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TECHNICAL OVERVIEW ON CIDER PRODUCTION

PROCEEDINGS OF

THE XXVIIes ENTRETIENS SCIENTIFIQUES LALLEMAND



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CIDER, AN ANCIENT ART IN A MODERN WORLD

Rebecca Mussell

Westons Cider, Herefordshire, U.K.

Cider has been produced in the Herefordshire village of Much Marcle since 1878. It represents a huge part of the region's heritage and, even in its modern form, still retains its traditional characteristics. Much Marcle is the home of Westons Cider, an independent, family-owned cider maker producing 42 million litres annually.

Cider in the U.K. has played a very special part in shaping the economic and social fabric of the nation and, while some features of today's production would be unrecognisable to the cider makers of the past, many would remain familiar. Three key areas have shaped the industry and created what we see today: research, technology, and collaboration.

Cider is the national drink of the U.K., and throughout its long history one thing has always remained constant – the apple. Over the years many aspects of cider production have changed and while certain aspects of the process would not be immediately recognisable to the cider makers of the past, the most consistent factor remains the fruit. It provides the flavour, colour, and aroma of the finished product. With over 10,000 varieties, there is no fear that the apple will be replaced.

West Country Cider is traditionally made using Frenchorigin apples known as bittersweets, bittersharps, sharps, and sweets. Bittersweets and bittersharps have high potential sugar, high acidity (that can be sour), a fibrous

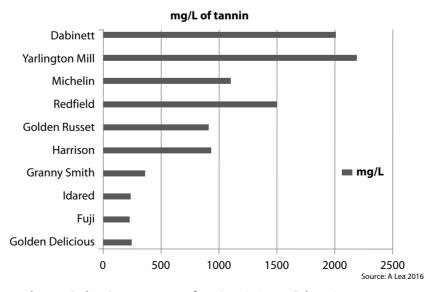


FIGURE 1. Key research by noted expert Andrew Lea, assessment of tannin variation mg/L, by variety.

structure that can feel woolly when eaten, the ability to continue maturing without degrading – thus allowing greater starch-to-sugar conversion – and also a high level of tannins. Tannins are complex flavonoid polyphenols that cause fruits to brown as they oxidise. They are the distinguishing feature of traditional English ciders, being both bitter and astringent. From a processing point of view, the polyphenols inhibit the breakdown of pectin, making the fruit easier to press.

U.K. cider making has had to become much more innovative to satisfy the complex and increasingly discerning palates of cider consumers. This has been achieved by blending ancient traditions with modern methods and technologies. Research has been crucial to better understanding the craft of cider making - for example, understanding the components of apple juice enables cider makers to ascertain where the flavour comes from. By better managing the variables to make a more consistent product, we can also understand what we may need to add to the press or the fermentation to produce the best possible results. While this new understanding of the raw components of the product has been revolutionary to the industry, understanding the global cider consumer, via consumer insight, has also had a major effect on the marketplace.

The industry has seen many periods of growth, and the most sustained period of growth began in 2000 with the 'cider over ice' renaissance fuelled by Magners. Today the U.K. cider industry is worth £2.88 billion per annum, which equates to 787 million litres and shows a year-on-year increase of 2.1%. The U.K. market consumes 45% of

the world's cider, with the remainder of Europe collectively consuming 21%, and North America and the Middle East both responsible for 10%, respectively.

Historically cider makers would use their instincts or even experiment with different methods or recipes. Cider was tasted, not tested, and the only discussions that took place were either between family members or at the local pub. National trade bodies such as the NACM (National Association of Cider Makers [U.K.]) or the AICV (European Cider & Fruit Wine Association) provide cider makers with an open forum for honest and industry-beneficial discussion where they can share best practices and co-invest in fundamental research projects.

In 2003 family cider maker Bulmers was purchased by Scottish & Newcastle Brewery. Shortly after this, in 2008, it was bought by Heineken, and in 2016 Rekorderlig cider was purchased by Molson Coors. The industry has become increasingly globalised with consolidation coming from the very top. It is likely that the coming years will see greater mergers and acquisitions. The input of the brewers has altered the way that some larger companies now produce their cider. Gone is the seasonal product with annual fermentation using freshly pressed juice. Producers are now opting to evaporate juice to create a year-round fermentation programme.

Brewers have also driven investment into the sector, and with investment we see greater developments in cider making technology. Hand harvesting has been replaced by machine harvesting, hand pressing has been replaced by hydraulic automated cider presses, and wild yeast has been replaced by specially selected and carefully nur-

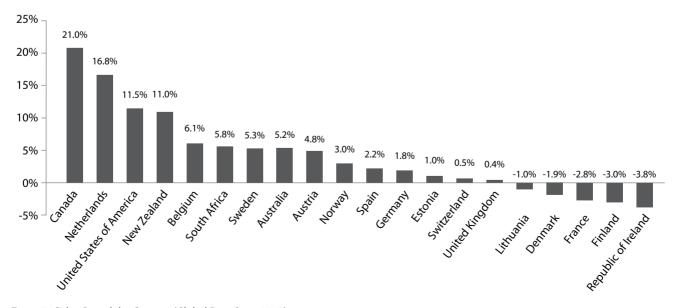


FIGURE 2. Cider Growth by Country (Global Data Sept. 2017)

tured yeast strains designed to increase efficiency and consistency. Filtration is believed to have benefited the most from technological advancement; The introduction of cross-flow filtration effectively eliminated 5 other process steps – centrifugation, fining, powder filtration, sheet filtration, and depth filtration.

The age-old cider industry is facing many threats and challenges, and there are now greater links between alcohol consumption and health. Our consumers have also changed. Millennials and Generation Xers have different views on how, when, and where they consume alcohol – if they do consume it at all. Major brands have seen serving sizes decrease, from 500 ml cans to 330 ml cans or from pints to half- or one-third-pints. This is a direct result of modern cider consumers' thirst for quality over quantity.

Various U.K.-based health lobbying groups are demonising cider, in the same way as tobacco. TV advertising and sports sponsorship is already being investigated. This,

combined with the recent introduction of the soft drinks levy, has enhanced requirements for more transparent product packaging, including full ingredient and nutritional declarations. And reductions in permitted allergens such as $\rm SO_2$ mean that the industry faces an uncertain and increasingly complex future.

The future may be unknown, but it appears to be bringing its fair share of opportunities and threats for this ancient industry. Through a combination of trend and threat analyses informed by consumer insight and innovation, we can create our own relevant and successful future that continually meets the needs of consumers in the 21st century and beyond. This is all possible thanks to countless hours of research, worthwhile cross-industry collaborations, and access to cutting edge technology. This will make cider making a modern beverage industry that retains all the character and quirky traditions of the past while embracing the opportunities of the future. No longer an art shrouded in myth and superstition, cider making has grown into a veritable science based on research and hard facts.

A TECHNICAL OVERVIEW OF FRENCH CIDER: FROM SPOILAGE CONTROL TO AROMATIC PROFILE CHARACTERIZATION

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I. Introduction

French cider is mainly produced in northwestern France, with pure fresh fruit juice accounting for a large part of the production. It is a naturally fermented product made from specific polyphenolic-rich cider apples, classified in 6 categories based on flavour. Cider contains between about 8 and 67 g/L residual sugar. This is mainly due to the particular process used in French cider making, which involves incomplete fermentation. The main steps of this process are milling, pressing, keeving, fermentation (with 3 main phases: oxidative, fermentative, and maturation), fining centrifugation or filtration operations, and bottling (with carbonation or secondary fermentation, also known as "prise de mousse"). All these parameters make it a distinct beverage compared to the cider or hard cider produced in other countries.

Cider is not an essential food in terms of nutritional contribution, despite the fact that it contains nutrients such as polyphenols, vitamins, and potassium. Good sensory quality is important as cider can be considered a festive beverage. This study looks at cider composition, especially aroma composition. Part of the aroma is already present in the apple juice, stemming from apple cultivar aromas. However, numerous molecules are produced during fermentation that can have an effect on aroma, including alcohols such as propanol, isobutanol, isopentanol, benzylic alcohols, and 2-phenylethanol (known for its rose aroma). Yeasts also produce esters during alcoholic fermentation (e.g., ethyl 3-methylbutyrate, 2-phenylethyl acetate, ethyl hexanoate, ethyl octanoate, ethyl decanoate, and many others). Other classes can also be detected as

terpene derivatives, sulfur-containing compounds, and lactones. All these compounds contribute to the aromatic complexity of cider, based on a subtle balance between all these volatile compounds.

II. French market

The French market posts about €400 million in sales and is divided into three main product categories: cider, Calvados, and juice. Cider volumes total over 100 million litres, with more than 90% sold in France. This segment is increasing in value thanks to innovating sectors such as *rosé* and small-bottle ciders, and effective communications efforts. However, table cider volumes are on the decline. The Calvados market represents around 15,000 hl of pure alcohol, with 50% reserved for export. Lastly, apple juice is a dynamic sector in France with more than 30 million litres produced and 60% processed in France. France also exports apples and apple musts to northern Europe and the U.S. The French market is seasonally driven with three peak sales periods: Epiphany, Candlemas, and summer.

III. Specificities of French orchards

French orchards were reorganized in the 1980s and 1990s, and new specialized orchards were introduced. Specific existing rustic cider varieties were selected to focus on alternating varieties with high phenolic or acid contents. These specialized orchards follow the table apple orchard model (Figure 1).



FIGURE 1. Changes in French orchards up to 1980 (source: IFPC)

The fruits are intended solely for processing, and there are no constraints with regard to appearance. As such, fewer pesticides and other such products are needed in orchard management. Harvest mechanization, with tree shaking and ground harvesting, reduces operating costs.

IV. Cider apple specificities

Apple ciders are classified based on their polyphenol content and acidity, as shown in Figure 2. There are 6 categories described in this classification. Sweet apples have low acidity (<60 meq.l-1) and low polyphenol (<2 g.l-1) content as evidenced by the *Dous Coëtligné* apple. Sour apples have a low polyphenol content (<2 g l-1), as do mildly sour (60 to 90 meq.l-1) apples such as the *Locard Blanc*. Sharp apples, such as the *Guillevic* or *Petit Jaune* cultivars, have a higher acid content (>90 and up to 240 meq.l-1) and low polyphenol concentration (<2 g l-1). Apples classified in the bittersweet category (e.g., *Bedan*) are considered intermediate cultivars due to their acidity (<60 meq.l-1) and high total polyphenol content (>2 g.l-1 and <3 g.l-1). Apples that are bitter, such as the *Marie Ménard*



and *Kermerrien* cultivars, have a high polyphenol content (3 to 9 g.l-1) and low acidity (<60 meq.l-1). Finally, bittersharp apples have a high content of acid (>60 meq.l-1 and up to 240 meq.l-1) and polyphenols (>2 g.l-1 and up to 6 g.l-1), but virtually no such cultivars are available in France. These varieties are very different from dessert, cooking, and eating apples due to their high polyphenol content, as shown in Figure 2. This diversity, as cider is always made with a blend of apples with different savory characteristics, allows for a well-balanced final product.

V. Cider making process

The cider making process starts with transporting the apples from the silo to be machine-washed in water. After the apples are sorted by appearance for quality to eliminate rotten fruits, they are ready for milling where they are crushed into small pieces (from 4 to 5 millimetres). In the French process, an extra step called "cuvage" can occur for 30 minutes to up to 5 hours, leading to oxidation. The pulp is then pressed with different materials, depending

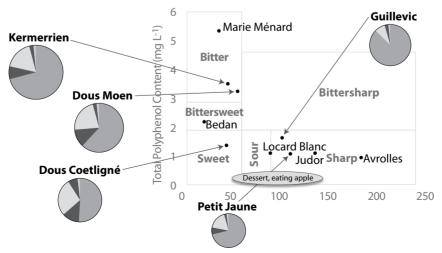


Figure 2. French apple classification by polyphenol content and acidity (from Sanoner and Guyot 1999)

TABLE 1. Common characteristics of must and contribution to the process and final product

Component	Content	Contribution
Sugars (fructose, glucose, saccharose	95 to 130 g/L	Sensory: sweet taste Technology: fermentation substrate
Acids (malic acid)	1 to 10 g/L expressed in g/L of H ₂ SO ₄	Sensory: acid taste Technology: conservation, limitation of spoilage
Polyphenols	1 to 5 g/L	Sensory: bitterness, astringency, colour Technology: oxygen consumption
Nitrogenous components (amino acids)	30 to 300 mg/L expressed in mg/L of nitrogen	Sensory: aroma generation Technology: stability in bottle
Pectin	0.3 to 1.3 g/L	Technology: participation in keeving process
Minerals	From a few mb/L to few g/L	Technology: precipitates (Fe: black; Cu: green)
Enzymes (PME, PPO)	Traces	Technology: PME; keeving PPO; polyphenol oxidation

on the size of the producer. The common characteristics of the must obtained are shown in Table 1.

1. Settling or keeving

In the French process, there is a specific step to make a gel after enzyme action based on pectin ability. The calcium needed for gel formation is either naturally occurring or can be added with the PME enzyme. Demethylation of the linear chain of homogalacturonan units in pectin enables bonding with calcium, which leads to gel formation. This step can occur after 2 to 6 days and is activated by CO₂ generation at the beginning of fermentation, which helps the gel rise to the top of the tank. This phenomenon leads to the formation of a complex, nitrogen-rich suspension called the "chapeau brun" (Figure 3). Dregs and mud also deposit on the bottom of the tank.

The clear must located between the gel and sediment layers is then transferred to a different tank to stop the fermentation step.

A new continuous dynamic system known as "flotation," where the juice is processed within 48 hours, can be also be used. The juice is placed in a specific device with nitrogen bubbling in the presence of CaCl₂.

2 Fermentation

The fermentation step (Figure 4), which in France relies on natural flora, begins with an oxidative phase where the main action is triggered by oxidative yeast in anaerobiosis. Oxygen flow is highly beneficial for this flora at the beginning of fermentation, leading to a limited growth of *Saccharomyces* during this step. This stage is considered very important because this is when fruity aromas are generated.

Alcoholic fermentation is a non-total process, meaning that the main goal is to yield a final product with a residual sugar content. This fermentation is conducted by *Saccharomyces* for 1 to 3 months at a moderate speed, as the level of population is relatively low at under 5x107 cfu/ml, and with oxygen deficiency. Fermentation speed is often slowed by biomass reduction. Yeast assimilable nitrogen (YAN) depletion is a technological goal to keep non-pasteurized products with different sugar levels stable all year long. As with wine, malolactic transformation can occur due to bacterial growth in cider. Since malic acid is the major acid at play, this step has a bigger impact on cider characteristics because the pH is altered. In this case, bacterial development can occur more easily. Dur-

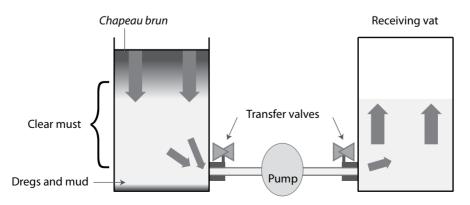


FIGURE 3: Keeving process in French cider production

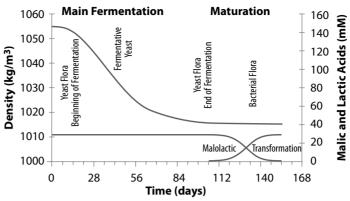


FIGURE 4: The different steps of cider fermentation

ing the maturation step after fermentation, other yeasts, such as *Brettanomyces anomalus* can grow, which have a negative influence on the aromatic quality of the cider. This has to be prevented.

3. Post-fermentation clarification

This is an important step because it makes it possible to have a clear product without turbidity and deposits at the end of the overall process. This stabilizes the cider and eliminates haziness due to the action of proteins or tannins. This is also an important step for eliminating microorganisms and ensuring better bacterial stability in the final product.

Clarification is done either by settling, centrifugation, or filtration. It can be completed by fining with the addition of protein. This helps improve filtration and stabilizes the final product by eliminating tannins. Other flocculants, such as bentonites or silica gel, can be used.

4. Packaging – second fermentation

As shown in Figure 5, after blending and final filtration, the cider is bottled with either carbonation or additional

yeast to trigger a second fermentation in the bottle. The choice of process to use depends on the producer's size. Small-scale to industrial producers usually opt for carbonation with or without pasteurisation, while very small-scale producers are more inclined toward second fermentation in the bottle.

VI. Cider aroma study

Cider aroma results in a subtle equilibrium of volatile compounds. As shown in Figure 6, volatile compounds of interest are generated either by oxidative yeasts or *Saccharomyces* fermentative yeasts. The latter flora can generate negative molecules like sulfur compounds under specific conditions. During the cider making process, spoiling yeasts, such as *Brettanomyces*, can develop as well, forming negative impact compounds like volatile phenols.

The different volatile compounds can interact. Volatile compounds can also interact with macro constituents like polyphenols, sugar, and alcohol. These different phenomena may change the final perception of the product.

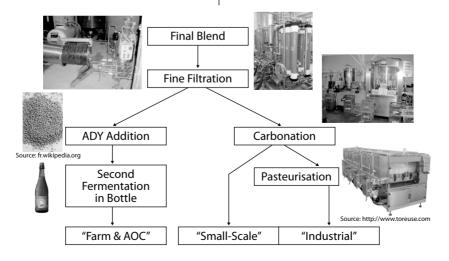


FIGURE 5. The final steps before cider commercialisation

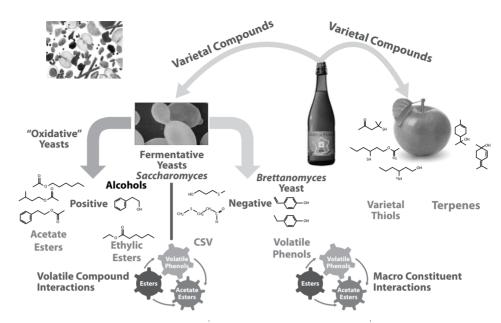


FIGURE 6. Origin of volatile compounds responsible for fruity aroma and compounds linked to defects in cider aroma

Lastly, varietal compounds may also have an impact on the overall aroma.

The work IFPC did on 66 ciders yielded the results presented in Figure 7, which shows that fruity ciders are richer

Variable Factor Map (PCA)

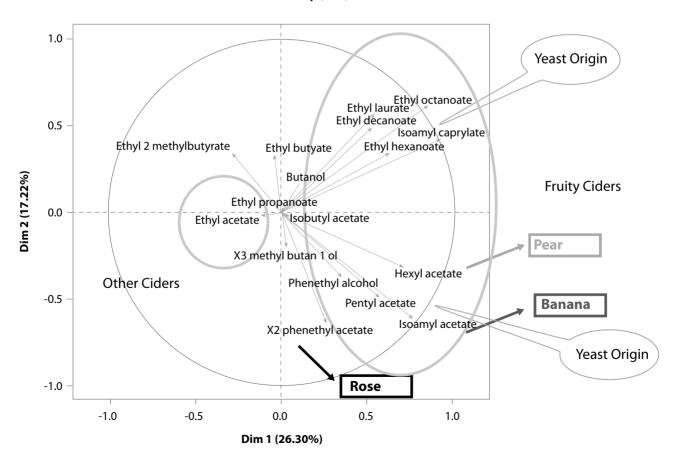


Figure 7. Principal Component Plot: Projection of volatile compounds, in relation to fruity ciders vs. others type of ciders, in the plane formed by the first and the second axis.

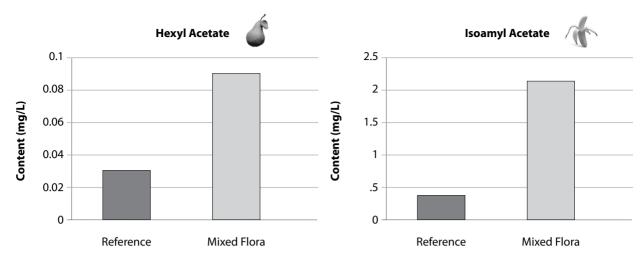


Figure 8. Acetate ester generation by oxidative yeast as a mixed flora with indigenous *Saccharomyces* strain vs. a reference done with only a *Saccharomyces* strain.

in esters originating from yeast metabolism. It seems that two different types of fruity ciders coexist, with one rich in ethylic esters and the second rich in acetate esters, giving specific fruity aromas.

Our research is aimed at meeting two main goals: 1) increasing the reactions that produce fruity aroma via technological flora; 2) avoiding spoilage flora that negatively impact overall flavour.

Research on promising yeast strains has demonstrated that an oxidative yeast is able to generate a significant amount of esters (Figure 8) when grown as a mixed flora with *Saccharomyces* vs. applications using *Saccharomyces* only. This demonstration of interest has been done on an industrial scale for a total volume of over 10,000 hectolitres.

Some Saccharomyces strains are also able to generate large amounts of acetate esters (Figure 9), resulting in

highly fruity products. Compared to the initial micro-filtered product, acetate esters are generated, which leads to an improvement in overall flavour.

VII. Brettanomyces impact

Moreover, the aroma balance can be altered by microorganism spoilage. *Brettanomyces anomala* is well known for its negative impact on the aroma of fermented products such as cider. Some studies have demonstrated a strong polarization with regard to the sensory attributes of cider, showing opposition between fruity ciders and ciders presenting phenolic aromas and gustatory characteristics such as bitterness and astringency due to polyphenols. A first hypothesis was proposed to explain this bi-polarization: that volatile phenols mask fruity aromas. Nevertheless, aroma analyses showed that high levels of volatile phenols in "phenolic-like" products are specifi-

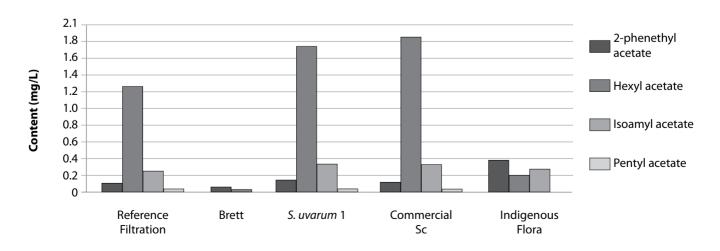


Figure 9. Aroma profile comparison between a cider made with different yeast strains and a cider made with indigenous flora after 52 days.

cally correlated with a lack of the acetate esters present in fruity ciders. These observations led us to the hypothesis that *Brettanomyces* causes acetate esters to degrade. The esterase activity potential of several cider *Brettanomyces* strains was then studied. This hypothesis was verified by measuring this activity during yeast growth in order to determine degradation conditions. Ester degradation was then experimentally studied in three different very fruity ciders inoculated with a *Brettanomyces* strain and compared to *Saccharomyces* and oxidative strains isolated from ciders.

The results obtained confirmed the degradation of specific acetate esters by *Brettanomyces* (ethylic esters not being degraded) with or without generation of volatile phenols.

Thus, even before volatile phenols likely to mask the fruity characteristic of ciders are produced, *Brettanomyces* can negatively impact cider aroma by degrading the esters that contribute to fruity notes.

This work can be used to provide producers with advice on equipment hygiene and cider protection against *Brett-anomyces* development. The goal is to gain a better understanding of the population level needed to trigger degradation in order to establish a maximum level to ensure cider aroma is protected.

This work was carried out with the financial support of Casdar, FranceAgrimer, and UNICID. The aroma analyses were carried out as part of the P2M2 analytical platform.

AUSTRALIAN CIDER: LEARNING FROM THE PAST, MOVING INTO THE FUTURE THROUGH INDUSTRY-EMBEDDED RESEARCH

Dr Fiona Kerslake, Dr Anna Carew, Dr Joanna Jones, Dr Bob Dambergs, Dr Nigel Swarts, A/Prof Dugald Close Lachlan Girschik, Madeline Way, Harriet Walker

Fermentation Research Group, Tasmanian Institute of Agriculture

The Fermentation Research Group (FRG) at the Tasmanian Institute of Agriculture (TIA) evolved from a strong, coolclimate viticulture focus with a grape-to-glass approach to a broader focus around fermented products. The group first began cider research in 2013 in response to industry-led demand for knowledge around the fermentation of apples and pears. Historical research outputs from research stations, such as Long Ashton in the U.K., have focussed on traditional cider-specific varietals, but the craft cider market in Australia is utilising out-of-specification fruit from the culinary market, or 'dessert' varietals. Thus, very little is known about the flavour and aroma profiles of ciders that result from this fruit, or how to handle the juice in the cidery.

Small-scale single variety trials – Drs Kerslake and Carew

In response to industry, the TIA FRG first began working with small-scale ferments (500 ml) of single varietals from commercially processed juice. We also engaged leading cider industry journalist, Max Allen, to participate in a cidermaker education session, at which we tasted the single-variety ciders, along with commercially available ciders from Tasmania, Australia, and around the world. Developing cidermaker vocabulary around ciders and their different styles was highlighted as an important area

for development alongside improving understanding of the contribution of single varieties to the final cider style. Word clouds were produced, which informed the Cider Australia cider style guide.

Cider Australia collaboration – Drs Kerslake and Carew

Collaboration with Cider Australia resulted in 230 entries being sampled from the 2014 and 2015 awards, and basic analysis of these ciders indicated that medal prediction could be based on cider compositional analysis in the dry and medium categories, but the prediction was not as accurate in the sweet category.

Honours project – The suitability of dessert apples for cider production - Lachlan Girschik

This project was developed through consultation with Tasmanian apple growers looking for potential markets for fruit that does not meet quality grades for the dessert apple market.

Key outcomes:

- Distinct phenolic profiles for the different varieties (Pink Lady, Red Delicious, Royal Gala)
- Pre-commercial harvest fruit had higher total phenolics and likely hydroxycinnamates than commercial harvest (over- or under-sized fruit) or post-commercial harvest fruit

• Apple size did not impact quality for any variety

Peer-reviewed paper - Girschik et al. (2017) "Apple variety and maturity profiling of base ciders using UV spectroscopy." Food Chemistry **228**: 323–329.

Tasmanian QA project – Drs Kerslake, Carew, and Jones

- The methyl cellulose precipitable tannin method for red wine (Mercurio, Dambergs, Herderich & Smith 2007) was proposed as a suitable alternative to the Folin-Ciocalteu method of measuring total phenolics and tannins (Singleton & Rossi 1965); however, after extensive analysis this is not the case.
- Distributed Industry Trial (DIT) with 3 commercial cideries and the TIA FRG fermentation facility with 4 treatments
 - 1.EC1118 + DAP
 - 2.EC1118 no DAP
 - 3. Opale + DAP
 - 4. Opale no DAP
 - 20 ciders tasted at the Cider Tasmania technical session
 - Opale had distinct banana ester aromas
 - Some perceived these more as tropical and fruity
 - Some producers thought this might be good in a commercial, fruity style cider
 - EC1118 had more yeast derived aromas and was perceived as having more acidity
 - EC1118 had a long, even palate, and Opale was broader, more fruit driven, with a slight hole in mid palate
 - DAP addition didn't seem to have a major influence, some 'harsh' acidity noted
 - In Opale + DAP, there were a few varnish-like notes
- Technical booklet The scientific principles underpinning inconsistencies in cider quality
- Faulty ciders were intentionally made to highlight to producers the specific character

Honours project - Maximising phenolic content of apple juice for cider making - Madeline Way

- Four varieties (Red Delicious, Pink Lady, Sturmer, Bulmer's Norman) were subjected to four treatments
 - 1. No maceration, free run juice
 - 2. No maceration, pressings juice

- 3. Maceration, free run juice
- 4. Maceration, pressings juice
- Varietal differences were observed in response to treatments
- In high phenolic varieties, pressure increased phenolic extraction
- For low phenolic varieties, maceration generally reduced phenolic extraction, likely due to adsorption of compounds to the solids

Current PhD project started 2017 - Interactions between apple variety, rootstock, and environment across different Australian regions

- Madeline Way
- Harvest method will also be analysed to determine the impact of mechanical harvesting on apple characteristics
- Yield and fruit quality attributes (sugar, pH, titratable acidity, yeast assimilable nitrogen, and phenolic content)
- Base cider quality analysis

Acknowledgements

Dickens Cider, Red Brick Road Cider, Red Sails Tasmania, Small Players Cider, Spreyton Cider Co., Brady's Lookout Cider, Lost Pippin Cider, Winemaking Tasmania, Willie Smith's Cider, Lucaston Park, Hansen orchards, Lallemand, Cider Australia, Cider Tasmania, Australian Wine Research Institute, Tasmanian State Government, Hanna Westmore, Jenn Lavers, Caroline Claye, Justin Direen

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CIDERMAKING VS. WINEMAKING: IS THERE A DIFFERENCE?

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Introduction

Cider production was widespread in the United States in the 1700-1800s. This industry was all but destroyed by Prohibition, which banned the production and sale of alcoholic beverages in the U.S. between 1920 and 1933, and did not rebound until 2010. Between 2011 and 2016, U.S. cider sales revenues increased at an average rate of 27.3% per year (Petrillo 2016), with retail sales reaching \$1.3 billion in 2016 (Brager et al. 2017). While the exponential increases in total cider sales and the sale of "big" cider brands in the U.S. leveled off from 2015 to 2016, sales of "craft/local" ciders have continued to increase steadily (Neilsen 2016). Furthermore, in 2016 sales of cider in the U.S. were only 1.3% of beer sales, whereas in the same year in Spain, France, and the U.K. cider sales were equal to 2%, 5%, and 22% of beer sales, respectively. After China, the U.S. is the world's second largest apple producer, with approximately two-thirds of apples being sold in the fresh market and one-third being further processed into juice, sauce, food ingredients, and cider (U.S. Apple 2017). Cider in the U.S. is currently made by big diversified beverage companies, large cider-focused producers, wineries of all scales, and small-scale specialized cider producers. These producers use a range of starting materials, including fresh apples of cider, dual-purpose, and dessert cultivars, as well as juice and concentrate, with feedstocks both domestically produced and imported. Clearly, there is potential for continued growth in craft/ local cider sales in the U.S. market. Recently, producers and industry suppliers have turned to the body of scientific knowledge on white wine production for guidance in cider process development. In some cases, strategies that have proven reliable and robust for white winemaking have not delivered similarly consistent results when applied to cider fermentation, particularly pertaining to preventing hydrogen sulfide production. The following report aims to summarize and highlight key differences in apple and grape juice biochemistry that may necessitate the development of strategies and products specific to cider fermentation.

Comparison of Cider Chemistry and Wine Chemistry

Biochemistry of the starting material will certainly affect yeast metabolism during fermentation, which, in turn, will affect the sensory attributes of wine or cider. In order to assess whether differences in apple and grape chemistries would necessitate differences in fermentation management strategies for the two materials, a comparison of some key fruit chemistry parameters is provided. Concentrations of several enologically relevant biochemical grape and apple components are compared in Table 1.

Beyond the differences in concentrations of some key fruit chemistry parameters noted in Table 1, the composition of each of these categories may differ across fruit species, as well. The compositions of several enologically relevant

Table 1. Comparison of concentrations of enologically relevant biochemical grape and apple components.

Component	Grape	Apple
Sugar	18 to 28%	8 to 15%
Acid	4 to 8 g/L	1 to 15 g/L
Polyphenols	100 to 2,500 mg/L	100 to 6,500 mg/L
YAN	0 to 300 mg/L	0 to 75 mg/L

Table 2. Comparison of composition of enologically relevant biochemical grape and apple components.

Component	Grape	Apple
Sugar	Glucose, Fructose	Glucose, Fructose, Sucrose, Sorbitol
Acid	Tartaric	Malic
Polyphenols	Color, Mouthfeel, Bitterness, Astringency	Bitterness, Astringency, Color
YAN (Yeast Assimilable Nitrogen)	Proline, Arginine, Ammonium	Asparagine, Aspartic Acid, Glutamine, Phenylalanine

biochemical grape and apple components are compared in Table 2.

Apples contain sucrose and sorbitol in addition to fructose and glucose, and the relative concentrations of these sugars vary significantly among apple cultivars. Depending on the yeast strain, these differences may result in differences in fermentation efficiency and residual sugar compared to the performance of the same yeast strain in grapes, where fermentable sugars consist of glucose and fructose. The fact that malic acid is the major organic acid constituent in apples is an important consideration in managing acidity to meet stylistic goals and maintain microbiological stability. In most cider making processes, polyphenols are not extracted from skins to an appreciable extent; however, this technique is being tested by some producers in an effort to improve the phenolic composition of ciders made from apples not exceptionally high in polyphenols. Depending on the intended cider style, production practices vary with regard to the promotion or prevention of oxidation during the process. Finally, differences in yeast assimilable nitrogen (YAN) and free amino acid composition in juice can translate into differences in fermentation rate, hydrogen sulfide production during fermentation, and production of volatile aromas during fermentation. The following section will explore these differences and their potential impacts on fermentation in more detail.

Yeast Assimilable Nitrogen and Cider Fermentation

Endogenous YAN in Apples

In Figure 1, the average concentration of YAN observed in apple juice samples analyzed by the Virginia Tech Enology Services Lab between 2010 and 2014 is compared to the average values for grape samples of different species. Relative to grapes, apples tend to have lower endogenous

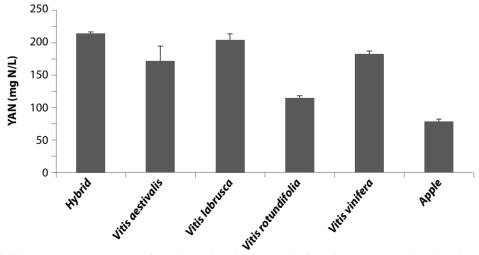


Figure 1. Yeast assimilable nitrogen concentrations of samples analyzed by Virginia Tech Enology Services Lab and Purdue Wine Grape Extension Program, 2010-2014, (Stewart, Hurley, Sandbrook, unpublished).

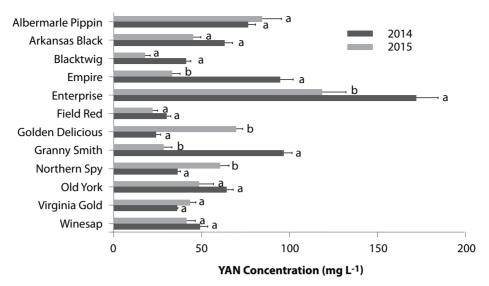


Figure 2. Yeast assimilable nitrogen concentrations observed in 12 apple cultivars grown in Virginia in 2014 and 2015. (Boudreau et al. 2016, unpublished)

YAN concentrations. As such, supplementation with yeast nutrients rich in YAN is a common practice for cider fermentation.

YAN variation is also to be expected within apple cultivars. Figure 2 presents observations of YAN by cultivar over two growing seasons. The cultivars in this study were grown in Virginia and represent dual-purpose and dessert cultivars currently utilized in cider production.

Figure 3 illustrates the low concentration of ammonium ions observed in apples grown in Virginia and the relatively minor contribution of ammonium ions to the total YAN concentration in apples. This differs from grapes, where ammonium ion concentration can contribute substantially to the total YAN concentration. These observations are in line with anecdotal reports from other growing regions. Factors influencing YAN concentration in apples and apple juice include orchard

management practices, crop load, cultivar, and juice clarification (Peck et al. 2016, Boudreau et al. 2017a). How much YAN is needed for cider fermentation? This question is often asked, with the underlying assumption that YAN is YAN, regardless of the composition, and that meeting a specific target value through YAN supplementation can ensure prevention of hydrogen sulfide production. Pre-fermentation YAN concentration targets for wine fermentation have been established and are routinely employed by winemakers and cider makers alike (Bisson et al., 2000, Scott Laboratories Fermentation Handbook, 2016). Recommendations on total pre-fermentation YAN concentration have evolved over time, taking into account starting sugar concentration and yeast strain nitrogen needs. However increasing evidence points to the importance of YAN composition and the influence interactive factors have on YAN requirements in meeting stylistic goals for wine and cider. While the current total YAN

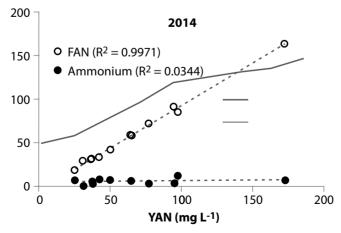


FIGURE 3. Free amino nitrogen and ammonium ion concentration in apple juices from 12 cultivars grown in Virginia in 2014 plotted against total YAN concentration for each sample. (Boudreau et al. 2016, unpublished)

recommendations for wine work well in general, many factors may influence their effectiveness, and merit consideration in terms of overall fermentation management strategies—especially in new, changing, or highly variable applications like cider making, where less empirical evidence is available. In this brief summary of recent research results from our lab, we provide evidence that in addition to the total initial YAN concentration, the composition of free amino acid and the presence of fungicide residues can influence fermentation kinetics and hydrogen sulfide production during fermentation.

Interactive effects of fungicide residues and YAN

The presence of residual fungicides on fruit can negatively impact beverage fermentation. For example, strict pre-harvest intervals for elemental sulfur applications are widely enforced in wine grape production because elemental sulfur on wine grapes has been demonstrated to cause hydrogen sulfide production during fermentation. In order to evaluate the potential for fungicide residues likely to be found in apples (but not grapes) to affect cider fermentation, we evaluated the effects of fenbuconazole (a common fungicide in apple orchards) and fludioxonil (a post-harvest storage fungicide used in apples) on hydrogen sulfide production and fermentation rate by yeast strain EC1118 in cider fermentation. Fludioxonil did not affect the fermentation rate or hydrogen sulfide production; however, fenbuconazole did have negative effects on fermentation at concentrations allowable by the USDA. A key result from this study is described in Figure 4, showing the interactive effects of total pre-fermentation YAN concentration and residual fenbuconazole concentration in apple juice. In Figure 4, the total hydrogen sulfide produced during fermentation is shown on the y-axis, and fenbuconazole concentrations are shown on the x-axis.

Within each fenbuconazole concentration level, three levels of total YAN were evaluated. For all fenbuconazole concentrations, increasing YAN from 153 mg/L to 253 mg/L resulted in decreased hydrogen sulfide production. Contrary to our expectations, for the 0.2 mg/L fenbuconazole concentration, increasing YAN concentration from 78 mg/L to 153 mg/L actually resulted in increased hydrogen sulfide production, although a further increase in total YAN to 253 mg/L alleviated that effect.

Interactive effects of methionine concentration and total YAN concentration

Variations in YAN composition, particularly free amino acid concentrations, have been demonstrated to influence volatile aromas produced during fermentation. To investigate how fundamental differences in the free amino acid composition of apples versus grapes might influence fermentation, we first compared the amino acid composition of apple juice and grape juice. We found that the most prevalent amino acids in apple juice differ from those found in grape juice. These differences were summarized previously in Table 2. While methionine is not one of the most prevalent amino acids in apple or grape, a deficiency of methionine (< 20 mg/L) can result in increased hydrogen sulfide production by certain yeast strains, due to the activation of the sulfate reduction sequence (SRS), a metabolic pathway in yeast. While grapes typically contain more than 20 mg/L of methionine, we observed < 5 mg/L methionine in 15 cultivars of apples grown in Virginia (Ma, unpublished). To determine whether low methionine concentration (<5 mg/L) in apple juice could contribute to the high prevalence of hydrogen sulfide production during cider fermentation even when minimum recommended YAN concentrations are met, we evaluated the interactive effect of methionine additions to juice at

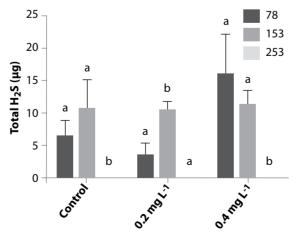


FIGURE 4. Interactive effects of total pre-fermentation YAN concentration and residual fenbuconazole concentration in apple juice. Values are expressed as mean ± SD. Means not labeled with the same lowercase letter are significantly different within the specified fenbuconazole concentration. (Boudreau et al. 2017b)

three initial total YAN concentrations on hydrogen sulfide production by yeast strain EC1118 during fermentation. In Figure 5, we highlight a key result of this study. The y-axis shows hydrogen sulfide production during fermentation, and the x-axis shows three levels of total YAN concentration (53 mg/L, 153 mg/L, and 253 mg/L, adjusted using DAP addition). Within each total YAN concentration, four methionine levels were evaluated (0 mg/L, 5 mg/L, 20 mg/L, and 50 mg/L). For the two lower total YAN concentrations (53 mg/L and 153 mg/L) addition of methionine resulted in decreased hydrogen sulfide production, and for the high total YAN condition, hydrogen sulfide production was very low and no differences in hydrogen sulfide were observed regardless of methionine concentration.

Finally, a triangle test was used to determine whether addition of methionine at 20 mg/L resulted in differences in aroma at a total YAN value of 150 mg/L. A significant difference in aroma was detected (Table 3).

Of note, contrary to our expectations, increasing total YAN from 53 mg/L to 153 mg/L resulted in increased hydrogen sulfide production for all methionine concentrations. This unexpected result led us to question our general recommendation to producers that apple juice be supplemented to 150 mg/L total YAN for cider fermentation. In the absence of more comprehensive studies into factors affecting YAN requirements for cider fermentation, in a practical sense, we continue to rely on current recommendations for total YAN values. However, taken together, our results on this topic highlight the complexity of cider fermentation management, and the potential for factors not generally measured by cider producers to influence the amount of YAN necessary to achieve the desired stylistic results from fermentation and to consistently avoid hydrogen sulfide production. For full methodological details used to obtain the key results highlighted in this summary, please refer to the hybrid open-access articles published in the *Journal of the Science of Food & Agriculture* (please see reference list for full citation, Boudreau et al. 2017b), and in the *Journal of the Institute of Brewing* (please see reference list for full citation, Boudreau et al. 2017c), respectively.

Conclusions and Directions for Future Work

The fruit chemistry comparisons and research results summarized in this report demonstrate the potential for differences in grape and apple chemistry to necessitate differences in fermentation management or fermentation products for cider making vs. winemaking. In addition, our results indicate that factors not often measured by cider makers, such as the presence of fungicide residues or the free amino acid composition, may influence the total YAN concentration required to avoid hydrogen sulfide production during fermentation. Further research will be required to fully characterize these applications and other potential interactive factors and to develop strategies and products with potential to promote consistent and successful cider fermentation.

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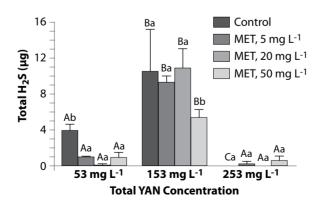


FIGURE 5. Interactive effect of pre-fermentation methionine and total YAN concentration. Means not labeled with a common lowercase letter are significantly different within the specified YAN concentration. Means not labeled with a common uppercase letter are significantly different across YAN concentrations. Values are expressed as mean ± standard deviation. (Boudreau et al. 2017c)

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IMPORTANCE OF LOCAL CIDER PRODUCTION IN ESTONIA

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Estonia is considered to be an "apple country." Although it might not be seen as ideal by the people living in it, the Estonian climate is very well suited to growing high-quality, premium-class cider apples. The relatively short and rather cold summer produces apples with more complex flavors and a higher content of polyphenolic compounds, and the long and rather cold winters give apple trees time to rest well before the new season. The craft beer boom that started in Estonia approximately five years ago was followed by a craft cider boom. This has been the trend all over the world – cider production figures and the number of cider producers have been steadily on the rise in recent years.

Cider research by CFFT in Estonia started in 2011 with a project looking at "The manufacture of premium-class fermented apple juice products based on apple cultivars grown in Estonia." The main aim was to classify local apple cultivars, compare them with well-known cider apple cultivars, and produce premium-class products – cultivar specific ciders – with local raw materials. The ultimate goal was to raise awareness of local apple cultivars so they are as well known as Cabernet Sauvignon, Syrah, Sauvignon Blanc, and other common grape varietals.

The three-year project consisted of monitoring the effect of different yeast strains on the fermentation of different apple juices obtained from different cultivars. Since juices of different cultivars were already different in terms of their sensory properties and chemical composition, understanding how to find the best match between apple cultivar and yeast strain was clearly important. The research was conducted during different seasons, and apples were analyzed at different stages of ripening. Fermentation tests were done on laboratory scale in 1 L volumes. The most successful results were upgraded to industrial scale in 500–1000 L volumes. Project partner OÜ Siidrikoda provided raw materials from a local apple orchard with approximately 8,000 apple trees and more than 20 cultivars. The cidery is interested in running different scale-up trials for various new yeast types. At the moment we see big potential in using non-*Saccharomyces* strains to add complexity to cider flavor.

Beverage producers, as well as yeast producers, have understood that wine and beer applications are not equally applicable to the cider industry. A new approach has already been successfully put into practice at OÜ Siidrikoda. As a result, the cidery is producing ciders that have won International Cider Challenge medals in the U.K. Since the U.K. is the most well-known country for cider, these awards can be taken as a sign of successful research put into practice. We are working with producers to build on that success so that our research can help increase cider quality worldwide.

CFFT CIDER RESEARCH

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Several extensive cider research projects have been conducted in Estonia through a partnership between the Center of Food and Fermentation Technologies and Lallemand.

The aim of the "Evaluation of different yeasts in cider fermentation" (July-August, 2016) project was to explore the differences, in terms of final product quality, in using apple juice concentrate vs. fresh apple juice in cider fermentation. The study looked at fresh apple juice and apple juice concentrate commercially available to cider producers. Both were fermented using a selection of yeasts where one was a brewing strain (Nottingham) and five were wine strains (BioDiva, Sensy, BC, DV10, Affinity ECA5). Per suggestion, Fermaid K was used as a nutritional supplement. The impact was characterized in terms of fermentation kinetics; sugar, ethanol, and malate content; nitrogen assimilation; and sensory properties of the final product.

Fermentation kinetics did not seem to be dependent on the fermentation matrix used but were rather a characteristic of the yeasts themselves. Based on the free amino nitrogen content, both pre- and post-fermentation, a complete depletion could not be observed with any of the studied strains. The residual concentration of FAN at the end of fermentation was similar in all of the yeasts studied, irrespective of the fermentation matrix.

When looking at the composition of residual sugars at the end of fermentation, yeasts were found to vary greatly in the consumption of fructose. Affinity ECA5 was shown to be the most fructophilic (1 g/L residual fructose) while BioDiva had close to 9 g/L of fructose left at the end of

fermentation. Ethanol formation in the samples corresponded with sugar assimilation – the more sugar was consumed, the more ethanol was formed.

With all yeasts used, a decrease in malic acid content was observed during the fermentation process. However, the consumption of malic acid by yeasts was higher in fresh apple juice where initial malic acid concentration was higher. The most logical explanation may have to do with the relatively low affinity to malic acid in the yeast malic enzyme (Mae1p) responsible for malic acid decarboxylation.

The fruity attribute (both in odor and taste) was what distinguished the samples most from a sensory perspective. In cider, yeasts have been shown to impart notes of yellow tropical fruits and stone fruits. According to the additional commentary by panel members, most of the samples made with apple juice had an unpleasantly overwhelming off-flavor, with descriptions such as "animal," "sweaty," "musty," and "sulfur," most likely attributed to the excessive production of H₂S. Since only the samples made with apple juice had the off-flavor, the question was posed whether the difference arose from the fact that fresh apple juice and concentrate were made from different apple cultivars, and/or whether the process of concentration contributed the most.

An ongoing project on the "Evaluation of cider fermentation under different conditions" (February-August 2017) was introduced to examine what factors in the cider fermentation process influence the final product the most. During the course of the project, the fermentation process

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of six commercial wine yeasts – BC, CEG, DV10, ECA5, OKAY, and Sensy – is observed under different conditions (fermentation matrix – apple juice vs. apple juice concentrate; temperature -18°C vs. 30°C; addition of nutrients – Fermaid O, Fermaid K, DAP). Both fresh apple juice and apple juice concentrate were made from the same apple variety. Characterization of the impact is similar to the previous project. Additionally, H₂S production is followed by placing lead acetate strips into the headspace of fermentation vessels.

Some of the preliminary results from the samples made with fresh apple juice have shown that, as expected, fermentation temperature primarily affects the speed of fermentation, i.e., at 30°C fermentations were about 2 times faster than at 18°C. In most cases, the impact of adding nutrients was found to depend on the yeast strain as well as the particular nutrient used. In general, Fermaid O was found to outperform the rest of the nutrients used.

In fresh apple juice, H₂S was more likely to be produced at 18°C, where the fermentation process was slower.

The results of extensive research on the subject of cider fermentation can be used to provide better knowledge to both the scientific and industrial cider communities. Since sensory perception plays a primary role in consumer acceptance of the final product, additional insight regarding the impact of cider production on the sensory and molecular levels enables us to understand and control the character of the finished product, or introduce interesting new flavors. Thus, given knowledge at a molecular level, it should be possible to maintain and expand the diversity of cider available to consumers.

Another practical application of the results includes the possibility of creating evidence-based guides on the use of various yeasts in cider making. Similar guides already exist for the production of wines and beers; however, the information obtained by other fermented beverage industries to achieve desirable properties in the finished product cannot necessarily be used to impart the same properties in cider. Cider needs to be approached separately and in great detail.

TALLINN, ESTONIA APRIL 25, 2017





