensities for multiple attributes, but also higher variability. Multiple sensory descriptors displayed interaction effects as the fermentation method produced different results in different cultivars. This study demonstrates that increasingly popular practices in the industry can produce significantly different ciders.
General Audience Abstract

The US cider market has grown rapidly in recent years, with many new products entering the market. However, there is limited research-based information available on cider fermentation management. This study investigates how production variables, namely apple cultivar and yeast inoculation, affect the chemical and sensory properties of the cider created. The overall goal of this project is to assess the chemical and sensory characteristics that come from cider production treatments. In this study, four experimental ciders were produced using combinations of two different apple cultivars – Harrison, a cider cultivar and GoldRush, a dessert/fresh market cultivar – and two fermentation management strategies – inoculated with dry active yeast strain EC1118 or indirect inoculation through a natural fermentation method. Ciders were analyzed for alcohol content, free and total SO₂, titratable acidity, volatile acidity, malic acid, pH and residual sugar. Sensory evaluation was conducted by a trained panel providing descriptive terms and intensities for each sample. Both chemical and sensory results were analyzed to reveal significant differences in samples based on not only apple type and inoculation method, but also the interactions between those two variables. This study demonstrates that increasingly popular practices in the cider industry – like natural fermentation or the use of cider-apple varieties – can produce significantly different ciders. This highlights the idea that producers need to treat each apple cultivar differently, as they behave differently throughout production.
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Chapter 1: Introduction

Cider, specifically hard apple cider, is a growing industry in both the USA and specifically the state of Virginia. As of 2018, cider had 1.3% production levels compared to beer (Ewing and Rasco, 2018; Gaille, 2018). Virginia is one of only 5 states in the country that has cider holding a 3% or larger share of alcohol sales compared to beer (Gaille, 2018). The cider industry is also one that lacks research about both production effects and sensory quality compared to its similar counterparts of wine or beer.

Cider has been fermented around the world for ages, especially in the UK and western Europe. Only in the last decade did modern cider reappear in the US and become a popular beverage (Jacobsen, 2014). Cider in the U.S. dates back to the Revolutionary era where many landowners had apple orchards which they then used for homemade ciders (Bandlamudi, 2017). When the time of Prohibition began, most of those apple orchards were then chopped down and cider was rare until recently. Cider is described as being in “a renaissance” in the modern era as it grows in popularity once again (Bandlamudi, 2017). Although the industry has been one of the fastest-growing categories of the alcohol industry (WSU Extension, 2019; Jamir et. al, 2020), the cider research field is still small and evolving. Most existing cider research has focused on classic techniques, like using cider apple juice or concentrate with a yeast inoculum, but the scope of interest has expanded to ask new questions and leaves many variables of the fermentation process unstudied.

A wide variety of apple cultivars can be used for cider production, including dessert, cider, and culinary apples (Ewing and Rasco, 2018). The effect of apple cultivar has been studied in terms of chemical properties, but there is still a lack of research on
what this means for sensory quality (Lea, 2015). Dessert apples are known to have a very distinct flavor that is easily recognizable by consumers in the apples themselves, but there is no research as to this effect in cider (Valois et. al, 2006). Existing research has found that the main aromas and flavors of the cider come from the characteristics of the apple variety used, but there is also large influence from compounds created by the yeasts and bacteria as well as through the fermentation and ageing processes (Riekstina-Dolge et. al, 2012).

1.1 Overall Objective

The overall objectives of this research project are (1) to determine if the variety of apple used and yeast inoculation have a relationship with how a consumer describes a hard cider and (2) to examine the influence of these production variables on cider chemistry.

1.2 Specific Objectives and Hypotheses

Specific Objective 1: Determine whether and how using dessert apples versus cider apples affects both chemical and sensory properties of ciders.

Working Hypothesis 1: Ciders created using cider apples will have chemical and sensory properties similar to each other but will differ from those made of dessert apples.

Specific Objective 2: Examine how yeast inoculation versus an ambient fermentation affects both the chemical and sensory properties of the cider created.

Working Hypothesis 2: Ciders fermented without employing inoculation will be more variable both within and between groups in their chemical and sensory properties than those that have been inoculated.
Chapter 2: Literature Review

2.1 Cider Definition and Virginia Production

Cider is defined as an alcoholic beverage made from the fermentation of apple juice (Thornton, 2013). More specifically, the TTB defines cider as a fruit wine from apples that is produced through normal, alcoholic fermentation of ripe apple juice (TTB, 2020). Virginia is the 6th largest producer of cider in the United States, and is the leading cider producing southern state. Cider has been produced in Virginia for centuries (Calhoun, 2010). It has not been until more recently, however, that cider became of similar production amounts and popularity as wine or beer. It has been suggested that the United States’ cider industry is not mature yet and has entered a phase of major quality growth (Fabien-Ouellet et. al, 2018).

Virginia is the sixth largest apple-producing state in the US with commercially viable growing regions located throughout the state from Southwest Virginia up to the Northern Shenandoah Valley (Virginia Apple Board, 2020; Virginia Cider, 2018). With this large apple industry comes many possible applications for the fruit, including fresh market apples, processing apples, and cider. Virginia has a rapidly growing cider industry with over 20 producers selling cider locally, regionally, and nationally in the United States (Virginia Apple Board, 2020). Over 30 different varieties of apples strictly for cider use are now grown in Virginia, and experimentation with new cultivars continues to gain interest (Virginia Cider, 2018). The United States’ cider sales grow at an average of about 73% each year, and Virginia’s sales parallel that trend (Virginia Cider, 2018).

2.2 Cider Apples

There are many different varieties of apples for both raw consumption as well as use in baking, cooking, or cider making. The main varietal types used for cider are cider and dessert apples. Cider apples have unique qualities like being bittersweet as well as
bittersharp and having a much higher level of astringency than those traditionally sold to consumers for snacking and baking purposes (Martin et. al, 2017). Cider apples won’t normally be found in a grocery store, as they are not typically palatable when consumed in raw apple form. Dessert apples are more palatable when fresh and cover a wide range of flavors (Jacobsen, 2014). Some researchers say that there is a need to educate and inform consumers about the different varieties of apples used for cider production and what the consumer should expect from each type (Martin et. al, 2017).

Cider has been traditionally made using “cider” apples. More ciders now are being produced using a blend of apples, with high amounts of dessert and culinary apples in the blend (Riekstina-Dolge et. al, 2012; Valois et. al, 2006; Virginia Cider, 2018). In fact, almost all ciders produced now are made from blends to get all the aspects that the producer is looking for. In the USA, the TTB does not control for or even consider the use of different apple cultivars, cider or dessert, when labeling ciders (Fabien-Ouellet et. al, 2018). This leaves it up to the producers to utilize their apples in a way that suits them and their ciders best. There has been little to no research looking into if there is any consumer demand for cider to be made only from cider apples (Fabien-Ouellet et. al, 2018). Cider apples have high levels of acidity and tannins, which can create quality ciders but are undesirable traits if the apples are consumed raw. Dessert apples tend to have lower acidity and tannin levels as well as a high nitrogen content (McKie, 2011; Proulx and Nichols, 2003; Valois et. al, 2006). Juices with large amounts of nitrogen ferment rather quickly and aggressively to the end, making drier ciders (Proulx and Nichols, 2003). While some countries, notably the UK and France, generally stick to just cider apples or blends primarily of them, North American countries have adopted cider
making practices that involve high proportionally dessert apple blends. This began as the majority of apple orchards in North America were dessert apples so when the idea of cider came to the continent, there was only one option to ferment with. As cider became a larger market, more cider apple orchards have been started and blends of cider and dessert apples have emerged (Jolicoeur, 2013; Calhoun, 2010).

A well-balanced cider is usually made up of a mix of bitter, bittersweet, sweet, and tart apples (Thornton, 2013). Although blends are the most commonly seen ciders today, single varietal ciders are still being made and sold. Using a single varietal limits the ability to pick and choose characteristics from different apples to make one cohesive cider with only desirable traits (Thornton, 2013; McKie, 2011).

Cider can really be made from almost any kind of apple, although there are some varieties that make better ciders than others (Lea, 2015). For positive cider outcomes, apples with higher sugar levels work best as the sugars help the fermentation process and will result in higher alcohol levels. This reasoning is why cider apples, which typically have higher sugar contents, are used in cider fermentations (Thornton, 2013). Dessert apples often suffer from pectin release which creates a cloudier cider (Lea, 2015). This can be seen as an undesirable trait to consumers. As consumers and producers’ opinions differ, there is no set definition for the best apples to make cider with (Proulx and Nichols, 2003). It is up to each individual producer to determine what works best for them and the outcome they are looking for.

Besides the differences in sensory attributes, different apple varieties produce ciders with different chemical compositions as well. The primary difference noted between varietals is the phenolic content (Rosend et. al, 2019). When looking at volatile
composition, apple variety has been found to be the primary influence (Rosend et. al, 2019). The choice of apple variety needs to be taken into account when a producer is wanting to create a certain cider with a specific profile.

2.3 Cider Fermentation

Cider fermentation involves the conversion of simple sugars like sucrose, fructose, glucose, and sorbitol in the apple juice into ethanol and carbon dioxide by yeast (Proulx and Nichols, 2003). This metabolism occurs by either the native yeasts already present in the apple juice, or by yeast that are added to the juice via targeted inoculation strategies.

Cider fermentations can experience a number of flaws, including becoming stuck or sluggish. These are caused by incomplete sugar utilization in the juice that is caused by a stress onto the yeast (Bisson and Butzke, 2000). These slow fermentations are related to fermentation kinetics and low amounts of yeast assimilable nitrogen (YAN) (Boudreau et. al, 2017). Fermentation problems related to YAN can be linked to common apple handling practices, like fungicides (Boudreau et. al, 2017). These stuck or sluggish fermentations can create reduced quality ciders (Boudreau et. al, 2017). Slow fermentation kinetics are often found in naturally fermented ciders, due to the competition of various yeasts and microorganisms to ferment the juice (Boulton et. al, 1997). Research into post-harvest management found that fruit storage treatment and handling impacted cider characteristics. It was noted that these results may be dependent on specific apple cultivar and that each cultivar may behave differently (Elwing et. al, 2019).
When it comes to the final product, the odor and the flavor are seen as the most important factors when looking at quality and these are both very closely related to the making procedure and how the fermentation is carried out (Antón et. al, 2014). A cider will contain compounds that contribute to and directly affect the flavor and come from both the original apples used as well as through fermentation (Williams, 1974).

2.4 Yeast Inoculation

Of the variables yet to be studied in depth, the lack of inoculation for fermentations is one of the more recent to spark interest. The idea of a “natural fermentation”, that is done solely by the native yeast already present in the juice, has yet to be explored much in cider although is established in wine and beer (Sánchez et. al, 2014).

Uninoculated fermentations studied in wines are more prone to being “stuck” or “sluggish”, or just simply take longer to start up or finish fermenting (Boulton et. al, 1997). Typically, an inoculated fermentation will begin with just a few hours of the yeast addition, where an uninoculated one will not begin for a few days. This occurs due to the fact that the various different native yeasts become involved in the fermentation in succession with one another instead of one dominant strain beginning fermentation all at once (Lea, 2015). On top of this, since the fermentative process is happening innately, the results of the final product can be variable, as native yeasts are unpredictable (Wang, 2018). Previous studies on wine have shown that sometimes wild yeast can contribute to the sensory aspects of the final product, either positively or negatively. Occasionally, off aromas or flavors can develop that are not desired (Boulton et. al, 1997). To help control for unwanted products, a more controlled fermentation with inoculation of specific and
known yeast strains are typically used. It has been found that to create a rapid and even rate fermentation with a product of consistent quality, inoculation is the best route (Heard and Fleet, 1985).

One of the main objectives behind yeast inoculation in a fermentation is the addition of a yeast strain with generally known characteristics and outcomes (Jolicoeur, 2013). This often leads to a predictable outcome with high reliability for the cider being made. When making an uninoculated cider, the outcome is unknown and hard to predict. The fermentation duration may also be difficult to predict. Native yeasts tend to be not as strong of fermenters as cultured strains (Vrooman, 2020). This often leads to slower fermentations or ones that may stop before being fully complete. A cider fermentation stopping early will lead to residual sugars in the final product (Jolicoeur, 2013). Since native yeasts are not as strong as a cultured yeast, they often die off in highly hygienic and amateur cideries (Proulx and Nichols, 2003). This makes an uninoculated fermentation almost impossible and not a viable option for many producers.

While the final product may be unpredictable, those who enjoy uninoculated fermentations note that the cider has more complex and interesting flavors than those produced by traditional inoculation of yeast (McKie, 2011). An inoculated fermentation singles out one strong yeast strain as the most dominant, which can create a flavor profile that is relatively one-note (McKie, 2011; Lea, 2015). The yeast strain most often used is *Saccharomyces cerevisiae*. Due to the competition of various native yeast strains in an uninoculated fermentation, each batch may have unique and complex qualities to it. Studies in cider have found that yeast strain is a variable that plays a large role in cider flavor (Riekstina-Dolge et. al, 2012). Yeast strains can impact the aroma profile as well,
mostly by raising the levels of higher alcohols and esters (Rosend et. al, 2019). The extent to how much a yeast strain can affect the volatile composition relies a lot on the apple variety used (Rosend et. al, 2019).

2.4.1 Yeast Inoculation in Wines

Most of the research on yeast inoculation in fermented food and beverage products has been done on wine. Considering the fact that wine has a similar production process to cider, these findings can be of importance when trying to fill the knowledge gap that exists in cider fermentation research.

Traditionally, wine was produced by the fermentation of the yeasts already native to the grape juice. This included many different types of yeasts that would begin the fermentation, but original beliefs thought, would eventually die off to leave *Saccharomyces cerevisiae* as the dominant species (Heard and Fleet, 1985). More recent evidence shows that may not be the case. Even in inoculated wine fermentations, the native yeasts can have a large contribution to the fermentation (Heard and Fleet, 1985). This supports the idea that all the yeast strains are important to the wine even when inoculating with a known and powerful starter culture. Research has suggested that even in inoculated fermentations, the major role of *S. cerevisiae* could be to influence the growth and development of other *Saccharomyces* strains rather than diminish others (Heard and Fleet, 1985). This evidence shows support for the idea that the native yeast strains heavily influence the wine in both inoculated and non-inoculated fermentations. Studies have shown that fermentations typically start with non-*S. cerevisiae* strains that come from both the grape juice and the surrounding environment to shape the flavor and
style of the wine. Next, the *Saccharomyces* strains begin to convert the sugars to alcohol and are the main strains in the fermentative process (Bezerra-Bussoli et. al, 2013; Ocón et. al, 2010). The native yeast strains may still become dominant in the fermentation and have a large impact as they are the best acclimated to the environmental conditions in which the grapes grow and the wine will come from (Bezerra-Bussoli et. al, 2013).

Research has shown that the non-*Saccharomyces* yeasts heavily impact the beginning phases of fermentation and use this time to compete with *Saccharomyces* strains for nutrients, which can possibly delay the onset of fermentation (Vrooman, 2020). These uninoculated fermentations can also possibly begin slower due to the increase in microbial activity that the native yeasts provide (Vrooman, 2020). As well as a slow start, these fermentations may also slow before an inoculated fermentation would as there are different nutrient needs and different activity levels (Vrooman, 2020).

Research in wine in Italy has suggested that the use of native yeasts can help improve the sensory characteristics of a wine as those yeast strains are the best adapted to the must (Tristezza et. al, 2014). Since the yeast is already a part of the grape juice, it should line up with its other properties and work well together, whereas an inoculated yeast strain may not be the best match (Rosend et. al, 2019). Native yeasts have also been found to provide unique flavor profiles that cannot be replicated through any other techniques (Ugliano and Henschke, 2009). Uninoculated fermentations can lead to a much more distinguished aroma in the wine as well (Wicklund et. al, 2020). Due to the increase in microbial diversity in an uninoculated fermentation, it has been noted that there is often an increase of both the complexity and intensity of aromatics in the wine (Vrooman, 2020). More information is needed to determine the exact impact of a yeast
strain on the flavor and aroma of a wine. This information can be used to help best choose a strain that will minimize off-flavors and maximize the desired characteristics of the product (Ugliano and Henschke, 2009). The use of native yeasts has been found to increase the potential for off-aromas and flavors as well as microbiological spoilage (Wicklund et. al, 2020; Vrooman, 2020), so further research into how different yeast strains react and ferment could help mitigate those risks.

The native yeasts, that are not always *Saccharomyces* strains, have been found to provide lower levels of alcohols, esters, and terpenes (Rosend et. al, 2019). These all differentiate the chemical composition from wines that have been inoculated with a *Saccharomyces* strain. There is, however, evidence that *Saccharomyces cerevisiae* strains can still be the main yeasts working in uninoculated fermentations and that they are the most important yeast in the conversion of sugars to ethanol (Bezerra-Bussoli et. al, 2013; Ocón et. al, 2010). In a consumer test on wines, it was found that the basic chemistry of both uninoculated and inoculated fermentations were very similar but that the panelists could easily distinguish the samples in a triangle test based off of differences in acidity levels (Vrooman, 2020). This evidence shows that although the chemical compositions may not appear very different, there can still be significant outcomes when looking at sensory data.

### 2.4.2 Pied de Cuvé

One method of performing a natural, or ambient, fermentation is the pied de cuvé (PDC) method. PDC is a form of indirect inoculation that can be done in two different ways. The first is by using must that is found already fermenting in the winery to make an
inoculum and the second is to use must that is found spontaneously fermenting out in the
vineyard or another location that doesn’t have direct effects from winery-resident yeast
(Morgan et. al, 2019). Both of these are methods of creating a subset of a natural
fermentation which can later be used as an inoculum with fresh juice. The second method
is used when it is desired to have more non-\textit{Sacchromyces cerevisiae} yeast strains since
the location allows for more variety of native yeast strains (Morgan et. al, 2019).

Utilizing a PDC method allows for a large variety of yeast strains and
microorganisms to be present in the cider or wine being made (Moschetti et. al, 2016).
Another advantage of a PDC fermentation is risk mitigation. Common issues that arise
with natural fermentations are spoilage microorganisms, stuck or sluggish fermentations,
and off-flavors. By only using an inoculum of spontaneously fermented juice, these risks
can be lowered (Moschetti et. al, 2016). One drawback to PDC methodology, is there is
an often a delay in fermentation by about 2 to 3 days (Morgan et. al, 2019).

After the PDC has spontaneously fermented for some (variable) time, it will be
used as an inoculum. Ratios for PDC methodology have differed as it has not been
heavily researched (Morgan et. al, 2019).

\textbf{2.5 Sensory Evaluation}

Sensory evaluation began in the United States when the government was
searching for information on food preferences within the armed forces (Stone et. al,
2012). The food and beverage industry noted the results that the government found and
began to implement the evaluation techniques to the field (Stone et. al, 2012). The
concept of sensory evaluation in food began in the 1940’s and evolved for some time
until it became how it is now (Stone et. al, 2012). Sensory evaluation is a set of
techniques to evoke, measure, analyze, and interpret human responses to a food or beverage through the senses (Lawless and Heymann, 2010). The idea of sensory evaluation is using humans as the measuring instrument to gather and obtain results.

There are three main classes of sensory tests that can be utilized: difference testing, descriptive analyses, and affective testing (Lawless and Heymann, 2010). Difference testing is seeing if a panelist can find any perceptible differences between products (Lawless and Heymann, 2010). Descriptive analysis studies use an interacting group of panelists to examine the perceived intensities of different product characteristics (Lawless and Heymann, 2010). Affective testing is a methodology to quantify how well a product is liked or disliked by the panelists in the study (Lawless and Heymann, 2010). One of the main principles in sensory evaluation is that the type of test used should be chosen based on the objectives that are being looked for (Lawless and Heymann, 2010).

2.5.1 Sensory Evaluation of Cider

While sensory testing is not new to the cider industry, it has traditionally not been utilized often or to its full extent. Sensory and consumer insights are collected rather sparingly and most often the innovation and product development is coming only from the cider makers without consulting consumer input (Jamir et. al, 2020). This can be seen as limiting to the cider industry as data from sensory evaluation can help shape a product to be what consumers are looking and willing to pay for. As the knowledge of cider flavor and aroma increases, it can become more possible to adapt the cider to meet new and specific consumer demands (Williams, 1974). There is a lot of sensorial data on both wine and beer and while some of these conclusions can map onto cider, there are still
major differences in the sensory properties of the different beverages (Le Quéré et. al, 2006).

**2.5.2 Descriptive Analysis**

Descriptive analysis (DA) is a method of obtaining fully complete sensory descriptions of a product using a trained panel (Lawless and Heymann, 2010). This technique is found to be most useful when looking at comparisons of sensory attributes across different products (Lawless and Heymann, 2010). In a DA, the participants are trained to use well-defined terms to describe the product in question and its characteristics (Heymann and Ebeler, 2017). The panelists may be trained in different ways, but each method involves the use of reference standards to ensure that all of the panelists will agree on the concept each descriptor is referring to (Heymann and Ebeler, 2017). These tests utilize a small panel of about 6 to 20 participants, with a typical group being 10-12 participants (Stone et. al, 2012). The panelists will endure group training sessions that are led by a non-participating leader to ensure that each of the participants fully understand each attribute and its reference point (Stone et. al, 2012). This helps create a consensus among the group so that the judging of the samples is as accurate as possible. The attributes used are ones that are generated by the panel themselves as descriptors for the product as they evaluate it in training sessions (Stone et. al, 2012). These attributes are used in the final testing of the product in question. Before the final evaluations can take place, the participants must be screened for accuracy in their use of descriptors as well as their ability to detect differences between products (Stone et. al, 2012). This determines whether or not a participant has been adequately trained and will be able to provide reliable results. Once all the panelists have been
trained, the complete final evaluations of the product using the list of descriptors created to assign attributes and their intensities to the samples (Stone et. al, 2012). Overall, the key to a successful DA study is the interactions among the panelists to reach a group consensus (Heymann and Ebeler, 2017).

Lexicons can be produced through DA testing. A flavor lexicon is a list of words that describe the flavor of the product in question (Drake and Civille, 2003). This list is created by the panelists as they evaluate the samples in their training sessions. Lexicons are important to have for food or beverage products as it is a set list of terms that can be used to describe the product in question, instead of using free description. Once a lexicon is created for a product, it can be utilized in a descriptive analysis study for panelists to rate the intensities of each of the characteristics according to the sample being evaluated (Stone et. al, 2012).

### 2.5.3 Consumer Acceptance of Cider

One factor often examined in sensory and consumer studies is willingness-to-pay. Willingness-to-pay (WTP) is looking at the amount that a consumer would pay for a product in regard to the overall experience, as well specific sensory or physical qualities (Tozer et. al, 2015). WTP can be tested in different ways and can help inform producers of how a consumer feels about their product in terms of valuation. A study on WTP in different cider samples showed positive correlation between liking of a product and WTP (Tozer et. al, 2015).

Besides overall hedonic liking, other attributes of a product have been found to affect a consumer’s willingness-to-pay and product valuation. In a study looking at the role of
production process and information on consumer perceptions of sparkling wines, it was concluded that both sensory and non-sensory attributes of the sparkling wines presented had an effect on consumer preferences. When consumers tasted both Charmat and Champenoise wines, both with and without information on the production process, the Charmat wines were preferred in terms of hedonic liking. However, when detailed information on the production was provided with no tasting, consumers preferred the Champenoise wines. From this, it can be suggested that production process plays a large role on liking expectations, even if not on informed liking (Vecchio et. al, 2019).
References


Who We Are | Virginia Cider. virginiacider.org, https://virginiacider.org/who-we-are/.


Chapter 3: Influence of Cider Cultivar and Fermentation Method on Chemical and Sensory Characteristics Determined by Descriptive Analysis

3.1 Abstract

The US cider market has expanded recently, but limited research-based information is available on cider fermentation management. This study investigates how apple cultivar and fermentation strategies affect the chemical and sensory properties of cider. Four experimental ciders were produced in triplicate using combinations of two different apple cultivars - Harrison (cider cultivar) and GoldRush (dessert cultivar), and two fermentation strategies - inoculation with active dry yeast strain EC1118 or Pied de Cuvé (PDC) ambient fermentation (inoculation with environmental microbiota from an orchard). Ciders were analyzed for alcohol content, free/total SO\(_2\), titratable acidity, volatile acidity, malic acid, pH, and residual sugar. Sensory evaluation was conducted using Descriptive Analysis with trained panelists. Results were analyzed via ANOVA and Principal Component Analysis. Apple cultivar and fermentation method resulted in significant differences for chemistry and sensory parameters. Malic acid concentration was greater in the inoculated ciders while concentrations of both residual sugar and volatile acidity were higher in the PDC ciders. The interaction effect of cultivar*fermentation method influenced both malic acid and residual sugar concentrations, where concentration differences between control and ambient ciders for these parameters was smaller for GoldRush than for Harrison, showing that fermentation strategies may yield different results in different apple cultivars. The opposite was observed for volatile acidity, where greater differences by fermentation method were observed in GoldRush. Harrison ciders displayed high intensities for multiple sensory attributes, but also higher variability. Multiple sensory descriptors also reflected a
significant interaction effect between our experimental treatments. This study demonstrates that the treatments evaluated, and the interaction between the treatments, produced significantly different ciders.

3.2 Introduction

Cider is defined as an alcoholic beverage made from the fermentation of apple juice (Thornton, 2013). More specifically, the United States Alcohol and Tobacco Tax and Trade Bureau defines cider as a fruit wine from apples that is produced through normal, alcoholic fermentation of ripe apple juice (TTB, 2020). It also must have the taste, aroma, and characteristics of a hard cider (Proulx and Nichols, 2003).

The recent rapid growth in the US cider industry has prompted new interest in cider production, and in research to inform cider-production decisions. Virginia ranks sixth in the United States for both apple production and number of cideries, with many of these producers primarily interested in production of Virginia-grown cider, or cider made from apples grown in one of the state’s 100+ orchards. (Virginia Apple Board, 2020; Virginia Cider, 2018; Garabelli, 2016). Virginia has multiple apple cultivars, like Harrison, once considered a “lost” apple, associated with the state that are of interest for producers (Albemarle Ciderworks, 2021). Although the cider industry has been one of the fastest-growing categories of the alcohol industry (WSU Extension, 2019; Jamir et. al, 2020), the cider research field is still small and evolving.

There is currently not enough research to understand how choices in the production process, like apple cultivar or fermentation method, contribute to overall cider quality or attributes. This is especially the case for Virginia and the USA in general, where there is not a continuous history of cidermaking.
One of the main factors that is thought to contribute to cider quality is the apple variety used for juice (Rosend et al., 2019). There are three main categories of apples: culinary, cider, and dessert. Culinary apples are best for cooking and are often used for foods like pies and sauces, dessert apples are usually the best to be eaten fresh, and cider apples are typically the best for fermenting into cider. However, both cider and dessert apples are used for cider production in the United States (WSU Extension, 2021). Cider apples have high levels of acidity and tannins, which are thought to create quality ciders but are undesirable traits if the apples are consumed fresh. Dessert apples are more palatable when consumed fresh out of hand (Jacobsen, 2014). Dessert apples tend to have lower acidity and tannin levels as well as a comparatively high nitrogen content (McKie, 2011; Proulx and Nichols, 2003; Valois et al., 2006). Traditionally ciders were made with cider apples, but more ciders are now being produced using a blend of apples, with high amounts of dessert and cider apples in the blend, as cider apples are of a limited supply (Riekstina-Dolge et al., 2012; Valois et al., 2006; Virginia Cider, 2018; Elwing et al., 2019).

Cider has a long, traditional history in many European countries, and through that there is generally more knowledge about apple cultivars. France is known for their ciders and have 11 main cultivars used for cidermaking. Some of these popular cultivars are Douce Moen, Douce Coet Ligne, Judor, and Petit Jaune (Merwin et al., 2008). These cultivars are heavily researched and are used for their high juice yields, pest and disease tolerance, replicability, and desired attributes that are contributed to cider (Merwin et al., 2008). Similarly, Spain uses apple cultivars that have been grown and studied for years. Some of these are Avrolles, Bedan, and Cidor (Merwin et al., 2008). These apples are
very often used because they are well-researched and reliable. England is also known to be a very large cider producer of Old-World ciders. Although less apples are grown in England compared to Spain or France, there are still many well-known apples for producers to choose from for cidermaking (Merwin et. al, 2008). A few of these are Kingston Black, Tom Putt, and Yarlington Mill (Orange Pippin, 2021). These three countries and their cider apples are so well established, that American producers often look to them for help on determining the validity of new American cultivars (Merwin et. al, 2008). There is a lot of research and production-based knowledge in Europe on their cultivars, but American knowledge has a long way to go.

**Choice of variety and blending of juices can have significant effects on cider fermentation.** For positive cider outcomes, apples with higher sugar levels work best as the sugars help the fermentation process and will result in higher alcohol level. Juices with large amounts of nitrogen ferment rather quickly and aggressively to the end, making drier ciders (Proulx and Nichols, 2003). Blends can help producers achieve a desired end product. Besides the differences in sensory attributes, different apple varieties produce ciders with different chemical compositions as well. One big difference noted between varietals is the phenolic content, but there is no conclusive evidence on how individual apple cultivar impacts chemical composition (Rosend et. al, 2019). Existing research looking into high-tannin apple cultivars found that both fermentation kinetics and cider aroma can be impacted by both type and concentration of phenolic compounds (Cairns et. al, 2019). Research has also been done looking into amino acid concentrations of Virginia apple cultivars, and it was found that relative amino acid profiles of different cultivars can potentially have impacts on cider flavor and aroma (Ma et. al, 2018).
Producers can take all of these variables into account when deciding if, or what, to blend for their ciders.

As a food product, the odor and flavor of cider are some of the most important quality indicators, and these are both thought to be influenced by the selection and blending of apples and how the fermentation is carried out (Antón et. al, 2014). Flavor compounds and precursors come from the apples as well as through fermentation (Williams, 1974). When looking at volatile composition of cider, apple variety has been found to be the primary influence, alongside the effects of yeast strain and the maturity of the apples (Rosend et. al, 2019).

Another key contributor to the final sensory quality of cider is the fermentation method. The idea of a “natural fermentation”, which is accomplished solely by the native yeast and bacteria already present on the fruit or in the environment, is fairly established in wine and beer (Sánchez et. al, 2014). Producers have utilized this method because it has the potential to increase the complexity of the aroma and flavors of their products, which is often valued in wine and beer (Sánchez et. al, 2014). However, uninoculated fermentations in wines are more prone to being “stuck” or “sluggish”, or just simply take longer to start up or finish fermenting (Boulton et. al, 1997). Typically, an inoculated fermentation will begin with just a few hours of the yeast addition, where an uninoculated one will not begin for a few days. This occurs due to the fact that the various different native yeasts become involved in the fermentation in succession with one another instead of one dominant strain beginning fermentation all at once (Lea, 2015). Native yeasts tend to be not as strong of fermenters as cultured strains (Vrooman, 2020). A cider fermentation stopping early will lead to residual sugars in the final product (Jolicoeur,
2013). On top of this, since the fermentative process is happening innately, the results of the final product can be variable, as native yeasts are unpredictable (Wang, 2018). Previous studies on wine have shown that sometimes wild yeast can contribute to the sensory aspects of the final product, either positively or negatively. Occasionally, off aromas or flavors can develop that are not desired (Boulton et. al, 1997). While the final product may be unpredictable, those who enjoy uninoculated fermentations note that the cider has more complex and interesting flavors than those produced by traditional inoculation of yeast (McKie, 2011). Due to the competition of various native yeast strains in an uninoculated fermentation, each batch may have unique and complex qualities to it.

Studies in cider have found that yeast strain is a variable that plays a large role in cider flavor (Riekstina-Dolge et. al, 2012). Yeast strains can impact the aroma profile as well, mostly by raising the levels of higher alcohols and esters (Rosend et. al, 2019). The extent to how much a yeast strain can affect the volatile composition relies a lot on the apple variety used (Rosend et. al, 2019). One variable in fermentation method is the use of yeast nutrients. Research has found that both yeast nutrient type as well as timing of nutrient addition, impact fermentation duration (Moore et. al, 2020). Yeast nutrient does not have any significant effect on H$_2$S, a negative fermentation by-product, production, but yeast strain does (Moore et. al, 2020). Both yeast strain and yeast nutrient are thought to work together to create many of the attributes found in cider (Moore et. al, 2020).

One method of performing a natural or ambient fermentation is the pied de cuvè (PDC) method. PDC is a form of indirect inoculation that involves creating a “starter” fermentation from ambient microbiota. PDC can be done in two different ways. The first is by using must that is found already fermenting in the winery to make an inoculum and
the second is to use must that is found spontaneously fermenting out in the vineyard or another location that doesn’t have direct effects from winery-resident yeast (Morgan et. al, 2019). Both of these are methods of creating a subset of a natural fermentation which can later be used as an inoculum with fresh juice. The second method is used when it is desired to have more non-\textit{Sacchromyces cerevisiae} yeast strains since the location allows for more variety of native yeast strains (Morgan et. al, 2019). Utilizing a PDC method allows for a large variety of yeast strains and other microorganisms to be present in the cider or wine being made (Moschetti et. al, 2016). Another advantage of a PDC fermentation is risk mitigation. By only using a subset of juice as an inoculum, risks, like spoilage microorganisms or stuck fermentations, can be lowered (Moschetti et. al, 2016).

There has been very little reported sensory evaluation of ciders, especially compared to the number and variety of ciders now on the market in the United States. Sensory and consumer insights are collected rather sparingly and most often the innovation and product development is coming only from the cider makers without consulting consumer input (Jamir et. al, 2020). In contrast, there is extensive published research into the sensory qualities of beers and wines. While these results can be sometimes generalized to cider, there are certain to be major differences in the sensory properties of the different beverages (Le Quéré et. al, 2006). Sensory evaluation of cider could lead to better understanding of the sensory impacts of production and processing. A key method in gathering information on sensory attributes and sensory differences is descriptive analysis (Lawless and Heymann 2010; Heymann, 2014). One descriptive analysis study done previously on cider looked at flavor profiles of ciders from three European countries. It was found that ciders from different countries produced different
flavor profiles. The research also looked into production methods and found that ciders made by the same methods were clustered together by both chemical and sensory qualities and were separate from ciders made by other methods (Qin et. al, 2017).

Therefore, in order to help fill the identified knowledge gaps on the relationships among apple variety and fermentation method on cider chemistry and sensory quality, this report presents research into the effects of apple cultivar and fermentation method on both the chemical and sensory properties of Virginia-grown cider. The objective of the reported research was to determine if apple cultivar and fermentation strategy have independent or interactive effects on cider, and if so, what those effects are. Ciders were created using two different apple cultivars with two different inoculation strategies. Once ciders were completed, sensory analysis was done in the form of a descriptive analysis study. Additionally, chemical analyses were run on the ciders to examine the chemical and sensory properties of the ciders and how they relate.

3.3 Materials and Methods

3.3.1 Apples and Juice

Two cultivars of apples commonly used for cider production in Virginia, USA were purchased from Silver Creek Orchards in Tyro, VA. Apples of cultivar Harrison (Albemarle Ciderworks, 2021) and cultivar GoldRush (Janick, 2001) were harvested at commercial maturity and transported from the orchard cold storage to our lab shortly after harvest. The apples were stored in bushel-sized cardboard boxes at 35º F for one week before being pressed in the Food Science and Technology Pilot Plant at Virginia Tech, Blacksburg, VA. The two cultivars, Harrison and Gold Rush, were pressed
separately using a Goodnature X-1 Industrial Cold Press Juicer (Goodnature, Buffalo, NY). Four bushel-sized boxes of each apple cultivar were pressed in batches with the press capacity being 25-30 pounds per batch.

### 3.3.2 Juice and Cider Chemistry

Prior to fermentation, primary juice chemistry parameters were determined using standard methods for fruit juice analysis. Juice samples for analysis were taken from the lot of pressed juice for each cultivar. Parameters evaluated were pH (probe, Accumet Ultra Triode Electrode Model 13-620-631, Thermo Fisher Scientific, Waltham, MA, USA); soluble solids (Brix Refractometer Model RF10, Extech Instruments Corporation, Nashua, NH, USA); Residual Sugar (Megazyme Glucose/Fructose Enzymatic Kit, Megazyme International, Wicklow, Ireland); Titratable Acidity, standard method as reported by Amerine and Ough 50 (Official Methods of Anaylsis, AOAC International, Rockville, MD, USA); malic acid (Megazyme Malic Acid Assay Kit, Megazyme International, Wicklow, Ireland); Total YAN was calculated using the nitrogen concentrations determined by two assays: Primary Amino Nitrogen, (Megazyme PANOPA Enzymatic Kit, Megazyme International, Wicklow, Ireland) and L-Arginine-Urea-Ammonia (Megazyme Ammonia (Rapid) Assay Kit, Megazyme International, Wicklow, Ireland). Using the two assays, YAN is calculated using:

\[
YAN_T = 1000 \times \left[ \frac{AM \times 14.01}{17.03} + \frac{UR \times 28.02}{60.06} + \frac{AR \times 28.02}{174.21} \right] + (129.74 \times \Delta A_{PAN})
\]

Where \(YAN_T\) = total YAN values, \(AM\) = ammonium ions, \(UR\) = urea, \(AR\) = arginine, and \(PAN\) = primary ammono nitrogen.
3.3.3 Fermentation Methods

All equipment used was cleaned before fermentation set-up began. The 1-gallon carboys were autoclaved. Any equipment that could not be autoclaved was sanitized prior to use to prevent contamination. The sanitizer is a solution made using 11.36 L water, 0.42 g/L potassium metabisulfite, and 0.63 g/L citric acid. Stoppers and airlocks were placed in sanitizer for 25 minutes then removed and air dried immediately prior to use.

Potassium metabisulfite was added to the juice 24 hours prior to initiation of fermentation in sufficient amount to obtain 25 mg/L free SO2: 161 mg of potassium metabisulfite powder was dissolved in a 8.05 mL of water to create a 0.02 g/mL solution and 1mL was added to each 2.52 L aliquot of juice. After both SO2 and juice were added to the container, they sat, loosely covered at 18ºC, for 24 hours before any yeast was added to prevent interference.

The experimental design of this project (Figure 1) involved creating four different treatment groups from two key variables: apple cultivar and fermentation method. Each of the two apple cultivar’s juice was split in half, with each half being used with a different method of inoculation, either control or PDC. Separate fermentations were initiated using cider apple juice (Harrison), while the other half was made using dessert apple juice (GoldRush).

The control fermentations used the EC1118 yeast strain (Lallemand, Montreal, Canada). Yeast was rehydrated following the manufacturer’s directions prior to use and added to the carboys of pressed juice at the rate recommended by the manufacturer.

The uninoculated fermentations used the pied du cuvé method to obtain an environmental culture of ambient microbiota in a small amount of juice, then used the
PDC inoculum at a 1:4 PDC:juice ratio to provide an orchard-derived inoculum to start the PDC fermentations (Moschetti et. al, 2016). The PDC inoculum was created by placing 1.80 L of juice out at Kentland Farms Orchard (Blacksburg, VA), lightly covered, under an apple tree for a total of 72 hours (Figure 2). Several practice fermentations were conducted at different time intervals to determine that 72 hours was the best time to allow for a natural fermentation to begin.

The fermentations were conducted at 18°C in a walk-in cooler. Fermentations took place in 1-gallon glass carboys enclosed by a one-hole, size 6, rubber stopper and a twin bubble water-filled airlock (The Vintage Shop, Delta, BC) wrapped in Parafilm (Sigma Aldrich, St. Louis, MO). The carboys were two-thirds full (2.52 L), leaving one-third of headspace. Each container was stirred one time, three days after the initial inoculation. Stirring was performed by vigorous swirling of each carboy to re-suspend the yeast without spilling juice out of the carboy or contaminating the airlock. Ciders were visually monitored for signs of completion. Residual sugar testing was used to confirm end of fermentation: a value of <10 g/L signified completion for dry ciders.

Once the ciders were finished fermenting, cider chemistry parameters were determined for each of the experimental replicates. The analyses completed were: pH (probe, Accumet Ultra Triode Electrode Model 13-620-631, Thermo Fisher Scientific, Waltham, MA, USA); soluble solids (Brix Refractometer Model RF10, Extech Instruments Corporation, Nashua, NH, USA); Residual Sugar (RS) (Megazyme Glucose/Fructose Enzymatic Kit, Megazyme International, Wicklow, Ireland); Titratable Acidity (TA), standard method as reported by Amerine and Ough 50 (Official Methods of Analytis, AOAC International, Rockville, MD, USA); malic acid (MAL) (Megazyme...
Malic Acid Assay Kit, Megazyme International, Wicklow, Ireland); Total YAN was calculated using the nitrogen concentrations determined by two assays: Primary Amino Nitrogen, (Megazyme PANOPA Enzymatic Kit, Megazyme International, Wicklow, Ireland) and L-Arginine-Urea-Ammonia (Megazyme Ammonia (Rapid) Assay Kit, Megazyme International, Wicklow, Ireland).

3.3.4 Sensory Analysis

A descriptive analysis (DA) sensory study was performed on the cider samples produced. DA was chosen as it provides the most complete set of descriptors for a set of samples and allows for comparisons among samples (Lawless and Heymann, 2010). This study was approved by the Virginia Tech Institutional Review Board (IRB 19-939) to ensure safe protocols for human subject research. COVID-19 safety precautions were taken, and this limited the number of panelists possible to a maximum of seven. For this study, a panel of six subjects (6 female, ages 21-60+) were recruited from the Virginia Tech campus and surrounding area to create a lexicon to describe the sensory characteristics of hard cider. Potential participants completed a screener to confirm they were at least 21 years of age, had consumed alcohol before, and had no allergies to apples, cider, or alcohol.

The descriptive lexicon was created through a series of training sessions with the panelists. The training involved panelists trying all 12 cider samples in a randomized order and providing sensory descriptions (aroma/flavor, taste, and mouthfeel) for each. During training, three samples per panelists were served each session as 1.5oz pours in black wine glasses with plastic watch glass lids at approximately 35ºF. Panelists were
asked to expectorate cider samples during training. There were ultimately six, one-hour group training sessions.

The panelists suggested and approved appropriate reference standards for descriptors to help group consensus and ensure uniformity. The panelists worked together in their training sessions to refine their lexicon down to 35 terms with recipes (Table 1). Following the lexicon generation, panelists were trained on a 15-point line scale to rate the intensity of the attributes.

After training and lexicon generation were complete, the panelists quantitatively evaluated the samples with the defined lexicon in order to describe the samples. These evaluation sessions were done individually in sensory testing booths. Each panelist took part in 4, 1-hour evaluation sessions in which they were presented with 6 ciders per session. Samples were randomized with 3-digit codes and counterbalanced using a balanced incomplete block design to reduce bias in serving order. Evaluation sessions took place in sensory booths and samples were served in the same manner as the training sessions. Each cider was evaluated using Compusense Cloud (Guelph, ON) software on an iPad. At the beginning of each new week, for the total of the two week evaluation study duration, panelists were presented with the reference standards in order to refresh themselves on the terms and references before evaluation of that day’s samples. A Williams Latin Square Design test was utilized to eliminate presentation order effects.

3.3.4 Data Analysis

The juice data were analyzed using one-way ANOVA, with apple cultivar as a factor, to evaluate if there were any significant differences in the multiple chemical parameters tested. Cider chemistry data were analyzed through a two-way ANOVA with
interactions to examine the effects of the processing methods on the chemical composition on the cider. ANOVA factors were apple cultivar and fermentation method. Tukey’s Honestly Significant Difference (HSD) was performed as post-hoc testing for the significant values.

The descriptive analysis results were analyzed using MANOVA to determine both if there were significant differences in the overall sample descriptions and to determine whether apple variety, fermentation, or their interactions could be responsible for that variation. Factors of interest for MANOVA were apple cultivar, fermentation method, and their interaction, and panelist, fermentation replicate, and panelist replicate were included to account for unwanted variation (Rencher and Christensen, 2012). Following this, two-way pseudomixed ANOVA (Heymann et. al, 2014) with interactions were conducted using each of the 35 descriptors as the dependent variable to determine if that term varied significantly by either apple, fermentation, or their interactions. Radar plots were created to visualize the flavor profiles of the ciders based on the significant descriptors for each sample. These results were further analyzed through Principal Component Analysis (PCA) plots to understand variation between samples and which attributes contribute most to this variation.

All statistical analyses were done using R, version 3.6.1, statistical software environment (R Core Team, 2021). Code and data are available from the corresponding author on request.

3.4 Results and Discussion
3.4.1 Chemistry Results

The starting juice from each apple cultivar was analyzed with a standard juice panel (Yeast Assimilable Nitrogen (YAN), Total Soluble Solids (TSS), pH, Titratable Acidity (TA), and Malic Acid (MAL)). Significant differences (Table 2) between the two juices were found for all analyses except YAN. Although YAN was not found to be statistically significant, there is a large difference in the measured values for GoldRush. This large variance may be a measurement error and likely caused the differences in YAN value between the apple varieties to not be analyzed as statistically significant. The other data indicates that the starting juices obtained from cultivars Harrison and GoldRush differed from one another in multiple chemical parameters, some of which have the capacity to affect fermentation rate and duration as well as cider sensory characteristics. Harrison apple juice contained higher amounts of sugar (Brix°), malic acid, and titratable acidity than the GoldRush juice. Prior research has found that cider apples contain noticeably higher amounts of sugar as well as higher acidity levels than dessert apples (Jacobsen, 2014). The results found here are consistent with these prior findings and can suggest that the cider apple ciders may end up with a higher alcohol content through fermentation (Thornton, 2013). The significantly higher amounts of acidity in the cider apples also lines up with existing research (Jacobsen, 2014).

The chemistry results (Table 3) of the ciders were analyzed to determine if the production treatments had any effect on the chemical properties of the ciders. Apple cultivar and inoculum were the variables studied, creating four treatment groups with three treatment replicates in each. Ciders were compared among treatment groups to see if there were any differences present in the parameters measured. Significant differences
(p < 0.05) were found between each of the treatment groups: the treatments created different ciders. Evaluation also showed significant differences between the two apple cultivars for Titratable Acidity (TA), pH, and Total Sulfur Dioxide (TSO₂) and significant differences between the two fermentation methods for Malic Acid (MAL), Residual Sugar (RS), TA, Volatile Acidity (VA), and pH. TA was higher for the Harrison variety ciders while pH and TSO₂ were higher in the GoldRush variety ciders. These data line up with the juice chemistry data found earlier. MAL and TA were higher values in the inoculated samples, while both VA, pH, and RS were higher in the PDC ciders. These findings may be related to malolactic fermentation where the environmental microbes in the PDC ciders may be causing more malolactic conversion. This would create less malic acid and TA in our PDC samples and a higher pH. Higher amounts of residual sugar in the ambient fermentations supports existing findings stating that ambient fermentations are often sluggish and don’t ferment to completion, leaving higher residual sugar in the cider (Vrooman, 2020). PDC ciders saw higher amounts of volatile acidity, which is consistent with research noting that ambient fermentations often have complex bouquets and are susceptible to off-aromas, which are often associated with VA and are caused by lack of control of lactic acid bacteria (Wicklund et. al, 2020; Vrooman, 2020). Volatile acidity is defined as the measure of the product’s gaseous acids and is found far below the legal limit of 0.14 g/100mL (Penn State Extension, 2015). Both alcohol percentage and Free Sulfur Dioxide (FSO₂) were not found to be significant. A prior study found that ambient fermentation resulted in lower alcohol concentration than Saccharomyces yeast-inoculated fermentations (Rosend et. al, 2019). However, in this study there were no
significant differences in alcohol concentration resulting from either fermentation type or apple cultivar.

Post-hoc testing reveals significant interactions of apple cultivar and fermentation method for MAL, TA, VA, and TSO$_2$. Comparisons of means revealed significant differences for MAL and VA between fermentations methods for both GoldRush and Harrison, as well as between GoldRush PDC and Harrison control and GoldRush control and Harrison PDC. For MAL, a magnitude effect can be seen, as the difference between PDC and control is smaller for GoldRush than it is for Harrison. The same effect can be seen in TA, RS, pH, and FSO$_2$. An opposite effect can be seen for VA and TSO$_2$, as the difference between control and PDC is larger in GoldRush. TA had significant values for the same variables as well as the difference between Harrison and GoldRush control samples. TSO$_2$ only saw significant differences between Harrison and GoldRush PDC samples. Both MAL and VA analysis highlight the importance of looking into how apple cultivar and fermentation method work together to create variably different ciders.

### 3.4.2 Descriptive Analysis Results

Results from the DA study were analyzed to look at any differences found in descriptive attributes for apple cultivar as well as fermentation style. MANOVA was done to control for family-wise error and found that there are significant differences in overall sensory profiles from not only apple cultivar and fermentation method, but also the interaction of the two (Table 4) (Rencher and Christensen, 2012). This lines up with existing research that found differences in cider relied on multiple processing conditions and how they interact (Le Quéré et. al, 2006). Individual variables that were significant
by 2-way ANOVA for apple, fermentation, or their interaction are in Table 5. Descriptors that differed by apple cultivar were tropical fruit, pine, full body, warm, fruity, and lingering. All of these descriptors were seen in higher amounts in the Harrison apples. This suggests that Harrison ciders created stronger flavor profiles than that from GoldRush. Descriptors that differed by fermentation method are alcohol, lemon, grassy, tart, sour, full body, and dry. Alcohol and full body had higher values in the PDC ciders. Lemon, grassy, tart, sour, and dry had higher values in the control samples. Dry mouthfeel was found to be significant for fermentation method, having higher ratings in the control ciders. These values may be associated with the amount of residual sugar in a cider, which had significant differences in terms of fermentation method (Table 5) with PDC ciders having higher amounts of residual sugar. The lingering descriptor was described by panelists in consensus training as “a sort of astringent and enduring mouthfeel”. This descriptor was significantly higher in the Harrison ciders (Table 5), lining up with existing research which notes that cider apples typically have higher astringency levels (Martin et. al, 2017). Alcohol was a significant descriptor, with higher values in the PDC ciders. This could be associated with the higher amounts of volatile acidity found in the PDC ciders as VA is often sensorially described as an alcohol aroma.

Interactive magnitude effects are visible here as tropical fruit, full body, lingering, alcohol, lemon, and dry all saw larger differences between control and PDC ciders in the GoldRush ciders. To the opposite effect, pine, warm, grassy, tart, and sour all saw larger differences between the PDC and control ciders in the Harrison samples. This indicates that the interaction of the two variables has an effect on the sensory properties of the ciders.
Radar plots (Figure 3) were created to show how the descriptors were used for each sample. Overall, it is notable that the attributes received stronger ratings in ciders created from Harrison apples and that there are larger differences in descriptor ratings between the conventional and pied de cuvè ciders in Harrison ciders. It can be seen that Harrison ciders were described as more sour than Gold Rush ciders, as well as more tart and with more of a warm mouthfeel. It is also noted the difference between the replicates is larger for the Harrison ciders, specifically the pied de cuvè.

PCA was used to explore the multivariate differences among the ciders in terms of their overall descriptive profiles (Figure 4a and 4b). Samples that are grouped more closely together will tend to have overall more similar descriptive profiles. There is one sample (HPC) much further from the others, denoting that it had different sensory attributes that could be causes by higher fermentation variation. This sample took the longest to finish fermenting and had higher residual sugars, so was expected to be perceived differently by panelists. All three Harrison PDC ciders seemed to vary from each other and from the other samples, which may be explained by the variability of ambient fermentations (Vrooman, 2020). The other three treatment groups were described more similarly within group. GoldRush did not see the same variation in the PDC ciders as Harrison. This could be due to cider apples having higher amounts of tannins present in the juice. Existing research has found that high amounts of tannins can impact both fermentation kinetics as well as sensory qualities of cider (Cairns et. al, 2019). PC1 separates all the conventional Harrison samples as well as one of the PDC Harrison and one of the conventional GoldRush from the other seven ciders. PC2
separates one PDC GoldRush, two PDC Harrison, and one conventional Harrison from the remaining samples.

Figures 4a and 4b help to explain which descriptors contributed to the sensory differences. Dimension 1 separates terms like dry, lemon, tart, sour, grassy, pine, watery, and warm from all other significant descriptors. Terms like tropical fruit, warm, watery, chemical, and strawberry are contributing heavily to Dimension 1. Dimension 2 separates descriptors like watery, chemical, alcohol, and nail polish remover from all other significant descriptors. Terms like nail polish remover, dry, sour, and lemon contribute heavily to Dimension 2. Longer vectors indicate higher influence of the descriptor towards the associated dimension.

Most Harrison ciders have positive Dimension 1 scores and most PDC ciders have positive Dimension 2 scores. GoldRush conventional are most associated with the term watery while GoldRush PDC are most associated with terms like chemical, watery, and alcohol. Harrison conventional are most associated with terms like tart and grassy while Harrison PDC are quite variable with the descriptor terms.

These findings point to noticeable effects in cider from apple cultivar used. Cider apples created ciders with stronger flavor profiles and attributes, which supports previous literature (Thornton, 2013). Stronger sensory attributes from cider apples could also mean stronger off-aromas or flavors, which is something to be considered when choosing an apple cultivar for cider production.

When looking at fermentation method, it is notable that the ambient fermentations have increased variability even among treatment groups. Overall interactions between cultivar and method show that apple cultivars respond differently to fermentation method.
This may pose problems for producers who want to mass-produce and market an ambient fermentation, as each bottle may differ from the others. On the contrary, producers could use market these variable ciders as being unique and distinct, which could be appealing to many consumers. More research needs to be done in terms of making ambient fermentations more replicable. Overall interactions between cultivar and method show that apple cultivars respond differently to fermentation method.

One of the Harrison PDC samples (HPC) is an outlier in both chemical and sensory analyses. Since this sample is one of the ambient fermentations, the differences in the cider could be caused by the production process. In general, the replicates of each cider were described similarly to one another, but different from the other ciders (Table 4). Overall, MANOVA is not significant for the fermentation rep or any of its interactions. This suggests that both the apple cultivar and fermentation methods are showing effects on the chemical and sensory properties of the cider. Findings here are consistent with existing research and what was expected.

These findings suggest that producers should take both apple cultivar and fermentation method into consideration when trying to achieve a cider with specific, desired attributes. These findings also note that single varietal ciders could be more of an option for producers as they are still able to provide unique flavor profiles without blending. However, cider apples still produced stronger overall flavors and aromas than dessert apples, which is consistent with past findings but could affect consumer’s perception of ciders.

Further, these findings suggest that apple cultivar and fermentation method together have a large impact on both chemical and sensory properties of cider. This is
consistent with existing research which has noted that compounds from fermentation microorganisms interact with compounds in the apple juice to create both the flavors and aromas of the cider (Riekstina-Dolge et al, 2012; Antón et al, 2014). These findings point to the potential for more work on ambient fermentation management, specifically in relation to apple cultivar being used as well as general replicability of the fermentation.

3.5 Conclusion

The objective of this research was to determine if apple cultivar and fermentation strategy have independent or interactive effects on cider, and if so, what those effects are. Ciders were created using two different apple cultivars with two different inoculation strategies. Chemical analyses were run on the ciders to examine the chemical properties of the ciders and how they relate, and sensory analysis was done in the form of a descriptive analysis study on the completed ciders.

Both juice and cider chemistry analysis revealed statistically significant differences in the apple juice and then the ciders created. Sensory testing revealed significant differences as well. Significant effects were found in chemical and sensory testing for not only apple cultivar and fermentation method, but the interaction of the two. This study confirms the fact that key aspects of the cider production process play large roles in the final product created. These results are important because they provide evidence that natural fermentations can fall within the parameters consumers expect from a cider but point out that there is need for more research on this fermentation management. Further, these results highlight the importance of treating each apple cultivar differently, as not all apples will behave similarly throughout fermentation or create comparable ciders.
3.6 References


Qin, Zihan, et al. “Flavor Profiling of Apple Ciders from the UK and Scandinavian Region.”

*Food Research International*, vol. 105, Dec. 2017. ResearchGate,


Chapter 4: Conclusions and Future Work

Cider is making a big comeback in both the United States as a whole and the state of Virginia (Virginia Apple Board, 2020; Virginia Cider, 2018; Garabelli, 2016). Cider sales are consistently growing, but the research-based knowledge of the product still needs improvement (WSU Extension, 2019; Jamir et. al, 2020). Without a continuous tradition of cidermaking and corresponding research, it is hard to know the impact of production strategies, like apple cultivar or fermentation method, on the chemical and sensory attributes of the cider.

The objective of this work was to examine possible effects of both apple cultivar and fermentation method on both chemical and sensory properties of cider. This research set out to determine not only if there were independent effects, but also interactive effects of the two production variables. Another main objective was to determine what those effects, if any, were on the chemical and sensory attributes. Overall, this project aimed to increase understanding of popular cider production methods and their effects as well as gain knowledge that could be useful for cider producers.
Ciders were created using two, Virginia-grown apple cultivars: GoldRush, a
dessert cultivar, and Harrison, a cider cultivar. Each apple cultivar was used to create two
treatment groups, one with a PDC inoculum and another with dry active yeast strain
EC1118. This made four treatment groups of cider, which were fermented in triplicate.
Chemical analyses were performed on both the apple juice and the cider. Sensory
evaluation was done using the Descriptive Analysis method on the completed ciders.

Chemical analyses revealed significant differences in both the juice and the cider.
The ciders had significant differences by not only apple cultivar and fermentation
method, but the interaction of the two. Analysis of the sensory results revealed significant
effects from the two variables and their interactions as well. Differences by interaction
show that each apple cultivar will behave differently, even if other processing conditions
remain the same. This highlights the idea that each apple cultivar should be treated
differently, and that not all methods will work for all apples.

These results also point out that ambient fermentations are a viable option for
producers as they create ciders that are within the parameters a consumer would expect
from a cider. The ambient fermentations were more variable than the conventional, so
replicability of these types of ciders would need much more research done. It is also still
unknown how consumers feel about natural ciders or how great the desire to have them in
the market is.

As far as future work, a Check-All-That-Apply (CATA) study is planned to be
performed in the following years. This study will look at the information effect on
hedonic liking and willingness-to-pay (WTP) for cider consumers. The study will use
both informed and non-informed groups to see if there is an effect on a consumer’s liking
and WTP of a cider if they are given information on how the cider was produced, specifically looking at the apple cultivar and fermentation method used.

More future work could include the use of yeast nutrients. This would make a third treatment variable but would be worth investigating to see the effect of addition of yeast nutrients to an ambient fermentation. The use of yeast nutrients could help some of the yeast strains present in the ambient fermentation to become more powerful and therefore, the main fermenting yeast strain. This would differentiate the flavor profile of the ambient fermentation from that of a cider that did not employ yeast nutrients.

Another example of future work could be setting up the PDCs in a different location. The use of the apple orchard was for the purpose that it was known that there was yeast there that was compatible with apples. Due to complications with animals and weather, it was suggested to possibly move the PDCs to other locations. This might change the microbial community present in the ciders, which in turn may affect the cider both chemically and sensorially.

Further studies could include analysis of the cider samples to determine which yeast strains are present. Daily samples could be taken throughout fermentation so that this future research could be possible.

Further sensory research could be done to determine why consumers might choose a natural product over the traditional or vice versa. Focus groups could be used to see what is appealing, or not, about naturally produced products and this could help inform cider producers on what is a preferred processing method or what information should be more heavily advertised to consumers.
Figures

Figure 1. Experimental design to create four treatment groups using apple cultivar (cider and dessert) and fermentation method (yeast inoculation or ambient) as production variables of interest.
Figure 2. Pied de cuvé set-up at Kentland Farms orchard. Set-up includes a bucket with fresh apple juice lightly covered in a layer of cheesecloth to prevent bugs from getting in.
Figure 3 Radar plots created to show mean intensities of significant attributes split by apple cultivar.
Figure 4a and Figure 4b. Principal Component Analysis (PCA) plot created of the samples (a) and variables (b).

Dimension 1 separates terms like dry, lemon, tart, sour, grassy, pine, watery, and warm from all other significant descriptors. Terms like tropical fruit, warm, watery, chemical, and strawberry are contributing heavily to Dimension 1. Dimension 2 separates descriptors like watery, chemical, alcohol, and nail polish remover from all other significant descriptors. Terms like nail polish remover, dry, sour, and lemon contribute heavily to Dimension 2. Longer vectors indicate higher influence of the descriptor towards the associated dimension.

Most Harrison ciders have positive Dimension 1 scores and most PDC ciders have positive Dimension 2 scores. GoldRush conventional are most associated with the term watery while GoldRush PDC are most associated with terms like chemical, watery, and alcohol. Harrison conventional are most associated with terms like tart and grassy while Harrison PDC are quite variable with the descriptor terms.
### Tables

<table>
<thead>
<tr>
<th>Attribute</th>
<th>Reference Standard</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Orthonasal Aroma by Nose/Sniffing</strong></td>
<td></td>
</tr>
<tr>
<td>Hay</td>
<td>½ c bunny hay bedding</td>
</tr>
<tr>
<td>White Vinegar</td>
<td>½ tsp white vinegar</td>
</tr>
<tr>
<td>Nail Polish Remover&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1 tbsp acetone</td>
</tr>
<tr>
<td>Vanilla</td>
<td>1 tsp vanilla flavoring</td>
</tr>
<tr>
<td>Oak</td>
<td>4 tbsp Oaked Dark Horse brand Buttery Chardonnay over 1 tbsp oak chips</td>
</tr>
<tr>
<td>Yeasty</td>
<td>1 packet dry active yeast in 250mL water</td>
</tr>
<tr>
<td>Maple Syrup</td>
<td>½ tsp imitation maple extract</td>
</tr>
<tr>
<td>Pine&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1 sprig from pine tree</td>
</tr>
<tr>
<td>Grassy&lt;sup&gt;c&lt;/sup&gt;</td>
<td>½ c grass</td>
</tr>
<tr>
<td>Floral</td>
<td>1 tsp rose water</td>
</tr>
<tr>
<td>Cooked Apple</td>
<td>1 tbsp Kroger brand apple pie filling</td>
</tr>
<tr>
<td>Bubblegum</td>
<td>1 in strip Hubba Bubba brand bubblegum</td>
</tr>
<tr>
<td>Musty</td>
<td>1 tbsp dirt</td>
</tr>
<tr>
<td>Chemical</td>
<td>Expo brand dry-erase marker</td>
</tr>
<tr>
<td><strong>Flavor by Mouth/Taste</strong></td>
<td></td>
</tr>
<tr>
<td>Apple&lt;sup&gt;b&lt;/sup&gt;</td>
<td>½ in slice Gala apple</td>
</tr>
<tr>
<td>Alcohol&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4 tbsp Gallo brand Extra Dry Vermouth</td>
</tr>
<tr>
<td>Strawberry&lt;sup&gt;a&lt;/sup&gt;</td>
<td>½ in wedge strawberry</td>
</tr>
<tr>
<td>Lemon&lt;sup&gt;ac&lt;/sup&gt;</td>
<td>½ in wedge lemon</td>
</tr>
<tr>
<td>Tropical Fruit&lt;sup&gt;b&lt;/sup&gt;</td>
<td>¼ c V8 brand Tropical Fruit Smoothie</td>
</tr>
<tr>
<td>Kiwi</td>
<td>½ in wedge kiwi</td>
</tr>
<tr>
<td>Tangy</td>
<td>1/8 c Oikos brand plain Greek yogurt</td>
</tr>
<tr>
<td>Bright</td>
<td>¼ c Sunny D brand</td>
</tr>
<tr>
<td>Fruity&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1 tbsp Naked Juice brand Rainbow Machine</td>
</tr>
<tr>
<td>Tart&lt;sup&gt;ac&lt;/sup&gt;</td>
<td>4 tbsp Kroger brand unsweetened cranberry juice</td>
</tr>
<tr>
<td>White Wine</td>
<td>4 tbsp Free Reign Sauvignon Blanc by Free Spirit Wines</td>
</tr>
<tr>
<td>Watery</td>
<td>Verbal Anchor – Watered down cider</td>
</tr>
<tr>
<td>Red wine</td>
<td>4 tbsp Yellowtail brand Cabernet Sauvignon</td>
</tr>
<tr>
<td><strong>Taste by Mouth/Taste</strong></td>
<td></td>
</tr>
<tr>
<td>Sweet&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10 g sucrose dissolved in 250mL distilled water</td>
</tr>
<tr>
<td>Sour&lt;sup&gt;ac&lt;/sup&gt;</td>
<td>¼ tsp citric acid dissolved in 500mL distilled water</td>
</tr>
<tr>
<td>Bitter</td>
<td>1000 mg caffeine dissolved in 500mL distilled water</td>
</tr>
<tr>
<td>Salty</td>
<td>20 g NaCl dissolved in 300mL distilled water</td>
</tr>
<tr>
<td><strong>Mouthfeel by Mouth/Taste</strong></td>
<td></td>
</tr>
<tr>
<td>Full Body&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>4 tbsp Mott’s brand apple juice</td>
</tr>
<tr>
<td>Warm&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1 g ground ginger dissolved in 400 mL of water in 1 tbsp:1 tbsp ratio with Lipton black tea brewed for 5 minutes</td>
</tr>
</tbody>
</table>
Table 1. Cider descriptor list and recipes for corresponding reference standards as determined by trained panel.

<table>
<thead>
<tr>
<th>Dry$^{ac}$</th>
<th>1 tsp Kroger brand allum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lingering$^b$</td>
<td>4 tbsp Gallo brand Extra Dry Vermouth</td>
</tr>
</tbody>
</table>

$^a$Significant differences in ratings for both apple and fermentation method  
$^b$Significant differences in ratings by apple  
$^c$Significant differences in ratings by fermentation method
<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Yeast Assimilable Nitrogen (mg/L N)</th>
<th>Brix (°)</th>
<th>Malic Acid (g/L)</th>
<th>pH</th>
<th>Titratable Acidity (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gold Rush</td>
<td>139.5 ± 24.5</td>
<td>14.2 ± 0</td>
<td>8.14 ± 0.06</td>
<td>3.28 ± 0.055</td>
<td>6.99 ± 0.02</td>
</tr>
<tr>
<td>Harrison</td>
<td>40.5 ± 1.5</td>
<td>16.75 ± 0.05</td>
<td>8.61 ± 0.06</td>
<td>3.26 ± 0</td>
<td>7.54 ± 0.04</td>
</tr>
<tr>
<td>P-value</td>
<td>0.056</td>
<td>&lt;0.001*</td>
<td>0.031*</td>
<td>0.0059*</td>
<td>0.0065*</td>
</tr>
</tbody>
</table>

Table 2. Values for juice chemistry results. Values listed are mean ± SD for analytical duplicates.

*Denotes a significant difference in a given juice chemistry parameter between cultivars
<table>
<thead>
<tr>
<th>Sample</th>
<th>Alc(%) (v/v)</th>
<th>MAL (g/L)</th>
<th>RS (g/L)</th>
<th>TA (g/L)</th>
<th>VA (g/L)</th>
<th>pH</th>
<th>FSO₂ (mg/L)</th>
<th>TSO₂ (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GoldRush Control</td>
<td>7.62 ± 0.06</td>
<td>6.893 ± 0.08</td>
<td>0.667 ± 0.707</td>
<td>6.37 ± 0.13</td>
<td>0.233 ± 0.005</td>
<td>3.52 ± 0.009</td>
<td>2.00 ± 0</td>
<td>11.67 ± 2.05</td>
</tr>
<tr>
<td>PDC</td>
<td>7.72 ± 0.21</td>
<td>1.87 ± 0.148</td>
<td>1.667 ± 1.25</td>
<td>4.68 ± 0.38</td>
<td>0.723 ± 0.021</td>
<td>3.61 ± 0.17</td>
<td>1.33 ± 0.9</td>
<td>16.67 ± 5.43</td>
</tr>
<tr>
<td>Harrison Control</td>
<td>8.82 ± 0.008</td>
<td>8.933 ± 0.02</td>
<td>0 ± 0</td>
<td>9.09 ± 0.009</td>
<td>0.293 ± 0.021</td>
<td>3.38 ± 0.012</td>
<td>1.33 ± 0.9</td>
<td>7.33 ± 0.94</td>
</tr>
<tr>
<td>PDC</td>
<td>7.97 ± 1.25</td>
<td>0.633 ± 0.37</td>
<td>3.667 ± 1.25</td>
<td>5.48 ± 0.18</td>
<td>0.597 ± 0.078</td>
<td>3.61 ± 0.02</td>
<td>0 ± 0</td>
<td>2.67 ± 0.47</td>
</tr>
<tr>
<td>p-value</td>
<td>0.15</td>
<td>0.37</td>
<td>0.37</td>
<td>&lt;0.001*</td>
<td>0.29</td>
<td>&lt;0.001*</td>
<td>0.067</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Fermentation</td>
<td>0.43</td>
<td>&lt;0.001*</td>
<td>0.01*</td>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
<td>0.067</td>
<td>0.94</td>
</tr>
<tr>
<td>Apple:Fermentation</td>
<td>0.32</td>
<td>0.01*</td>
<td>0.09</td>
<td>&lt;0.001*</td>
<td>0.014*</td>
<td>0.24</td>
<td>0.50</td>
<td>0.049*</td>
</tr>
</tbody>
</table>

Table 3. Cider chemistry results for each of the four treatment groups created by the use of two different apple cultivar and two different inoculation methods. Values are mean ± SD.

*Denotes a value that is significant difference in a given parameter due to one of the treatments applied, where the treatments are apple cultivar, fermentation method, or the interaction of the two.
<table>
<thead>
<tr>
<th></th>
<th>Degrees of Freedom</th>
<th>Wilk’s Lambda</th>
<th>Approximate F Value</th>
<th>Numerator Degrees of Freedom</th>
<th>Denominator Degrees of Freedom</th>
<th>Pr(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apple</td>
<td>1</td>
<td>0.39</td>
<td>2.80</td>
<td>36</td>
<td>64.00</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Fermentation</td>
<td>1</td>
<td>0.29</td>
<td>4.40</td>
<td>36</td>
<td>64.00</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Fermentation Rep</td>
<td>2</td>
<td>0.37</td>
<td>1.13</td>
<td>72</td>
<td>128.00</td>
<td>0.26</td>
</tr>
<tr>
<td>Panelist Name</td>
<td>5</td>
<td>&lt;0.001</td>
<td>13.53</td>
<td>180</td>
<td>322.82</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Session Name</td>
<td>1</td>
<td>0.54</td>
<td>1.54</td>
<td>36</td>
<td>64.00</td>
<td>0.07</td>
</tr>
<tr>
<td>Apple:Fermentation</td>
<td>1</td>
<td>0.49</td>
<td>1.85</td>
<td>36</td>
<td>64.00</td>
<td>0.02</td>
</tr>
<tr>
<td>Apple:Fermentation Rep</td>
<td>2</td>
<td>0.36</td>
<td>1.19</td>
<td>72</td>
<td>128.00</td>
<td>0.20</td>
</tr>
<tr>
<td>Apple_Panelist Name</td>
<td>5</td>
<td>0.06</td>
<td>1.41</td>
<td>180</td>
<td>322.81</td>
<td>0.004*</td>
</tr>
<tr>
<td>Apple_Session Name</td>
<td>1</td>
<td>0.57</td>
<td>1.37</td>
<td>36</td>
<td>64.00</td>
<td>0.14</td>
</tr>
<tr>
<td>Fermentation:Fermentation Rep</td>
<td>2</td>
<td>0.33</td>
<td>1.31</td>
<td>72</td>
<td>128.00</td>
<td>0.09</td>
</tr>
<tr>
<td>Fermentation:Panelist Name</td>
<td>5</td>
<td>0.03</td>
<td>1.82</td>
<td>180</td>
<td>322.81</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Fermentation:Session Name</td>
<td>1</td>
<td>0.56</td>
<td>1.37</td>
<td>36</td>
<td>64.00</td>
<td>0.13</td>
</tr>
<tr>
<td>Fermentation Rep: Panelist Name</td>
<td>10</td>
<td>0.02</td>
<td>0.96</td>
<td>360</td>
<td>646.27</td>
<td>0.68</td>
</tr>
<tr>
<td>Fermentation Rep:Session Name</td>
<td>2</td>
<td>0.43</td>
<td>0.92</td>
<td>72</td>
<td>128.00</td>
<td>0.65</td>
</tr>
<tr>
<td>Panelist Name:Session Name</td>
<td>5</td>
<td>0.06</td>
<td>1.35</td>
<td>180</td>
<td>322.81</td>
<td>0.010*</td>
</tr>
<tr>
<td>Residuals</td>
<td>99</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</table>

Table 4. MANOVA table for the descriptive analysis results to examine whether or not there was variation between samples and if so, what factors were causing it. * Denotes a significant value.
<table>
<thead>
<tr>
<th>Attribute</th>
<th>Gold Rush</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>Harrison</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>PDC</td>
<td>Control</td>
<td>PDC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tropical Fruit^b</td>
<td>1.24 ± 0.11</td>
<td>1.47 ± 0.19</td>
<td>1.62 ± 0.18</td>
<td>1.11 ± 0.19</td>
<td>1.04 ± 0.18</td>
<td>1.08 ± 0.16</td>
<td>2.07 ± 0.19</td>
<td>1.69 ± 0.17</td>
<td>1.91 ± 0.17</td>
</tr>
<tr>
<td>Pine^b</td>
<td>0.01 ± 0.03</td>
<td>0.22 ± 0.04</td>
<td>0.1 ± 0.02</td>
<td>0.17 ± 0.05</td>
<td>0.43 ± 0.07</td>
<td>0.01 ± 0.03</td>
<td>0.6 ± 0.14</td>
<td>0.53 ± 0.06</td>
<td>0.35 ± 0.06</td>
</tr>
<tr>
<td>Full Body^bc</td>
<td>3.17 ± 1.19</td>
<td>2.11 ± 1.07</td>
<td>2.55 ± 1.54</td>
<td>3.24 ± 1.38</td>
<td>3.48 ± 1.77</td>
<td>2.78 ± 1.74</td>
<td>3.58 ± 1.67</td>
<td>4.18 ± 1.32</td>
<td>3.57 ± 1.78</td>
</tr>
<tr>
<td>Warm^b</td>
<td>1.64 ± 1.97</td>
<td>2.19 ± 1.85</td>
<td>1.81 ± 1.97</td>
<td>1.74 ± 1.6</td>
<td>2.1 ± 2.24</td>
<td>2.16 ± 1.81</td>
<td>3.34 ± 1.21</td>
<td>2.58 ± 1.27</td>
<td>2.39 ± 1.83</td>
</tr>
<tr>
<td>Linger Lingering^b</td>
<td>2.62 ± 1.99</td>
<td>2.63 ± 1.96</td>
<td>2.7 ± 2.39</td>
<td>2.96 ± 1.26</td>
<td>1.78 ± 1.16</td>
<td>2.61 ± 1.51</td>
<td>3.79 ± 2.56</td>
<td>3.17 ± 1.95</td>
<td>3.22 ± 2.21</td>
</tr>
<tr>
<td>Alchohol^c</td>
<td>2.11 ± 2.63</td>
<td>2.42 ± 2.01</td>
<td>2.36 ± 1.94</td>
<td>2.88 ± 2.91</td>
<td>2.73 ± 2.07</td>
<td>3.19 ± 2.07</td>
<td>1.92 ± 2.39</td>
<td>2.54 ± 2.3</td>
<td>2.9 ± 2.82</td>
</tr>
<tr>
<td>Lemon^ac</td>
<td>1.96 ± 1.66</td>
<td>1.55 ± 1.77</td>
<td>1.15 ± 1.38</td>
<td>0.99 ± 1.32</td>
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Table 5. Each of the agreed upon descriptors used to evaluate the cider samples. Values are mean ± SD.

| Salty     | 0.77 ± 1.00 | 0.64 ± 0.79 | 0.70 ± 0.93 | 0.56 ± 0.66 | 1.18 ± 1.26 | 0.97 ± 1.48 | 0.77 ± 0.98 | 0.98 ± 1.17 | 0.79 ± 1.11 | 1.01 ± 1.28 | 0.49 ± 0.69 | 0.18 ± 0.39 |

*Significant differences in ratings for both apple and fermentation method
*Significant differences in ratings by apple
*Significant differences in ratings by fermentation method
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Supplementary Table 1. Coding used for each treatment group as referenced. Fermentations done in experimental triplicates and using two production variables to create four treatment groups.