FOLIAR NITROGEN APPLICATION INCREASES FLAVOR VOLATILES AND PRECURSORS, SENSORY PERCEPTION, AND YEAST ASSIMILABLE NITROGEN CONCENTRATION IN HARD APPLE CIDER

A Thesis

Presented to the Faculty of the Graduate School

of Cornell University

In Partial Fulfillment of the Requirements for the Degree of

Master of Science

by

Brittany Sarah Cook

August 2023

ABSTRACT

Aromatic volatile compounds are a crucial component of hard apple (*Malus ×domestica* Borkh.) cider aroma and flavor. Nitrogen is a key element in the biosynthesis of amino acids and ammonium which are the primary precursors of aromatic volatile compounds. In this experiment, nitrogen-rich urea fertilizer was applied to cider apple trees of the cultivars ‘Ellis Bitter’ and ‘Harry Masters Jersey’ to create Low (3 weekly applications), High (5 weekly applications), and Control (0 applications) treatments. Fermentation kinetics, initial yeast assimilable nitrogen (YAN) content, amino acid content, ester content, and fatty acid content in ciders increased with the number of applications; however, higher alcohols showed a mixed relationship in the cultivar ‘Ellis Bitter’. Sensory differences among treatments were discernable by untrained panelists. Foliar urea applications can grant additional avenues for cider customization but fertilization with the intention to maintain tree health or YAN content can influence aromatic properties.

BIOGRAPHICAL SKETCH

Brittany Cook was born in March 1997 in Los Angeles County, California to James and Maria Cook. Brittany was homeschooled from K-12 with an unstructured curriculum that encouraged passion pursuits, autonomy, and inquiry-based learning. She graduated high school at 15 years old which allowed her to pursue a wide variety of interests before starting school at California State University, Fullerton in 2015. She became interested in plant biology and environmental sustainability during her first year at CSU Fullerton, and her later interest in agriculture stemmed from a desire to learn more about food insecurity and climate change. Brittany developed her skills in bioinformatics at her undergraduate institution and graduated in May 2020 but yearned for a greater understanding of agricultural systems and began the M.S./Ph.D. program in Horticulture at Cornell University the following fall semester.

To my family, Andrew, and all my mentors at CSU Fullerton who brightened my darkest days and helped me feel at home in S.T.E.M.

To those taken by COVID-19 and those who still live with the invisible scars.

ACKNOWLEDGMENTS

This research project would not be possible without the hard work, intellect, and guidance of so many individuals. I give so much gratitude to my advisor Dr. Gregory Peck for not only his guidance, mentorship, and patience on this project but going above and beyond to help me feel welcome and become the best version of myself. I also thank my committee members, Drs. Anna Katharine Mansfield and Misha Kwasniewski for coaching me through brand new disciplines as well as their support and enthusiasm for this project and my academic growth. A huge thank you to Yanxin Lin from the Kwasniewski Lab and Drs. Amanda Stewart and Merlon Ac-Pangan from the Stewart Lab for the tireless efforts in processing the dozens of samples we sent your way. Thank you to Alina Stelick and Adriana Cardenas Bonilla at the Cornell University Sensory Evaluation Center for helping me to interpret and improve my sensory data, and the many helping hands from the Peck Lab during all different steps of this project: Mike Brown, David Zakalik, Aly Mashek, Jules Hart, Shanthanu Krishna Kumar, Kamal Tyagi, Brent Arnoldussen, Adam Karl, and Kate Brown.

This project was made possible with funding from the College of Agriculture and Life Sciences, the School of Integrated Plant Science, and the Horticulture Section at Cornell University. The Angry Orchard Hard Cider Company and the Arthur Boller Research Award also provided funding critical to this project's success.

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

Ala	Alanine
Arg	Arginine
Asn	Asparagine
Asp	Aspartate
Cys	Cysteine
DAP	Diammonium phosphate
FC	Folin-Ciocalteu total polyphenols
GAE	Gallic acid equivalent
Glu	Glutamate
Gln	Glutamine
Gly	Glycine
His	Histidine
H_2S	Hydrogen sulfide
Ile	Isoleucine
Leu	Leucine
Lys	Lysine
Met	Methionine
N	Nitrogen
PAN	Primary amino nitrogen
Phe	Phenylalanine
Pro	Proline
Ser	Serine
SPI	Starch pattern index
SSC	Soluble solids concentration
TA	Titratable acidity
Thr	Threonine
Trp	Tryptophan
Tyr	Tyrosine
Val	Valine
YAN	Yeast assimilable nitrogen

CHAPTER 1

LITERATURE REVIEW

Hard Cider Production and Industry Projections

Increasingly, apple (*Malus ×domestica* Borkh.) growers in the United States are planting high-tannin apple cultivars to supply the expanding alcoholic "hard" cider industry. Hard cider accounts for less than 2% of alcoholic beverage sales in the US, but is one of the fastest-growing categories, demonstrating a 500% growth in sales from 2011 to 2020 (American Cider Association, 2020; Snyder, 2018). Globally, the hard cider market was valued at $14.6 billion in 2020—and with a projected annual growth of 5%—its value is expected to reach over $26 billion by the year 2031 (Allied Market Research, 2021). Additionally, the popularity of high-value artisan products has increased within the craft brewing industry, paving the way for hard apple cider to enter the craft beverage market (Smith & Lal, 2022). However, as of 2020 hard cider's market growth rate has recently been surpassed by hard seltzer and ready-to-drink cocktail sales (Grand View Research, 2020; Kelley, 2022; Lin, 2021).

Maintaining adequate fruit supply has been identified as a major obstacle to cider makers expanding their operations (Becot et al., 2016). Roughly half of Northeastern cider producers procure fruit or juice from external sources and have expressed significant interest in purchasing specialty high-tannin and dual-purpose hard cider cultivars—presenting an opportunity for apple growers to diversify their

production with the surging demand and market gap (Becot et al., 2016; Mello-Klein, 2022; Peck & Miles, 2015; Zakalik, 2021).

The desirable qualities of hard cider apples differ greatly from the fresh-market apples that dominate the US market. Fresh-market apples are typically prioritized for appearance, size, firmness, and flavor attributes (Musacchi & Serra, 2018), while apples used in hard cidermaking are prioritized for a balance of sweetness, bitterness, astringency, acidity, and aroma (Symoneaux et al., 2015). High-tannin hard cider apples can often incur premium prices (Zakalik & Peck, 2023). However, any apple can be used to manufacture hard cider. Fresh-market and processing apples are commonly used in hard cider blends to reach desired acidity and sugar content and to reduce costs, but typically have low concentrations of tannins and thus contribute little to bitterness and astringency (Thompson-Witrick et al., 2014). Cultivars with high sugar content can be desirable for hard cider to increase ethanol concentration through fermentation, and residual sugars and unfermentable sugar-alcohols (i.e., sorbitol) determine the sweetness of a finished cider. Optimal hard cider apple qualities vary depending on a producer's preference, although, apples that are high in tannins are among the most difficult to source (Pashow, 2018). Producing specific aroma profiles is also dependent on a cidermaker's goals, yet developing the desired aroma profile is a complex process that remains a major challenge to the hard cider industry.

Olfactory Perception and Consumer Preference for Aromatic Volatile Compounds

Flavor is the result of taste and olfaction sensations, with a majority of the flavor in fermented beverages coming from aromatic (also referred to as odorous)

volatile components residing in the headspace region above the liquid (Burdock, 2009). The aroma of a fruit wine, such as hard cider, is perceived by both orthonasal and retronasal olfaction, and is one of the most influential characteristics that determine its quality and value (Pino & Queris, 2011; Satora et al., 2008). There are three definitions for human odor/aroma threshold: an absolute threshold is the first concentration at which a person can reliably detect an odorant, a recognition threshold is the lowest concentration at which its reliably discerned, and a difference threshold is the smallest amount and increment to cause a perceptibly weaker or stronger odor difference (Doty, 2002). Humans demonstrate tremendous variability in odor detection, intensity, quality, and liking, and these differences are due to unique individual variation in olfactory perception which can be affected by gender, age, culture, environment, and health. Ultimately, genetics is the greatest contributing factor for affecting olfactory perception, since approximately 400 functional genes (each with an average of five segregating variants per gene) are believed to alter the function of olfactory receptors. Olfactory receptor gene functionalities between any two humans will vary by an average of 30% (Oleszkiewicz et al., 2020; Trimmer & Mainland, 2017).

Consumer liking of hard cider aromatics is inherently variable due to genetics, physiological effects, and context, but there are often clear subgroups of preferences in cider and other alcoholic beverages (Francis & Williamson, 2015). Demographics, consumption experience (i.e., beverage education and diversity of exposures) and frequency, neophobia (aversion to trying new foods), and personality traits play a rather weak role in the separation of preference subgroups in blind acceptance tests.

However, the aforementioned attributes do influence informed purchase intent with drinking experience having the greatest impact (Yegge, 2001).

Hard cider aroma is comprised of hundreds of aromatics, and consumer perception and preference is significantly affected when there are variations even in small quantities (King et al., 2010). It is important to consider minor compounds in addition to higher concentrated or abundant volatiles since many aromatics—such as ethyl hexanoate, 1-octen-3-ol, and beta-damascenone—can be easily perceived even at extremely low concentrations (<1 ng/L) yet have a great aromatic impact on hard ciders (Abrodo et al., 2010; Acree & Arn, 2004; Falqué et al., 2001; Jagatić Korenika et al., 2022). Additionally, alcoholic beverage producers must not only take into account that certain volatile aromatics can mask other aroma components, but that aroma—both perceived intensity and identity—can differ broadly based on the other volatile compounds present (Hein et al., 2009).

Because of the extremely low concentration of many volatile aromatics, it can be challenging to extract and isolate volatile aromatics for quantitative analysis (Gerhardt, 1990; Steffen & Pawliszyn, 1996); however, the steady development and accessibility of gas chromatographic systems and their detector components is consequently improving the quality of volatile analysis. Gas chromatography–mass spectrometry (GC-MS) is relatively fast, precise, and accurate making it one of the most popular methods to analyze volatiles (Abrodo et al., 2010). The two main approaches to GC-MS employed to analyze foods and beverages are targeted and untargeted analysis. Targeted analysis is intended for the quantitative measurement of predefined compound(s), and untargeted analysis aims to identify nearly all

compounds present in a sample. A major challenge to untargeted analysis is data processing, but improvements of analytical platforms and bioinformatic tools have greatly streamlined this process (Gehlenborg et al., 2010; Patti et al., 2012). Liquid chromatography–mass spectrometry (LC-MS) is another common method used to separate, examine, and identify chemicals in a mixture, but it is more expensive to operate, requires specialized operator training, and more frequent maintenance. LC-MS has the advantage of increased sensitivity and can be used to detect thermally fragile and nonpolar compounds, but a vast majority of the volatiles compounds found in fermented fruit beverages, such as hard cider, are suitable for GC-MS analysis and thus GC-MS is often preferred over LC-MS for its lower cost and convenience (Perez et al., 2016).

Major Factors that Influence Aromatic Volatile Composition

Given the importance of hard cider aroma to consumer desirability, it is crucial for hard cider-producers to both qualitatively and quantitatively understand the formation of volatile aromatics in order to produce a finished cider with the desired aroma (Ye et al., 2014). Apple cultivar and yeast strain are the two most powerful predictors of aroma composition in finished hard cider, and fruity aromas (both fruit and yeast derived) are the most significant factors that will influence perceived cider quality amongst consumers (Lorenzini et al., 2019; Nogueira et al., 2012; Rosend et al., 2019; Tarko et al., 2019). However, hard cider makers generally consider hard cider aroma to be most dependent on technological parameters and fermentation conditions since cultivar-specific apple odors can be easily and rapidly lost during milling and pressing through oxidation (e.g., thiols) or potency loss via diffusion into

the air since many cultivar-specific volatiles exist in very small concentrations (Abrodo et al., 2010; Villière et al., 2015).

Yeast

Hard cider aromatic compounds develop de novo most prominently during the fermentation process in which yeast produce secondary aromas. Primary aromas—also called varietal aromas—originate directly from the fruit while secondary aromas arise from the process of yeast fermentation, malolactic fermentation, other microbial activity (such as that of lactic acid bacteria and acetobacter), oxidation, and aging. Fatty acids, higher alcohols (6–16% of total cider volatiles), esters (78–92% of total cider volatiles), and carbonyls have been identified as the most important volatile aromatics in hard cider, and are formed during yeast fermentation as secondary metabolites (Rita et al., 2011; Roberto et al., 2005; Vidrih & Hribar, 1999).

Saccharomyces cerevisiae is the most common yeast species used in fruit wine fermentations, and non-*Saccharomyces* yeasts are often considered contaminants to be eliminated via pasteurization, the addition of sulfite, and/or sanitization (Ciani & Maccarelli, 1997; Loureiro & Malfeito-Ferreira, 2006). Diverse yeasts are naturally present on the surface of plants and fruits and participate in the decomposition of ripening fruit and fermentation process, but most hard cider producers add cultured, commercially available yeast strains in order to obtain uniform products that meet desired characteristics in the finished cider (Escalante, 2018). *Torulaspora*, *Metschnikowia*, and *Lachancea* are three non-*Saccharomyces* yeast genera that yield largely positive results for hard cider fermentations, but it is still recommended to add

Saccharomyces yeasts towards the middle or end of fermentation. *Rhodotorula*, *Pichia*, and *Candida* will also naturally develop at low levels in freshly extracted apple juice but die off very quickly after the start of alcoholic fermentation (Fleet et al., 1984; Stewart et al., 2018).

The relationship between yeast species, as well as strains within a species, and the formation of volatile aromatics is not fully understood. A sensory analysis (Leguerinel et al., 1989) of cider fermented using 12 different strains from *Saccharomyces uvarum* indicated that various strains produced significantly different concentrations of volatile aromatics, acetic acid, isobutanol, and isoamyl alcohol, but these differences were not perceptible in sensory tests with trained human subjects. Different strains of yeast will produce distinct aromatic profiles when fermenting the same pomace. This is due to each strain's individual ability to release different cultivar-specific volatiles from the pomace in addition to de novo yeast-derived compounds (Molina et al., 2009; Vilanova & Sieiro, 2006; Wondra & Berovic, 2001). For example, Molina et al., 2009, describes how a neutral grape (*Vitis vinifera*) juice fermented by two different *S. cerevisiae* strains differed in both sensory perception and descriptive analysis by trained panelists; strain EC1118 produced more pineapple, fatty, and solvent aromas while VIN13 had more banana, green, yeasty, and fruity aromas. The negative perception of non-*Saccharomyces* yeast is not universal—some hard cider producers and consumers enjoy the "off-flavors" produced by these yeasts such as with *Brettanomyces*—and several recent studies have attributed non-*Saccharomyces* yeast to contributing positively to typically desirable sensory qualities (Estela-Escalante et al., 2012; Fleet, 2003; P.-T. Liu et al., 2016; Steensels &

Verstrepen, 2014). In some instances, non-*Saccharomyces* yeast can outperform *S. cerevisiae* in aromatic volatile production. For example, a study comparing 23 volatile compounds and 12 species of non-*Saccharomyces* yeast species to *S. cerevisiae* in 'Malvar' white grape wine found higher ester, higher alcohol, and fatty acid production in several of the non-*Saccharomyces* yeast species. In particular, *Torulaspora delbrueckii* produced positive flowery and fruity aromas, while *Hanseniaspora guilliermondii* and genera *Candida* and *Pichia* produced high quantities of extracellular enzymes responsible for early fermentation biotransformations that contribute to organoleptic composition—such as the clarification and phenolic and aromatic extraction (Cordero-Bueso et al., 2013).

Temperature

The temperature of fermenting apple juice can influence secondary aromas produced by yeast. At lower juice temperatures (13 to 15 °C), yeast produce higher concentrations of esters during fermentation. Conversely, production of higher alcohols is observed with higher fermentation temperatures (25 to 28 °C). This is presumably due to enhanced yeast metabolic and biosynthetic activity rates, but the exact mechanisms are unknown (Beltran et al., 2006; Molina et al., 2007). High fermentation temperatures can lead to significant loss of volatile aromas due to compound volatility and hydrophobicity—as observed with fatty acid esters in 'Merlot' wines fermented at 25 °C (Killian & Ough, 1979; Mouret et al., 2014). Temperature conditions during pre-fermentative maceration have shown little to no effect on organoleptic attributes in 'Cabernet Sauvignon' wines; however, 'Cabernet

Sauvignon' wine fermentations subjected to a 10 °C to 20 °C temperature gradient over 33 days yielded lower tannin content, loss of red color intensity, and greater fruit aroma intensity (Ruiz-Rodríguez et al., 2021). Similar studies have not yet been conducted in hard cider.

Yeast Nutrition

A heavily researched and influential area for aromatic volatiles in hard cidermaking and other fruit wines is yeast metabolism and nutrition. Yeast assimilable nitrogen (YAN) is comprised of ammonia, ammonium, and primary amino nitrogen (PAN) and is metabolized by yeast for reproduction and growth. Second to sugars, nitrogen is the most important macronutrient for yeast development and is frequently the limiting factor in fruit wine fermentations (Bell & Henschke, 2005). While *S. cerevisiae* is not as nutritionally demanding as other microbes such as lactic acid bacteria (Maicas, 2020; Walker & Stewart, 2016), it requires fermentable sugars and YAN for growth—YAN being the limiting factor in fermentations across the spectrum of fermenting fruit juices (Escalante, 2018). YAN deficiencies can result in slow and incomplete fermentations, and the composition of YAN is known to impact both chemical and sensory profiles in wines and ciders (Bell & Henschke, 2005; Boudreau IV et al., 2017; Herraiz & Ough, 1993; Vilanova et al., 2007).

Low YAN concentration increases hydrogen sulfide production: a foul, rotten-egg smelling compound that is an extremely undesirable, yet common, problem in hard cider (Boudreau IV et al., 2017). Hydrogen sulfide is generally formed as an intermediary compound during the formation of the sulfur amino acids methionine and

cysteine in the sulfate/sulfite assimilation pathway (Ono et al., 1999). When there is insufficient YAN to provide amino acid precursors to sequester sulfide during fermentation, excess hydrogen sulfide is expelled outward through yeast cell membranes (Jiranek et al., 1995; Ugliano et al., 2011). However, the activity of sulfite reductase is highly yeast species and strain-dependent (Cordente et al., 2009; Song et al., 2020). *S. cerevisiae* is known to increase hydrogen sulfide synthesis in cases of excess YAN concentration: the excess nutrient results in unsustainably rapid growth and reproduction and hydrogen sulfide is released as a yeast starvation stress response (Ugliano et al., 2009).

Sufficient YAN is also crucial for the synthesis of higher alcohols, esters, aldehydes, and fatty acids which are produced as byproducts in yeast metabolic pathways (Saerens et al., 2010). The diversity and concentrations depend not only on yeast species and fermentation conditions, but the ratio of ammonia to amino acid concentration, which can yield very different volatile aromatic profiles—yet, the casual mechanism(s) are not well understood (Tahim & Mansfield, 2019; Torrea et al., 2011).

Aromatic Volatile Synthesis

There is a strong relationship between the availability and composition of YAN in juice and the production of fermentative aromas, and several trends have been observed in grape-based wine. When ketoacids are carboxylated, aldehydes are produced that form higher alcohols within the yeast cytosol when reduced. These higher alcohols are then exported outside of the cell and accumulate to contribute to the organoleptic quality, either positively in adequate concentrations or negatively in

excess (Romano et al., 1992; Vidrih & Hribar, 1999). Precursor ketoacids can originate from glucose metabolism or amino acid catabolism taken in by the yeast cell from the surrounding juice (Webb & Ingraham, 1963). Any factors that increase sugar metabolism or amino acids—such as temperature, amino acid concentration, or amino acid composition—promote the synthesis of higher alcohols. Esters are similarly synthesized inside yeast via the activation of specific enzymes which catalyze the reaction between a higher alcohol and a volatile fatty acid (Nordström, 1965).

When the juice YAN is relatively low (75 mg N/L), the addition of YAN during fermentation will increase the production of higher alcohol production. When starting YAN is relatively high (400 mg N/L), the addition of YAN can sometimes decrease higher alcohol production (Bell & Henschke, 2005; Carrau et al., 2008; Jiménez-Martí et al., 2007; Vilanova et al., 2007). For ester production, high initial YAN during fermentation yields higher ester concentrations. However, for some yeast strains and juice chemical compositions, YAN addition can impair ester synthesis. It is believed that nitrogen availability in the juice impacts yeast carbon flux and the excretion of carbon metabolic byproducts that serve as the precursors to higher alcohol and ester compounds (Garde-Cerdán & Ancín-Azpilicueta, 2008; Hernandez-Orte et al., 2006; Jiménez-Martí et al., 2007). Since ester compounds frequently have the most profound impact on sensory perception, it is generally considered that greater juice YAN content will increase the final concentrations of the most influential volatile compounds for a fermentation (Rollero et al., 2015; Villière et al., 2015). The redox status within yeast cells effects the availability of acetyl-CoA, which is the intermediate of central carbon metabolism and a precursor to α-keto acid. It is believed

that the nitrogen content in a fermenting juice influences this redox, ultimately impacting in the concentrations of yeast-produced volatiles since central carbon metabolism and α-keto acids are essential to ester, aldehyde, higher alcohol, and fatty acid biosynthesis pathways (Bloem et al., 2016; Godoy et al., 2020; Tehlivets et al., 2007).

Yeast Assimilable Nitrogen Supplementation and Management Strategies

Yeast assimilable nitrogen is present in apple juice primarily as ammonium ions and free amino nitrogen (Boudreau IV et al., 2018). In order to both enhance hard cider aroma and ensure fermentation completion, YAN can be added before and/or during fermentation as either single amino acids (Fairbairn et al., 2017), ammonium salts (Chen et al., 2019; Wang et al., 2017), or a combination of amino acids and ammonium (Hu et al., 2019)

Adequate nitrogen content (a minimum of 45 mg N/L in apple juice) is desirable for several aspects of fermentation and is often supplemented to either maintain or increase yeast growth and metabolism throughout the fermentation process (Bisson & Butzke, 2000; Kelkar & Dolan, 2012). In winemaking, 140 mg/L YAN is considered the minimum level for fermentation completion, but the recommended range is 200 to 350 mg/L depending on the desired wine style, yeast strain(s) used, and initial sugar content (Bell & Henschke, 2005; Torrea et al., 2011; Ugliano et al., 2011). Apple juice is far below these thresholds, ranging between 10 to 100 mg/L YAN depending on the cultivar and rootstock (Boudreau IV et al., 2018; Ma et al., 2018; Peck et al., 2016). Recent research has contributed to identifying appropriate juice YAN concentrations for cider fermentation, but the topic requires additional exploration in order to

establish commercial recommendations (Boudreau IV et al., 2018; Cairns et al., 2022; Ma et al., 2018).

Commercially, nitrogen can be supplemented at one or multiple stages of production to address de novo YAN deficiencies. Inorganic or organic nitrogen supplementation to juice are the most common methods, with diammonium phosphate (DAP) being the most ubiquitously used because of its high nitrogen content, low cost, and availability. Sugar content and yeast strains should first be assessed to determine appropriate nitrogen needs, since excessive additions can lead to ester taint formation, increased fermentation temperature, acidification, excessive phosphate content, and rapid, unsustainable growth in yeast populations (Bell & Henschke, 2005; Mansfield, 2019). To avoid these potential issues, many wine and hard cider producers prefer high-PAN, low-ammonia yeast nutrients composed of vitamins, minerals, and sterols from inactivated dry yeast cells to supplement metabolism (Ángeles Pozo-Bayón et al., 2009).

Pre-harvest methods to increase fruit nitrogen and thus juice YAN, such as soil and foliar nitrogen fertilization, selecting rootstock genetics for improved nutrient uptake, and reducing crop load are known to increase final fruit and must nitrogen content in winemaking (Bell & Henschke, 2005; Moss, 2016; Neilsen et al., 2010). There has been limited research on these applications in apples, but previous research (Karl et al., 2020a, 2020b; Plotkowski & Cline, 2021) has shown that both soil and foliar nitrogen fertilization can increase YAN in apple juice.

In an apple orchard, both ground and foliar applications of nitrogen fertilization are can be applied to increase or sustain tree nitrogen content (Merwin & Stiles, 1994).

Apple trees utilize nitrogen reserves in the spring stored in the woody tissue to support flowering, vegetative growth; once there is sufficient leaf area and transpiration, usually around fruit set, apple trees will then utilize nitrogen acquired from the soil (Cheng & Raba, 2009). Apple leaves can also absorb nitrogen, and in commercial orchards it is common practice to apply foliar nitrogen fertilizer in the form of urea.

In a study using the winegrape cultivars 'Sauvignon blanc' and 'Petit Manseng,' must YAN content was more than doubled by foliar urea applications and was more effective at increasing YAN than soil fertilization via nitrogenous calcium nitrate (Moss, 2016). Foliar fertilization alleviates deficiencies faster than soil application as nutrients applied to the leaves are absorbed faster; additionally, it gives growers the opportunity to combine other agrochemicals and reduce labor, machinery, and energy costs (Fageria et al., 2009; Niu et al., 2021). Low nitrogen content in apple trees has been linked to poor vegetative growth, as well as decreased fruit yield, size, and soluble solid concentrations (Cheng & Fuchigami, 2002; Xia et al., 2009). Alternatively, excessive nitrogen increases green fruit coloration and tree vulnerability to diseases—such as apple scab (*Venturia inaequalis*) and fireblight (*Erwinia amylovora*)—and reduces flesh firmness, cold hardiness in buds, red fruit coloration, and long-term storage quality (Fallahi, 1997; Fallahi et al., 1997; Raese et al., 2007; Rühmann et al., 2002; Stiles & Reid, 1991; Wargo et al., 2003, 2004). Total nitrogen content in leaf tissue can be used to estimate overall tree nitrogen status. The ideal leaf nitrogen content for young apple trees is between 2.4 and 2.6% and 2.2 to 2.4% for mature trees when fertilizing fresh market and processing apple cultivars (Cheng & Raba, 2009). However, there are currently no standard nitrogen fertilizer guidelines

for orchards or cultivars specifically intended for hard cider production (Cheng & Raba, 2009; Cheng & Schupp, 2004; Stiles & Reid, 1991).

Conclusion

There is a lack of management information for how to manage hard cider-specific apple cultivars in the US. Understanding nitrogen and its potential influence at all stages of the hard cidermaking process allows producers and growers the opportunity to maximize the quality of their products. Thus, the goal of the research described within this thesis is to discover how nitrogen applications can affect juice and cider volatile aromatic profiles and hopefully enhance overall cider quality. The two areas of research interest include: the potential impact of foliar urea fertilization on tree and fruit physiology, fermentation kinetics, aromatic volatile and amino acid composition and concentration, and YAN content; and if different levels of foliar urea fertilization are sensorily distinct and discernable by consumers. Experiments were carried out using foliar urea fertilizers applied to two different hard cider cultivars to further investigate these research questions; juice from these experiments was fermented to track pre- and post-fermentation characteristics, and the YAN contents, aromatic volatile compositions, and sensory attributes of the finished hard ciders were accessed. The following thesis describes how foliar urea nitrogen applications influence hard cider quality and aromatic volatiles.

REFERENCES

Abrodo, P. A., Llorente, D. D., Corujedo, S. J., de la Fuente, E. D., Álvarez, M. D. G., & Gomis, D. B. (2010). Characterisation of Asturian cider apples on the basis of their aromatic profile by high-speed gas chromatography and solid-phase microextraction. *Food Chemistry*, *121*(4), 1312–1318. https://doi.org/10.1016/j.foodchem.2010.01.068

Acree, T. E., & Arn, H. (2004, June 25). *Flavornet and human odor space*. Flavornet. https://www.flavornet.org/

Allied Market Research. (2021). *Cider market by type, packaging and distribution channel: Global opportunity analysis and industry forecast, 2022-2031*. Allied Market Research. https://www.alliedmarketresearch.com/cider-market

American Cider Association. (2020). *Off-premise sales aide*. https://ciderassociation.org/resources/

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

Becot, F. A., Bradshaw, T. L., & Conner, D. S. (2016). Apple market expansion through value-added hard cider production: Current production and prospects in Vermont. *HortTechnology*, *26*(2), 220–229. https://doi.org/10.21273/HORTTECH.26.2.220

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

Beltran, G., Novo, M., Leberre, V., Sokol, S., Labourdette, D., Guillamon, J.-M., Mas, A., François, J., & Rozes, N. (2006). Integration of transcriptomic and metabolic analyses for understanding the global responses of low-temperature winemaking fermentations. *FEMS Yeast Research*, *6*(8), 1167–1183. https://doi-org /10.1111/j.1567-1364.2006.00106.x

Bisson, L. F., & Butzke, C. E. (2000). Diagnosis and rectification of stuck and sluggish fermentations. *American Journal of Enology and Viticulture*, *51*(2), 168–177. https://doi-org /10.5344/ajev.2000.51.2.168

Bloem, A., Sanchez, I., Dequin, S., & Camarasa, C. (2016). Metabolic impact of redox cofactor perturbations on the formation of aroma compounds in Saccharomyces cerevisiae. *Applied and Environmental Microbiology*, *82*(1), 174–183. https://doi.org/10.1128/AEM.02429-15

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

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

Burdock, G. A. (2009). *Fenaroli's Handbook of Flavor Ingredients* (6th ed.). CRC Press. https://doi.org/10.1201/9781439847503

Cairns, P., Hamilton, L., Racine, K., Phetxumphou, K., Ma, S., Lahne, J., Gallagher, D., Huang, H., Moore, A. N., & Stewart, A. C. (2022). Effects of hydroxycinnamates and exogenous yeast assimilable nitrogen on cider aroma and fermentation performance. *Journal of the American Society of Brewing Chemists*, *80*(3), 236–247. https://doi.org/10.1080/03610470.2021.1968171

Carrau, F. M., Medina, K., Farina, L., Boido, E., Henschke, P. A., & Dellacassa, E. (2008). Production of fermentation aroma compounds by *Saccharomyces cerevisiae* wine yeasts: Effects of yeast assimilable nitrogen on two model strains. *FEMS Yeast Research*, *8*(7), 1196–1207. https://doi.org/10.1111/j.1567-1364.2008.00412.x

Chen, D., Toussaint, S., Huang, W., Zhan, J., & Liu, S.-Q. (2019). Effects of diammonia phosphate addition on the chemical constituents in lychee wine fermented with *Saccharomyces cerevisiae*. *LWT*, *105*, 224–232. https://doi.org/10.1016/j.lwt.2019.02.018

Cheng, L., & Fuchigami, L. H. (2002). Growth of young apple trees in relation to reserve nitrogen and carbohydrates. *Tree Physiology*, *22*(18), 1297–1303. https://doi.org/10.1093/treephys/22.18.1297

Cheng, L., & Raba, R. (2009). Accumulation of macro- and micronutrients and nitrogen demand-supply relationship of 'Gala'/'Malling 26' apple trees grown in sand culture. *Journal of the American Society for Horticultural Science*, *134*(1), 3–13. https://doi.org/10.21273/JASHS.134.1.3

Cheng, L., & Schupp, J. (2004). *Nitrogen fertilization of apple orchards*.

Ciani, M., & Maccarelli, F. (1997). Oenological properties of non-*Saccharomyces* yeasts associated with wine-making. *World Journal of Microbiology and Biotechnology*, *14*(2), 199–203. https://doi.org/10.1023/A:1008825928354

Cordente, A. G., Heinrich, A., Pretorius, I. S., & Swiegers, J. H. (2009). Isolation of sulfite reductase variants of a commercial wine yeast with significantly reduced hydrogen sulfide production. *FEMS Yeast Research*, *9*(3), 446–459. https://doi.org/10.1111/j.1567-1364.2009.00489.x

Cordero-Bueso, G., Esteve-Zarzoso, B., Cabellos, J. M., Gil-Díaz, M., & Arroyo, T. (2013). Biotechnological potential of non-*Saccharomyces* yeasts isolated during spontaneous fermentations of *Malvar* (*Vitis vinifera* cv. L.). *European Food Research and Technology*, *236*(1), 193–207. https://doi.org/10.1007/s00217-012-1874-9

Doty, R. L. (2002). Olfaction. In V. S. Ramachandran (Ed.), *Encyclopedia of the Human Brain* (pp. 717–727). Academic Press. https://doi.org/10.1016/B0-12-227210-2/00259-4

Escalante, W. D. E. (2018). Perspectives and uses of non-*Saccharomyces* yeasts in fermented beverages. In *Frontiers and New Trends in the Science of Fermented Food and Beverages*. IntechOpen. https://doi.org/10.5772/intechopen.81868

Estela-Escalante, W. D., Rychtera, M., Melzoch, K., & Guerrero-Ochoa, M. R. (2012). Influence of aeration in the fermentative activity of *Kloeckera Apiculata* during fermentation of apple juice. *Acta Biológica Colombiana*, *17*(2), 309–322. https://api.semanticscholar.org/CorpusID:94725856

Fageria, N. K., Filho, M. P. B., Moreira, A., & Guimarães, C. M. (2009). Foliar fertilization of crop plants. *Journal of Plant Nutrition*, *32*(6), 1044–1064. https://doi.org/10.1080/01904160902872826

Fairbairn, S., McKinnon, A., Musarurwa, H. T., Ferreira, A. C., & Bauer, F. F. (2017). The impact of single amino acids on growth and volatile aroma production by *Saccharomyces cerevisiae* strains. *Frontiers in Microbiology*, *8*. https://www.frontiersin.org/articles/10.3389/fmicb.2017.02554

Fallahi, E. (1997). Preharvest nitrogen optimization for maximizing yield and postharvest fruit quality of apples. *Acta Horticulturae*, *448*, 415–420. https://doi.org/10.17660/ActaHortic.1997.448.77

Fallahi, E., Conway, W. S., Hickey, K. D., & Sams, C. E. (1997). The role of calcium and nitrogen in postharvest quality and disease resistance of apples. *HortScience*, *32*(5), 831–835. https://doi.org/10.21273/HORTSCI.32.5.831

Falqué, E., Fernández, E., & Dubourdieu, D. (2001). Differentiation of white wines by their aromatic index. *Talanta*, *54*(2), 271–281. https://doi.org/10.1016/S0039-9140(00)00641-X

Fleet, G. H. (2003). Yeast interactions and wine flavour. *International Journal of Food Microbiology*, *86*(1), 11–22. https://doi.org/10.1016/S0168-1605(03)00245-9

Fleet, G. H., Lafon-Lafourcade, S., & Ribéreau-Gayon, P. (1984). Evolution of yeasts and lactic acid bacteria during fermentation and storage of *Bordeaux* wines. *Applied and Environmental Microbiology*, *48*(5), 1034–1038. https://doi.org/10.1128/aem.48.5.1034-1038.1984

Francis, L. l., & Williamson, P. O. (2015). Application of consumer sensory science in wine research. *Australian Journal of Grape and Wine Research*, *21*(S1), 554–567. https://doi.org/10.1111/ajgw.12169

Garde-Cerdán, T., & Ancín-Azpilicueta, C. (2008). Effect of the addition of different quantities of amino acids to nitrogen-deficient must on the formation of esters, alcohols, and acids during wine alcoholic fermentation. *LWT - Food Science and Technology*, *41*(3), 501–510. https://doi.org/10.1016/j.lwt.2007.03.018

Gehlenborg, N., O'Donoghue, S. I., Baliga, N. S., Goesmann, A., Hibbs, M. A., Kitano, H., Kohlbacher, O., Neuweger, H., Schneider, R., Tenenbaum, D., & Gavin, A.-C. (2010). Visualization of omics data for systems biology. *Nature Methods*, *7*(3), Article 3. https://doi.org/10.1038/nmeth.1436

Gerhardt, K. O. (1990). Gas chromatography—Mass spectrometry. In M. H. Gordon (Ed.), *Principles and Applications of Gas Chromatography in Food Analysis* (pp. 59–85). Springer US. https://doi.org/10.1007/978-1-4613-0681-8_2

Godoy, L., Acuña-Fontecilla, A., Catrileo, D., Godoy, L., Acuña-Fontecilla, A., & Catrileo, D. (2020). Formation of aromatic and flavor compounds in wine: A perspective of positive and negative contributions of non-*Saccharomyces* yeasts. In *Chemistry and Biochemistry of Winemaking, Wine Stabilization and Aging*. IntechOpen. https://doi.org/10.5772/intechopen.92562

Grand View Research. (2020). *Hard seltzer market size & share report, 2022-2030*. https://www.grandviewresearch.com/industry-analysis/hard-seltzer-market

Hein, K., Ebeler, S. E., & Heymann, H. (2009). Perception of fruity and vegetative aromas in red wine. *Journal of Sensory Studies*, *24*(3), 441–455. https://doi.org/10.1111/j.1745-459X.2009.00220.x

Hernandez-Orte, P., Bely, M., Cacho, J., & Ferreira, V. (2006). Impact of ammonium additions on volatile acidity, ethanol, and aromatic compound production by different *Saccharomyces cerevisiae* strains during fermentation in controlled synthetic media. *Australian Journal of Grape and Wine Research*, *12*(2), 150–160. https://doi.org/10.1111/j.1755-0238.2006.tb00055.x

Herraiz, T., & Ough, C. S. (1993). Formation of ethyl esters of amino acids by yeasts during the alcoholic fermentation of grape juice. *American Journal of Enology and Viticulture*, *44*(1), 41–48. https://doi.org/10.5344/ajev.1993.44.1.41

Hu, K., Jin, G.-J., Xu, Y.-H., Xue, S.-J., Qiao, S.-J., Teng, Y.-X., & Tao, Y.-S. (2019). Enhancing wine ester biosynthesis in mixed *Hanseniaspora uvarum/Saccharomyces cerevisiae* fermentation by nitrogen nutrient addition. *Food Research International*, *123*, 559–566. https://doi.org/10.1016/j.foodres.2019.05.030

Jagatić Korenika, A.-M., Preiner, D., Tomaz, I., Skendrović Babojelić, M., & Jeromel, A. (2022). Aroma profile of monovarietal <i>Pét-Nat<i> ciders: The role of Croatian traditional apple varieties. *Horticulturae*, *8*(8), Article 8. https://doi.org/10.3390/horticulturae8080689

Jiménez-Martí, E., Aranda, A., Mendes-Ferreira, A., Mendes-Faia, A., & del Olmo, M. lí. (2007). The nature of the nitrogen source added to nitrogen depleted vinifications conducted by a *Saccharomyces cerevisiae* strain in synthetic must affects gene expression and the levels of several volatile compounds. *Antonie Van Leeuwenhoek*, *92*, 61–75. https://doi.org/10.1007/s10482-006-9135-1

Jiranek, V., Langridge, P., & Henschke, P. A. (1995). Regulation of hydrogen sulfide liberation in wine-producing *Saccharomyces cerevisiae* strains by assimilable

nitrogen. *Applied and Environmental Microbiology*, *61*(2), 461–467. https://doi.org/10.1128/aem.61.2.461-467.1995

Karl, A. D., Brown, M. G., Ma, S., Sandbrook, A., Stewart, A. C., Cheng, L., Mansfield, A. K., & Peck, G. M. (2020a). Foliar urea applications increase yeast assimilable nitrogen concentration and alcoholic fermentation rate in 'Red Spy' apples used for cider production. *HortScience*, *55*(8), 1356–1364. https://doi.org/10.21273/HORTSCI15029-20

Karl, A. D., Brown, M. G., Ma, S., Sandbrook, A., Stewart, A. C., Cheng, L., Mansfield, A. K., & Peck, G. M. (2020b). Soil nitrogen fertilization increases yeast assimilable nitrogen concentrations in 'Golden Russet' and 'Medaille D'or' apples used for cider production. *HortScience*, *55*(8), 1345–1355. https://doi.org/10.21273/HORTSCI15028-20

Kelkar, S., & Dolan, K. (2012). Modeling the effects of initial nitrogen content and temperature on fermentation kinetics of hard cider. *Journal of Food Engineering*, *109*(3), 588–596. https://doi.org/10.1016/j.jfoodeng.2011.10.020

Kelley, K. (2022). *Alcoholic beverage consumption statistics and trends 2022*. PennState Extension. https://extension.psu.edu/alcoholic-beverage-consumption-statistics-and-trends-2022

Killian, E., & Ough, C. S. (1979). Fermentation esters—Formation and retention as affected by fermentation temperature. *American Journal of Enology and Viticulture*, *30*(4), 301–305. https://doi.org/10.5344/ajev.1979.30.4.301

King, E. S., Kievit, R. L., Curtin, C., Swiegers, J. H., Pretorius, I. S., Bastian, S. E. P., & Leigh Francis, I. (2010). The effect of multiple yeasts co-inoculations on *Sauvignon Blanc* wine aroma composition, sensory properties and consumer preference. *Food Chemistry*, *122*(3), 618–626. https://doi.org/10.1016/j.foodchem.2010.03.021

Leguerinel, I., Mafart, P., Cleret, J. J., & Bourgeois, C. (1989). Yeast strain and kinetic aspects of the formation of flavour components in cider. *Journal of the Institute of Brewing*, *95*(6), 405–409. https://doi.org/10.1002/j.2050-0416.1989.tb04645.x

Lin, M. (2021). *Hard seltzer industry: Unlikely to fizzle out | Toptal®*. Toptal Finance Blog. https://www.toptal.com/finance/market-research-analysts/hard-seltzer-industry

Liu, P.-T., Lu, L., Duan, C.-Q., & Yan, G.-L. (2016). The contribution of indigenous non-*Saccharomyces* wine yeast to improved aromatic quality of *Cabernet Sauvignon* wines by spontaneous fermentation. *LWT - Food Science and Technology*, *71*, 356–363. https://doi.org/10.1016/j.lwt.2016.04.031

Lorenzini, M., Simonato, B., Slaghenaufi, D., Ugliano, M., & Zapparoli, G. (2019). Assessment of yeasts for apple juice fermentation and production of cider volatile compounds. *LWT*, *99*, 224–230. https://doi.org/10.1016/j.lwt.2018.09.075

Loureiro, V., & Malfeito-Ferreira, M. (2006). Spoilage activities of *Dekkera/Brettanomyces* spp. *Food Spoilage Microorganisms*, 354–398. https://doi.org/10.1533/9781845691417.3.354

Ma, S., Neilson, A. P., Lahne, J., Peck, G. M., O'Keefe, S. F., & Stewart, A. C. (2018). Free amino acid composition of apple juices with potential for cider making as determined by UPLC-PDA. *Journal of the Institute of Brewing*, *124*(4), 467–476. https://doi.org/10.1002/jib.519

Maicas, S. (2020). The role of yeasts in fermentation processes. *Microorganisms*, *8*(8), 1142. https://doi.org/10.3390/microorganisms8081142

Mansfield, A. K. (2019). Feed your yeast: Managing YAN in fermentations. *Cornell AgriTech*.

Mello-Klein, C. (2022, October 19). *Don't call it a comeback. Hard cider's rise in popularity is a return to form for one of America's most historic drinks*. Northeastern Global News. https://news.northeastern.edu/2022/10/19/hard-cider-popularity/

Merwin, I. A., & Stiles, W. C. (1994). Orchard Groundcover Management Impacts on Apple Tree Growth and Yield, and Nutrient Availability and Uptake. *Journal of the American Society for Horticultural Science*, *119*(2), 209–215. https://doi.org/10.21273/JASHS.119.2.209

Molina, A. M., Guadalupe, V., Varela, C., Swiegers, J. H., Pretorius, I. S., & Agosin, E. (2009). Differential synthesis of fermentative aroma compounds of two related commercial wine yeast strains. *Food Chemistry*, *117*(2), 189–195. https://doi.org/10.1016/j.foodchem.2009.03.116

Molina, A. M., Swiegers, J. H., Varela, C., Pretorius, I. S., & Agosin, E. (2007). Influence of wine fermentation temperature on the synthesis of yeast-derived volatile aroma compounds. *Applied Microbiology and Biotechnology*, *77*, 675–687. https://doi.org/10.1007/s00253-007-1194-3

Moss, J. R. (2016). *Evaluation of nitrogen management schemes in cover cropped vineyards* [Thesis, Virginia Tech]. https://vtechworks.lib.vt.edu/handle/10919/80510

Mouret, J. R., Camarasa, C., Angenieux, M., Aguera, E., Perez, M., Farines, V., & Sablayrolles, J.-M. (2014). Kinetic analysis and gas–liquid balances of the production of fermentative aromas during winemaking fermentations: Effect of assimilable nitrogen and temperature. *Food Research International*, *62*, 1–10. https://doi.org/10.1016/j.foodres.2014.02.044

Musacchi, S., & Serra, S. (2018). Apple fruit quality: Overview on pre-harvest factors. *Scientia Horticulturae*, *234*, 409–430. https://doi.org/10.1016/j.scienta.2017.12.057

Neilsen, G. H., Neilsen, D., Bowen, P., Bogdanoff, C., & Usher, K. (2010). Effect of timing, rate, and form of N fertilization on nutrition, vigor, yield, and berry yeast-assimilable N of grape. *American Journal of Enology and Viticulture*, *61*(3), 327–336. https://doi.org/10.5344/ajev.2010.61.3.327

Niu, J., Liu, C., Huang, M., Liu, K., & Yan, D. (2021). Effects of foliar fertilization: A review of current status and future perspectives. *Journal of Soil Science and Plant Nutrition*, *21*(1), 104–118. https://doi.org/10.1007/s42729-020-00346-3

Nogueira, A., Wosiacki, G., Hui, Y., & Özgül, E. (2012). *Handbook of plant-based fermented food and beverage technology*.

Nordström, K. (1965). Possible control of volatile ester formation in brewing. *Proceedings of the 10th Congress of the European Brewing Convention*, *10*, 195–208.

Oleszkiewicz, A., Alizadeh, R., Altundag, A., Chen, B., Corrai, A., Fanari, R., Farhadi, M., Gupta, N., Habel, R., Hudson, R., Hughes, J. L., Joshi, A., Kamrava, S. K., Luckett, C., Mahmut, M. K., Masala, C., Mori, E., Pellegrino, R., Piras, R., … Hummel, T. (2020). Global study of variability in olfactory sensitivity. *Behavioral Neuroscience*, *134*, 394–406. https://doi.org/10.1037/bne0000378

Ono, B. I., Hazu, T., Yoshida, S., Kawato, T., Shinoda, S., Brzvwczy, J., & Paszewski, A. (1999). Cysteine biosynthesis in *Saccharomyces cerevisiae*: A new outlook on pathway and regulation. *Yeast (Chichester, England)*, *15*(13), 1365–1375. https://doi.org/10.1002/(SICI)1097-0061(19990930)15:13<1365::AID-YEA468>3.0.CO;2-U

Pashow, L. (2018). *Hard Cider Supply Chain Analysis*. Cornell Cooperative Extension.

Patti, G. J., Yanes, O., & Siuzdak, G. (2012). Metabolomics: The apogee of the omics trilogy. *Nature Reviews Molecular Cell Biology*, *13*(4), Article 4. https://doi.org/10.1038/nrm3314

Peck, G., McGuire, M., Boudreau, T., & Stewart, A. (2016). Crop Load Density Affects 'York' Apple Juice and Hard Cider Quality. *HortScience*, *51*(9), 1098–1102. https://doi.org/10.21273/HORTSCI10962-16

Peck, G., & Miles, C. (2015). Assessing the production scale and research and extension needs of U.S. hard cider producers. *The Journal of Extension*, *53*(5). https://tigerprints.clemson.edu/joe/vol53/iss5/18

Perez, E. R., Knapp, J. A., Horn, C. K., Stillman, S. L., Evans, J. E., & Arfsten, D. P. (2016). Comparison of LC–MS-MS and GC–MS analysis of benzodiazepine compounds included in the drug demand reduction urinalysis program. *Journal of Analytical Toxicology*, *40*(3), 201–207. https://doi.org/10.1093/jat/bkv140

Pino, J. A., & Queris, O. (2011). Analysis of volatile compounds of mango wine. *Food Chemistry*, *125*(4), 1141–1146. https://doi.org/10.1016/j.foodchem.2010.09.056

Plotkowski, D. J., & Cline, J. A. (2021). Seasonal and postharvest changes in amino acid composition in 'Crimson Crisp' apple (*Malus Domestica* Borkh.) in response to summer foliar urea applications. *HortScience*, *56*(9), 1041–1052. https://doi.org/10.21273/HORTSCI15982-21

Raese, J. T., Drake, S. R., & Curry, E. A. (2007). Nitrogen fertilizer influences fruit quality, soil nutrients and cover crops, leaf color and nitrogen content, biennial bearing and cold hardiness of 'Golden Delicious.' *Journal of Plant Nutrition*, *30*(10), 1585–1604. https://doi.org/10.1080/01904160701615483

Rita, R.-D., Zanda, K., Daina, K., & Dalija, S. (2011). Composition of aroma compounds in fermented apple juice: Effect of apple variety, fermentation temperature and inoculated yeast concentration. *Procedia Food Science*, *1*, 1709–1716. https://doi.org/10.1016/j.profoo.2011.09.252

Roberto, R. M., García, N. P., Hevia, A. G., & Valles, B. S. (2005). Application of purge and trap extraction and gas chromatography for determination of minor esters in cider. *Journal of Chromatography A*, *1069*(2), 245–251. https://doi.org/10.1016/j.chroma.2005.02.019

Rollero, S., Bloem, A., Camarasa, C., Sanchez, I., Ortiz-Julien, A., Sablayrolles, J.-M., Dequin, S., & Mouret, J.-R. (2015). Combined effects of nutrients and temperature on the production of fermentative aromas by *Saccharomyces cerevisiae* during wine fermentation. *Applied Microbiology and Biotechnology*, *99*, 2291–2304. https://doi.org/10.1007/s00253-014-6210-9

Romano, P., Suzzi, G., Comi, G., & Zironi, R. (1992). Higher alcohol and acetic acid production by apiculate wine yeasts. *Journal of Applied Bacteriology*, *73*(2), 126–130. https://doi.org/10.1111/j.1365-2672.1992.tb01698.x

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

Rühmann, S., Leser, C., Bannert, M., & Treutter, D. (2002). Relationship between growth, secondary metabolism, and resistance of apple. *Plant Biology*, *4*(2), 137–143. https://doi.org/10.1055/s-2002-25727

Ruiz-Rodríguez, A., Palma, M., & Barroso, C. G. (2021). Influence of temperature during pre-fermentative maceration and alcoholic fermentation on the phenolic composition of 'Cabernet Sauvignon' wines. *Foods*, *10*(5), 1053. https://doi.org/10.3390/foods10051053

Saerens, S. M. G., Delvaux, F. R., Verstrepen, K. J., & Thevelein, J. M. (2010). Production and biological function of volatile esters in *Saccharomyces cerevisiae*. *Microbial Biotechnology*, *3*(2), 165–177. https://doi.org/10.1111/j.1751-7915.2009.00106.x

Satora, P., Sroka, P., Duda-Chodak, A., Tarko, T., & Tuszyński, T. (2008). The profile of volatile compounds and polyphenols in wines produced from dessert varieties of apples. *Food Chemistry*, *111*(2), 513–519. https://doi.org/10.1016/j.foodchem.2008.04.007

Smith, M., & Lal, P. (2022). Environmental and economic assessment of hard apple cider using an integrated LCA-LCC approach. *Sustainable Production and Consumption*, *32*, 282–295. https://doi.org/10.1016/j.spc.2022.04.026

Snyder, C. (2018). *Hard cider business benchmark survey*. Pennsylvania State University Extension. https://extension.psu.edu/hard-cider-business-benchmark-survey

Song, Y., Gibney, P., Cheng, L., Liu, S., & Peck, G. (2020). Yeast assimilable nitrogen concentrations influence yeast gene expression and hydrogen sulfide production during cider fermentation. *Frontiers in Microbiology*, *11*. https://doi.org/10.3389/fmicb.2020.01264

Steensels, J., & Verstrepen, K. J. (2014). Taming wild yeast: Potential of conventional and nonconventional yeasts in industrial fermentations. *Annual Review of Microbiology*, *68*(1), 61–80. https://doi.org/10.1146/annurev-micro-091213-113025

Steffen, A., & Pawliszyn, J. (1996). Analysis of flavor volatiles using headspace solid-phase microextraction. *Journal of Agricultural and Food Chemistry*, *44*(8), 2187–2193. https://doi.org/10.1021/jf950727k

Stewart, A. C., Ma, S., Peck, G. M., McGuire, M. N., Boudreau IV, T. F., & O'Keefe, S. F. (2018). Yeast assimilable nitrogen and cider fermentation. In *Scott Laboratories Cider Handbook*.

Stiles, W. C., & Reid, W. S. (1991). Orchard nutrition management. *Cornell Cooperative Extension*, *Information Bulletin 219. Ithaca, NY*. https://ecommons.cornell.edu/handle/1813/3305

Styger, G., Prior, B., & Bauer, F. F. (2011). Wine flavor and aroma. *Journal of Industrial Microbiology and Biotechnology*, *38*(9), 1145. https://doi.org/10.1007/s10295-011-1018-4

Symoneaux, R., Chollet, S., Patron, C., Bauduin, R., Le Quéré, J.-M., & Baron, A. (2015). Prediction of sensory characteristics of cider according to their biochemical composition: Use of a central composite design and external validation by cider professionals. *LWT - Food Science and Technology*, *61*(1), 63–69. https://doi.org/10.1016/j.lwt.2014.11.030

Tahim, C. M., & Mansfield, A. K. (2019). Yeast assimilable nitrogen optimization for cool-climate *Riesling*. *American Journal of Enology and Viticulture*, *70*(2), 127–138. https://doi.org/10.5344/ajev.2018.17087

Tarko, T., Sroka, P., Duda-Chodak, A., & Januszek, M. (2019). The influence of cultivar of apple-tree and yeasts used for fermentation on the concentration of volatile compounds in ciders and their sensory properties. *Journal of Food and Nutrition Research*, *58*(4), 370–381. https://doi.org/10.1007/s11274-004-4490-4

Tehlivets, O., Scheuringer, K., & Kohlwein, S. D. (2007). Fatty acid synthesis and elongation in yeast. *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids*, *1771*(3), 255–270. https://doi.org/10.1016/j.bbalip.2006.07.004

Thompson-Witrick, K. A., Goodrich, K. M., Neilson, A. P., Hurley, E. K., Peck, G. M., & Stewart, A. C. (2014). Characterization of the polyphenol composition

of 20 cultivars of cider, processing, and dessert apples (*Balus × domestica* Borkh.) grown in Virginia. *Journal of Agricultural and Food Chemistry*, *62*(41), 10181–10191. https://doi.org/10.1021/jf503379t

Torrea, D., Varela, C., Ugliano, M., Ancin-Azpilicueta, C., Leigh Francis, I., & Henschke, P. A. (2011). Comparison of inorganic and organic nitrogen supplementation of grape juice–effect on volatile composition and aroma profile of a *Chardonnay* wine fermented with *Saccharomyces cerevisiae* yeast. *Food Chemistry*, *127*(3), 1072–1083. https://doi.org/10.1016/j.foodchem.2011.01.092

Trimmer, C., & Mainland, J. D. (2017). Chapter 17—The olfactory system. In P. M. Conn (Ed.), *Conn's Translational Neuroscience* (pp. 363–377). Academic Press. https://doi.org/10.1016/B978-0-12-802381-5.00029-4

Ugliano, M., Fedrizzi, B., Siebert, T., Travis, B., Magno, F., Versini, G., & Henschke, P. A. (2009). Effect of nitrogen supplementation and *Saccharomyces* species on hydrogen sulfide and other volatile sulfur compounds in *Shiraz* fermentation and wine. *Journal of Agricultural and Food Chemistry*, *57*(11), 4948–4955. https://doi.org/10.1021/jf8037693

Ugliano, M., Kolouchova, R., & Henschke, P. A. (2011). Occurrence of hydrogen sulfide in wine and in fermentation: Influence of yeast strain and supplementation of yeast available nitrogen. *Journal of Industrial Microbiology and Biotechnology*, *38*(3), 423–429. https://doi.org/10.1007/s10295-010-0786-6

Vidrih, R., & Hribar, J. (1999). Synthesis of higher alcohols during cider processing. *Food Chemistry*, *67*(3), 287–294. https://doi.org/10.1016/S0308-8146(99)00136-3

Vilanova, M., & Sieiro, C. (2006). Contribution by *Saccharomyces cerevisiae* yeast to fermentative flavour compounds in wines from cv. *Albariño*. *Journal of Industrial Microbiology and Biotechnology*, *33*(11), 929–933. https://doi.org/10.1007/s10295-006-0162-8

Vilanova, M., Ugliano, M., Varela, C., Siebert, T., Pretorius, I. S., & Henschke, P. A. (2007). Assimilable nitrogen utilisation and production of volatile and non-volatile compounds in chemically defined medium by <i>Saccharomyces cerevisiae<i> wine yeasts. *Applied Microbiology and Biotechnology*, *77*(1), 145–157. https://doi.org/10.1007/s00253-007-1145-z

Villière, A., Arvisenet, G., Bauduin, R., Quéré, J.-M. L., & Sérot, T. (2015). Influence of cider-making process parameters on the odourant volatile composition of hard ciders. *Journal of the Institute of Brewing*, *121*(1), 95–105. https://doi.org/10.1002/jib.197

Walker, G. M., & Stewart, G. G. (2016). Saccharomyces cerevisiae in the production of fermented beverages. *Beverages*, *2*(4), Article 4. https://doi.org/10.3390/beverages2040030

Wang, S.-Y., Li, Y.-Q., Li, T., Yang, H.-Y., Ren, J., Zhang, B.-L., & Zhu, B.-Q. (2017). Dibasic ammonium phosphate application enhances aromatic compound concentration in *Bog Bilberry* syrup wine. *Molecules*, *22*(1), Article 1. https://doi.org/10.3390/molecules22010052

Wargo, J. M., Merwin, I. A., & Watkins, C. B. (2003). Fruit size, yield, and market value of `Goldrush' apple are affected by amount, timing and method of nitrogen fertilization. *HortTechnology*, *13*(1), 153–161. https://doi.org/10.21273/HORTTECH.13.1.0153

Wargo, J. M., Merwin, I. A., & Watkins, C. B. (2004). Nitrogen fertilization, midsummer trunk girdling, and avg treatments affect maturity and quality of `Jonagold' apples. *HortScience*, *39*(3), 493–500. https://doi.org/10.21273/HORTSCI.39.3.493

Webb, A. D., & Ingraham, J. L. (1963). Fusel oil. In W. W. Umbreit (Ed.), *Advances in Applied Microbiology* (Vol. 5, pp. 317–353). Academic Press. https://doi.org/10.1016/S0065-2164(08)70014-5

Wondra, M., & Berovic, M. (2001). Analyses of aroma components of *Chardonnay* wine fermented by different yeast strains. *Food Technology and Biotechnology*, *39*(2), 141–148. ISSN 1330-9862.

Xia, G., Cheng, L., Lakso, A., & Goffinet, M. (2009). Effects of nitrogen supply on source-sink balance and fruit size of 'Gala' apple trees. *Journal of the American Society for Horticultural Science*, *134*(1), 126–133. https://doi.org/10.21273/JASHS.134.1.126

Ye, M., Yue, T., & Yuan, Y. (2014). Changes in the profile of volatile compounds and amino acids during cider fermentation using dessert variety of apples. *European Food Research and Technology*, *239*(1), 67–77. https://doi.org/10.1007/s00217-014-2204-1

Yegge, J. M. (2001). *Influence of sensory and non-sensory attributes of* Chardonnay *wine on acceptance and purchase intent* [Ph.D., University of California, Davis]. https://www.proquest.com/docview/304684825/abstract/6FD5B870A74C4E4APQ/1

Zakalik, D. (2021). *Crop load management of seven European cider apple cultivars: Effects on biennial bearing and fruit quality* [Master's Thesis, Cornell University]. https://doi.org/10.7298/van0-xz83

Zakalik D. and Peck, G.M.. 2023. High-tannin apple supply and demand in North America: results from a 2021 cider industry survey. *Fruit Quarterly*. 29(1):30–34. https://nyshs.org/fruit-quarterly/

CHAPTER 2

FOLIAR NITROGEN APPLICATION INCREASES FLAVOR VOLATILES AND PRECURSORS, SENSORY PERCEPTION, AND YEAST ASSIMILABLE NITROGEN CONCENTRATION IN HARD APPLE CIDER

Abstract

Volatile compounds are a crucial component of hard apple (*Malus ×domestica* Borkh.) cider aroma, flavor, and consequent marketability. The biosynthesis of aromatic volatile compounds involves amino acids and ammonium as the main precursors which are used by *Saccharomyces cerevisiae* yeast to produce aldehydes, higher alcohols, volatile acids, sulfates/sulfites, fatty acids, esters, and ethyl esters. The quantity and composition of aromatic compounds is affected by both pre- and postharvest factors. In particular, nitrogen is a key element in the production of volatiles and their precursors; therefore, the application of nitrogen fertilizer has a potential influence on cider aromatics. In this experiment, nitrogen-rich urea fertilizer was applied to hard cider apple trees of the cultivars 'Ellis Bitter' and 'Harry Masters Jersey' to create Control (0 applications), Low (3 weekly applications), and High (5 weekly applications) treatments. Total leaf nitrogen content increased with the number of foliar urea applications, demonstrating plant uptake and distinctions among treatment groups. The total fruit yield and fruit efficiency (total harvested fruit mass relative to trunk cross-sectional area) did not differ among treatments within the same cultivar. Similarly, total phenolics, pH, soluble solids concentration, fruit ripeness, and fruit size did not differ among treatments. Red peel color was reduced with the number of foliar urea applications on average by 5-10% in the Low treatment and 20% in High treatment compared to the Control. Fermentation kinetics, amino acid content, and yeast assimilable nitrogen (YAN) content in juices increased with the number of foliar

urea applications. Relative to the Control, the High treatment increased YAN by 130% in ‘Ellis Bitter’ and 145% in ‘Harry Masters Jersey’. Over 90% of the YAN in all juice samples was composed of primary amino nitrogen (PAN), and the majority of the PAN among all treatments was asparagine. Ester and fatty acid content in hard ciders increased with the number of urea applications; however, higher alcohols showed a mixed relationship with the number of applications in the cultivar ‘Ellis Bitter’. Aroma and flavor differences among treatments were discernable by triangle discrimination test panelists; however, the sensory differences were not easily identifiable with untrained panelists. This study demonstrated that foliar urea applications can provide a management strategy that maintains general tree health while simultaneously increasing YAN and aromatic compounds.

Introduction

The growth of the hard cider (fermented apple juice) industry offers apple growers an opportunity to diversify their orchards to produce low in supply and high in demand hard cider cultivars that can be sold for a premium (Zakalik & Peck, 2023). Continued research is required to optimize hard cider quality, in particular, how pre-harvest orchard management techniques may impact the fruit quality, fermentation kinetics, and sensory attributes of the finished product. Nitrogen fertilization is a standard management practice in apple orchards, since nitrogen is an essential macronutrient for plant metabolism, and its role in the fresh-market apple cultivars has been well-studied (Cheng & Fuchigami, 2002; Merwin & Stiles, 1994; Wargo et al., 2003). In the spring, apple trees utilize nitrogen reserves stored in the woody tissue to support flowering and vegetative growth (Cheng & Raba, 2009). Once there is sufficient leaf area and transpiration, usually around fruit set, apple trees will utilize nitrogen acquired from the soil. Apple leaves can also absorb nitrogen, and in commercial orchards it is common practice to apply foliar nitrogen fertilizer in the form of urea during the late summer and fall to ensure sufficient reserves in the tree for the following spring (Merwin & Stiles, 1994).

Insufficient nitrogen concentration in apple trees has been linked to poor vegetative growth, decreased fruit yield, size, and soluble solid concentrations (Cheng & Fuchigami, 2002; Xia et al., 2009). In contrast, excessive nitrogen decreases red fruit peel coloration, flesh firmness, cold hardiness in buds, and long-term storage quality retention and increases disease susceptibility to apple scab (*Venturia inaequalis*) and fireblight (*Erwinia amylovora*) (Fallahi, 1997; Fallahi et al., 1997; Raese et al., 2007; Rühmann et al., 2002; Stiles & Reid, 1991; Wargo et al., 2003, 2004). Total nitrogen concentration in leaf tissue can be used to estimate overall tree nitrogen status. Target leaf nitrogen concentration for young apple trees is between 2.4 and 2.6% and 2.2 to 2.4% for mature trees when fertilizing fresh market and

processing apple cultivars, but there are no standard nitrogen fertilization guidelines for orchards and cultivars intended for use hard cider (Cheng & Raba, 2009; Cheng & Schupp, 2004; Stiles & Reid, 1991). Understanding nitrogen and its potential influence at all stages of the hard cidermaking process allows producers and growers the opportunity to maximize the quality of their products. Increasing fruit nitrogen content may impact cider fermentation both via changes to yeast assimilable nitrogen content (YAN) and composition of volatile aromatic compounds.

Yeast assimilable nitrogen is composed of primary amino nitrogen (PAN) and ammonia ions and is the source of nitrogen used by *Saccharomyces cerevisiae* and other yeast species during alcoholic fermentation for growth and reproduction (Bell & Henschke, 2005). In winemaking, 140 mg/L YAN is considered the minimum concentration for successful fermentation; apple juice is typically far below this threshold, ranging between 10 to 100 mg/L YAN depending on the cultivar and rootstock (Boudreau IV et al., 2018; Ma et al., 2018; Peck et al., 2016; Tahim & Mansfield, 2019). In addition to slow and or incomplete fermentations, low YAN content during fermentation is known to increase hydrogen sulfide production which is an undesirable compound in wines and ciders (Bell & Henschke, 2005; Boudreau IV et al., 2017; Herraiz & Ough, 1993; Vilanova et al., 2007).

Volatile aromatic compounds exist in extremely low concentrations with a majority of the flavor in fermented beverages coming from odorous volatile components residing in the headspace region above the liquid (Burdock, 2009; Gerhardt, 1990). Hard cider aroma is derived from hundreds of aromatic volatiles, and consumer perception and preference can be significantly affected when there are even in small variations (King et al., 2010). The concentration of a volatile aromatic must be above its absolute threshold (first concentration at which a person can reliably detect an odorant) in order to contribute to cider aroma, but certain aromatic volatiles can mask other aroma components and alter both perceived intensity and identity of a

beverage's aromatic profile (Doty, 2002; Hein et al., 2009). Fatty acids, higher alcohols (6–16% of total cider volatiles), esters (78–92% of total cider volatiles), and carbonyls have been identified as the most important volatile aromatics in hard cider, which are primarily formed during yeast fermentation as secondary metabolites (Rita et al., 2011; Roberto et al., 2005; Vidrih & Hribar, 1999).

Aroma can be perceived by both orthonasal and retronasal olfaction and is one of the most influential attributes of the quality and value of a fruit wine, such as hard cider (Pino & Queris, 2011; Satora et al., 2008). While the dominant perceived aroma of hard cider is broadly defined as "fruity," rarely does hard cider smell of ripe, fresh apples that consumers may have experienced from unfermented apple juices. crisp and tart to sweet and juicy. Subtle earthy or spiced undertones and yeast-like or bread-like notes can vary greatly in ciders and are an indication of fermentation or storage process (Cristea et al., 2019; Qin et al., 2018). Consumer liking of hard cider aromatics is inherently variable due to genetics, physiological effects, and context, but there are often consumer groups of preferences in cider and other alcoholic beverages (Francis & Williamson, 2015). In blind acceptance tests, demographics, consumption experience (i.e., beverage education and diversity of exposures) and frequency, neophobia (aversion to trying new foods), and personality traits play a rather weak role in the separation of consumers, but do influence informed purchase intent (Yegge, 2001). Given that aroma is one of the most value-determining components of a cider and yet perceptions vary greatly among individual consumers, it is crucial for hard cider-producers to both qualitatively and quantitatively understand the formation of volatile aromatics in order to produce a finished cider with the desired aroma (Ye et al., 2014).

Yeast assimilable nitrogen is critical for catalyzing the metabolic pathways of volatile aromatics (Saerens et al., 2010). Precursor ketoacids can originate from yeast glucose metabolism and amino acid catabolism. These precursor amino acids form

aldehydes when carboxylated and higher alcohols when the aldehydes are reduced within the yeast cytosol (Vidrih & Hribar, 1999; Webb & Ingraham, 1963). Any factors that increase sugar metabolism rates—such as fermentation temperature, amino acid concentration, and amino acid composition—promote the synthesis of higher alcohols. Esters are similarly synthesized by enzyme-catalyzed reactions between higher alcohols and volatile fatty acids inside yeast cells (Nordström, 1965). Nitrogen availability in the juice impacts yeast excretion of carbon metabolic byproducts that serve as the precursors to higher alcohol and ester compounds (Garde-Cerdán & Ancín-Azpilicueta, 2008; Hernandez-Orte et al., 2006; Jiménez-Martí et al., 2007). Since ester compounds frequently have the most profound impact on cider aroma, it is desirable to increase YAN content in juice to increase the final concentration of esters (Rollero et al., 2015; Villière et al., 2015).

Both soil and foliar nitrogen fertilization have been found to be effective methods of increasing fruit YAN concentrations in both grapes and apples (Bell & Henschke, 2005; Karl et al., 2020a, 2020b; Neilsen et al., 2010; Plotkowski & Cline, 2021). Foliar urea application in particular has been observed as faster, more convenient to growers, and more effective at increasing YAN than nitrogenous calcium nitrate via soil fertilization for grapes (Fageria et al., 2009; Moss, 2016; Niu et al., 2021). There is minimal information for how to manage hard cider-specific apple cultivars in the US. Thus, the two areas of research interest described within this study include: the potential impact of foliar urea fertilization on tree and fruit physiology, fermentation kinetics, aromatic volatiles, amino acid composition and concentration, and YAN content; and whether different levels of foliar urea fertilization produce sensorily distinct ciders discernable by consumers.

A more detailed understanding of specific aromatic volatile changes and their sensory distinguishability by consumers in hard apple cider would prove beneficial to the hard cider industry. Most notably, traditional hard ciders where aromatics can be

enhanced rather than overwhelmed by adjuncts such in flavored and some mass-market ciders. This research investigated how foliar nitrogen applications to common hard cider varieties impact finished cider's aroma profiles, along with their underlying precursors. The aim of this study was to provide additional and more precise information about how nitrogen applied to apple trees might affect cider quality. The hypothesis of this study is that increased foliar nitrogen application rates would increase juice YAN which would increase cider aromatic volatiles that would be discernable by consumers.

Materials and Methods

Research Site

This experiment took place in 2021 at the Cornell University Agricultural Experiment Station in Ithaca, NY (42.444, -76.463) on Hudson silty clay loam, 6 to 12 percent slopes (Soil Survey Staff, 2022). The two apple (*Malus ×domestica* Borkh.) cultivars investigated were 'Ellis Bitter' and 'Harry Masters Jersey'. Both cultivars were grafted on 'Geneva 11' dwarfing rootstock and planted in 2018. The trees were trained to follow the high-density tall spindle system with 1.2 m between trees and 3.7 m between rows. All trees were visibly healthy during the trial with standard pest control practices used for the region throughout the growing season (Agnello et al., 2021).

Experimental Design

The study was a randomized complete block design with four replications. There were three treatment groups—Control, Low, and High—which differed by the number of urea applications—none, three, and five, respectively. Foliar applications began on 22 July 2021 and ended on 2 September 2021. Urea was applied to the Low treatment at 5, 4, and 3 weeks before harvest and the High treatment at 6, 5, 4, 3, and 2 weeks before harvest. Urea granular fertilizer (The Andersons, Maumee, Ohio) was dissolved in water to a concentration of 10 g/L (4.6 g of N/L) and applied with a Solo 451 backpack mist blower (Newport News, VA). The urea mixture was applied to the point of drip to each tree during each application, and a minimum of two buffer trees were located between independent treatment groups. 'Ellis Bitter' required a greater

volume of spray due to the comparatively larger size of the trees. Three to four trees were within each experimental unit to obtain sufficient fruit yield for the desired fermentation volume. Average wind speeds were no higher than 2.25 m/s during applications to avoid inconsistencies and prevent applications from drifting to far neighboring trees.

Tree/Vegetative Quality: Nitrogen and Carbon Assessment in Leaves, Trunk Measurement, and Return Bloom

Thirty to forty healthy, mid-shoot leaves were sampled randomly across all trees within each experimental unit. Total nitrogen and carbon percentage were measured by the Cornell Nutrient Analysis Laboratory (Ithaca, NY) 6 weeks after harvest for 'Ellis Bitter' and 2 weeks for 'Harry Masters Jersey' by combustion following standard protocols (VarioMax CNS, Elementar Analysensysteme GmbH, Langenselbold, Germany). Trunk circumference of all experimental trees was measured 30 cm above the graft union and utilized in conjunction with the circle area formula to estimate trunk cross sectional area. The number of flower clusters per tree was counted in May 2022 to assess return bloom.

Harvest and Fruit Measurements

Fruit from 'Ellis Bitter' was harvested on 30 Aug 2021 and 'Harry Masters Jersey' was harvested on 21 Sep 2021. For 'Ellis Bitter', mature fruit that had dropped prior to harvest was counted but was not weighed or used in fermentation. 'Harry Masters Jersey' is very susceptible to pre-harvest drop, so dropped fruit was counted and weighed, and then juiced for fermentation.

Ten fruits were sub-sampled from each experimental unit to measure the peel blush percentage, flesh firmness, size, weight, chlorophyll a, and starch pattern index (SPI). Peel blush was visually assessed by estimating the total surface area of red coloration across the peel. Flesh firmness was measured with a penetrometer equipped with an 11.1 mm probe along the equator of both the sun-exposed side and the shaded side of the peeled apples (Güss GS Fruit Texture Analyzer, Strand, South Africa). A Turoni 53500 DA meter (Forli, Italy) was used to measure chlorophyll a content on both the sun-exposed and shaded side of fruits, and SPI was rated on an 8-point scale (1 = 0% starch degradation; 8 = 100% starch degradation) from equatorial cross-sections sprayed with an iodine (2.2 g/L iodine, 8.8 g/L potassium iodide) solution (Sigma-Aldrich, St. Louis, MO) (Blanpied & Silsby, 1992).

Juice Extraction and Fermentation

The fruit harvested from each experimental unit was milled then pressed by hydraulic press using polypropylene cider press cloths (Conway, MA) to extract juice samples by the traditional rack and cloth press method. Two to three buffer fruits of the next experimental unit were milled and discarded when transitioning between experimental units to minimize the amount of flesh residue transferred from the previous milling. Two replicate fermentations were conducted for each experimental unit.

A minimum of 19 L of juice was collected for each fermentation, with the exception of one block from ‘Harry Masters Jersey’ which only produced sufficient juice for fermentation in 1 L Erlenmeyer flasks. The juice extractions were aliquoted

into 3.8 L glass containers and a potassium metabisulfite (Fulkerson Winery, Dundee, NY) solution (100 μL of a 175 g/L solution) was added to each carboy or 1 L flask prior to overnight storage at 17 ºC. SafCider™ AB-1 (Fermentis, Muncy Valley, PA) *Saccharomyces cerevisiae* yeast was inoculated approximately 24 hours later. The yeast was first rehydrated with 100 mL of water at 40 °C, followed by an additional 100 mL of a mixed juice sample at the same temperature. This process lasted for 20 minutes and 5 minutes respectively. Subsequently, 1 mL of this yeast mixture was introduced for every 100 mL volume of a given juice sample. The 3.8 L containers were each fitted with stoppers and fermentation airlocks, stored at 17 ºC, and weighed approximately every 24 hours to track fermentation rate via the loss of mass (mostly from CO_2) from metabolized sugars. When the containers lost less than 1 g per day, the fermentations were considered complete. The fermented juice was then racked from the lees and either bottled for sensory analysis or stored at -80 ºC for chemical analyses.

Juice Chemistry

Juice samples were measured for soluble solids concentration, pH, total phenolic concentration, and primary amino nitrogen concentration (PAN). Soluble solid concentrations were measured by an Atago PAL-1 digital refractometer (Tokyo, Japan); pH was measured by a Metrohm Unitrode pH meter (Herisau, Switzerland); total phenolic concentration was measured using the Folin Ciocalteu method (Singleton and Rossi, 1965) on a Spectramax 384 Plus microplate spectrophotometer and SoftMax Pro 7 Microplate Data Acquisition & Analysis Software (Molecular

Devices, San Jose, CA). Juice samples frozen at -80° C were thawed, vortexed, then centrifuged for 8 min at 500 g. Assay mixtures consisted of 1.5 µL of juice sample, 34.9 µL of water and 90.9 µL of Folin-Ciocalteu reagent [reagents supplied by Sigma-Aldrich (St. Louis, MO)]. Three minutes after addition of Folin-Ciocalteu reagent, 72.7 µL of 7% w/v sodium carbonate buffer was added and then incubated in the dark at room temperature. Reactions took place in Cellistar 96-well microplates (Greiner Bio-One, Monroe, NC, USA). A seven-point standard curve was generated at λ 765nm, with gallic acid from 0-3 g/L. Total polyphenol content was inferred by linear regression from the standard curve.

Primary amino nitrogen was measured by Megazyme Primary Amino Nitrogen and Ammonia (Rapid) spectrophotometric assay kits (Bray, Ireland) using manufacturer specifications. Juice samples, stored at -80° C, were thawed, vortexed, then centrifuged at 1,100 g for 5 minutes. The standard curve was created using serial dilutions of 140 mg N/L as isoleucine to 0, 20, 40, 60, 80, 100, 120, and 140 mg N/L concentrations. Standards were analyzed in duplicate on a Molecular Devices Spectramax 384 Plus spectrophotometer at λ 340 nm, and 3.33 µL sample aliquots were suspended in 200 µL buffer solution and 3.33 µL Milli-Q deionized water. The cells were measured again after 15 min and addition of 6.67 µL o-phthaldialdehyde (OPA). The difference in absorbances was used to calculate PAN content.

Cider Chemistry

The total phenolic concentration and PAN was measured for fermented samples following the same protocol as for the juice samples. The residual H_2S of the

cider samples was measured using a method modified from Jastrzembski et al. (2017). Thirty mL of fermented cider was put into a 250 mL flask with a single-hole stopper fitted with a Kitigawa 120SB H_2S detector tube (Pompton Lakes, New Jersey). Two Alka Seltzer Gold™ (Bayer, Elkhart, Indiana) tablets were placed in flask and immediately sealed with the stopper. The amount of residual H_2S sparged from the carbon dioxide generated by the Alka Seltzer™ tablet was recorded once the tablet had dissolved completely.

Amino Acid Quantification and Characterization

Amino acid concentrations were quantified using a Waters Corporation AccQ-Tag Ultra Derivatization Kit on an Acquity UPLC (Milford, MA) following the protocol of Ma et al. (2018). Juice samples were centrifuged at 3,500 g for 10 min, filtered through PTFE 0.22 μm membrane filters (Micro Solv, Eatontown, NJ) and spiked with an internal standard of L-(+)-norvaline (Acros Organics, NJ) to a final concentration of 2.5 mM. A working standard was made of Waters Amino Acid Hydrolysate Standard and four stock solutions of L–norvaline, L-glutamine, GABA, and L-asparagine (Sigma-Aldrich, St. Louis, MO) dissolved in 0.1 N HCl.
The working standard contained 0.25 mM for L-alanine, L-arginine, L-aspartic acid, L-glutamic acid, L-glycine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tyrosine, and L-valine, and 0.125 mM for L-cysteine. Juice samples and standards were derivatized using an AccQ-Tag Ultra Derivatization Kit following manufacturer instructions to generate amino acid derivatives with stable UV absorbance characters. A Waters

AccQ-Tag Ultra Amino Acid Analysis Column (BEH C18 1.7 µm column) on a Waters H-Class UPLC/PDA system was used. Each run had a total run time of 10 min using the following mobile phases (A-D): A- 100%AccQ-Tag Ultra eluent A concentrate; B- 90:10 water-AccQ-Tag Ultra eluent B; C-100% HPLC-grade water; D- 100% AccQ-Tag Ultra eluent B. Amino acids were detected at λ 260 nm. Empower™ Software was used to integrate and quantify peaks using the ApexTrack function (Waters Corporation, Milford, MA).

Volatile Organic Compound Quantification and Characterization

Samples for volatile quantification and characterization were prepared by adding 2 g of NaCl (Spectrum, New Brunswick, New Jersey) to 5 ml aliquots of each juice or cider sample in individual 20-ml amber SPME glass vial to inactivate enzymatic activity and improve headspace partitioning (Canuti et al., 2009). An internal standard solution of 2-octanol (Spectrum, New Brunswick, New Jersey) was added at a volume of 10 µl to each vial. The vials were then sealed and analyzed using headspace solid-phase microextraction gas chromatography mass spectrometry (HSSPME-GC-MS). The HS-SPME-GC-MS system consisted of a prep and load autosampler (Varian, Palo Alto, California) mounted on an 7890B gas chromatograph with a 5977A mass selective detector (Agilent, Santa Clara, California). The extraction process was modified from a method developed by Hampel et al. (2014). A 65-µm polydimethylsiloxane/divinylbenzene 1-cm solid-phase microextraction (SPME) fiber was used to extract samples (Supelco, Bellefonte, Pennsylvania), and they were preincubated for 15 min at 45° C to ensure consistent temperature. The

SPME fiber was then exposed for 45 min at 45° C in the headspace above the sample, and the vials were agitated at 6 g during extraction. The SPME fiber was desorbed in the inlet at 250° C for 14.7 min, with the inlet in a splitless mode for 2 min [inlet glass liner/SPME direct, 0.75 mm I.D. (Supelco, Bellefonte, Pennsylvania)], after which spit flow was turned on (50 mL/min) for the remainder of the GC-MS run.

All samples were run in two analytical replications and blanks were run after every 5-6 samplings to mitigate potential carryover effects. The quality and consistency of the data were monitored by the area of the internal standards not varying from the mean by more the 20%. Compound identification and confirmation were performed using the NIST MS Search v2.2, NIST 14 Mass Spectral Library Database (Scientific Instrument Services, Ringoes, New Jersey). Confirmation was achieved by comparing mass spectra obtained from the sample with those from the pure standards injected under the same conditions. A selection of identified compounds of interest was then quantified using calibration curves for each compound at five different concentration levels. For compounds for which standards were not available, semi-quantitative analysis was done using the internal standard 2-octanol.

Sensory Evaluation

To source panelists, a recruitment email was sent by the Cornell Sensory Evaluation Center (Ithaca, NY, USA) and through the Cornell Food Science undergraduate and graduate listservs. Digital flyers were distributed in Stocking Hall, Cornell University (Ithaca, NY, USA). The sensory evaluations took place at the Cornell Sensory Evaluation Center in individual booths where participants inputted

their responses digitally on a computer. All participants were untrained, above 21 years of age, and provided informed consent. Ninety-five percent of participants reported consuming hard cider at least monthly. A blind discrimination test and a blind descriptive test were conducted. The discrimination test was three triangle tests differentiating treatments within the same cultivar with open-responses for choice-selection reasoning; 98 participants—58 female, 32 male, and 3 genderqueer/non-conforming—aged 21 to 72, were recruited for this trial. The descriptive test included liking, aftertaste, mouthfeel, aroma, and flavor intensity ratings for all three treatments within the same cultivar. This trial occurred over two sessions across two days and both sessions included a mixture of new and repeat participants. There were 186 unique participants—129 female, 51 male, and 6 genderqueer/non-conforming—aged 21 to 62 years of age. Each participant received a $5 cash reward per session for their participation in this study.

Discrimination Test

In the discrimination test, participants were given three separate triangle tests. In each test, participants were asked to identify which sample was different from the other two identical samples. The order of the triangle tests a participant received was randomly assigned, and each participant only evaluated triangles from one cultivar. Participants were also asked to explain their reasoning for selection after each triangle test. All samples were labeled with a random 3-digit code and were presented to the participants in a randomized order generated by the RedJade program (RedJade Software Solutions, Redwood City, California).

Descriptive Test

Based on results from the discriminatory trial and measured changes in volatiles, a series of sensory descriptors were selected for and used in the questionnaire administered to participants via RedJade. Participants were given one sample from each of the three treatments within the same cultivar and asked to rate the intensity of sensory attributes for each sample on a scale of 0–100, with 0 denoting "none" and 100 denoting "extremely intense." Participants were also asked to rate their overall liking of the samples on a 9-point hedonic scale with 1 representing "disliked extremely" and 9 representing "liked extremely". All samples were labeled with a random 3-digit code and presented to the participants in a randomized order. 'Ellis Bitter' and 'Harry Masters Jersey' ciders were evaluated on separate days; both days included new participants in addition to panelists who participated in both testing days.

Sample Preparation

For all sensory trials, hard cider samples were served at room temperature, covered immediately after pouring, and served in under 20 min. Participants were encouraged to drink water to cleanse their palate in between tastings. Served samples were approximately 30 mL. Samples in the discrimination trial were served in clear polystyrene cups, while the samples in the descriptive trials were served in International Organization for Standardization (ISO) standard size wine glasses.

Statistical Analysis

All statistical analysis was conducted in R (R Core Team, 2022). Data were compared using linear mixed effects models with the number of foliar urea applications as a continuous response variable. The number of treatment applications was included in the models as a fixed effect while block was included as a random effect. All regressions were analyzed as mixed models with a random block term, using the lmer function from the lme4 package (Bates et al., 2021). Principal component analyses and correlation heatmaps were generated using the prcomp, princomp, fviz_elg, and fviz_pca, functions from the factoextra package (Kassambara & Mundt, 2020) and the ggbiplot function from the ggbiplot package (Wickham, 2016). Analysis of variance (ANOVA) was performed for exploratory data analysis to identify the potentially significant features to discriminate the treatments under study. Responses to sensory trial questionnaires were recorded and analyzed using the software RedJade. Standard deviations, ANOVA, and Tukey's honestly significant difference post-hoc tests (Tukey's HSD) were conducted using R; for the ANOVA and Tukey's HSD analyses, the alpha value was established at 0.05.

Results

Fruit and Tree Characteristics

Leaf nitrogen concentration in ‘Ellis Bitter’ was greater than the Control by 20% for the Low treatment, but there was no difference observed in High treatments. High treatment ‘Harry Masters Jersey’ trees had a 27% increase from the Control in leaf nitrogen concentration, but the Low treatment was not different from the Control or High treatment. Leaf carbon concentration differences were negligible. ‘Ellis Bitter’ Low treatment leaves has 2% more carbon than the Control; ‘Harry Masters Jersey’ leaf carbon was 1% greater in the High treatments than the Control. Fruit blush decreased with increasing foliar nitrogen applications in both cultivars (Table 2.1). ‘Ellis Bitter’ Control apples had 18% and 12% more fruit blush than the High and Low treatments, respectively. Control treatment ‘Harry Masters Jersey’ apples had 31% more blush than the High and 18% more than Low treatment apples.

Table 2.1 Leaf nitrogen and carbon concentration and fruit yield measurements of 'Ellis Bitter' and 'Harry Masters Jersey' cultivars after three (Low) and five (High) weekly foliar urea spray treatments in an experiment conducted in 2021 in Ithaca, NY. Values are mean ± standard error (n=4). Mean separation among treatments at $P \leq 0.05$ indicated by different lower-case letters within each column for each cultivar.

Treatment	Leaf Nitrogen (g/kg)	Leaf Carbon (g/kg)	Fruit Blush (%)	TCSA[Z] (cm^2)	Average Fruit Weight (kg)	Yield (Fruit no./Tree)	Crop Density (Fruit no. /TCSA[Z])	Fruit Efficiency (Fruit mass g/TCSA[Z])
Ellis Bitter								
Control	11.0 ± 0.0 **a**	452.7 ± 16.2 **a**	86.8 ± 7.5 **a**	12.3 ± 2.34	0.25 ± 0.02	48.2 ± 10.7 **a**	3.9 ± 0.6 **a**	0.92 ± 1.70 **a**
Low	13.7 ± 1.2 **b**	463.3 ± 3.6 **b**	76.4 ± 10.9 **b**	12.6 ± 3.4	0.25 ± 0.02	59.1 ± 9.8 **b**	4.7 ± 0.3 **b**	1.05 ± 1.07 **b**
High	12.7 ± 1.1 **ab**	447.9 ± 17.3 **ab**	71.6 ± 13.7 **c**	13.7 ± 2.4	0.25 ± 0.01	44.6 ± 13.7 **a**	3.3 ± 0.5 **a**	0.78 ± 1.54 **a**
Harry Masters Jersey								
Control	14.2 ± 1.8 **a**	466.4 ± 1.9 **a**	54.1 ± 4.7 **a**	7.7 ± 1.2 **a**	0.12 ± 0.01	55.5 ± 14.5 **b**	7.2 ± 1.0 **b**	0.29 ± 1.06 **b**
Low	16.4 ± 0.5 **a**	461.3 ± 15.1 **a**	46.8 ± 7.8 **b**	5.7 ± 1.0 **b**	0.12 ± 0.01	42.3 ± 10.2 **a**	7.4 ± 0.7 **b**	0.21 ± 0.94 **b**
High	19.4 ± 1.3 **b**	470.5 ± 7.3 **b**	37.2 ± 6.6 **c**	5.9 ± 2.3 **b**	0.13 ± 0.02	48.3 ± 23.0 **ab**	8.1 ± 0.8 **a**	0.15 ± 0.19 **a**

[Z]TCSA = trunk cross sectional area

Juice Chemistry

Primary amino nitrogen (PAN) did not differ among 'Ellis Bitter' juice treatments (Table 2.2). For 'Harry Masters Jersey', PAN was 96% greater in High treatments compared to the Control. All juice samples contained ammonia and arginine-urea below the assay's minimum threshold for accurate detectability.

Total phenolic concentration in juice differed among 'Ellis Bitter' treatments: Low treatment juices contained 12% less total phenolic concentration than the Control and High treatment juices had 22% more than the Control. Sorbitol, glucose, fructose, and sucrose concentrations did not differ among treatments for 'Harry Masters Jersey' juice. For 'Ellis Bitter' juice glucose and fructose concentrations in the High treatment juices were 25% greater than the Low treatment.

Fermentation Characteristics and Cider Chemistry

Maximum fermentation rates increased with the number of foliar urea applications (Table 2.2). The maximum fermentation rate for the Low and High treatments were 23% and 45% greater than the Control for 'Ellis Bitter' and 21% and 100% greater than the Control for 'Harry Masters Jersey'. All ciders fermented to <1 g/L for the fermentable sugars glucose, fructose, and sucrose. Sorbitol concentration did not change after fermentation. There was no difference among treatments for the residual hydrogen sulfide or total phenolic concentration in the finished cider.

The PAN of 'Ellis Bitter' hard cider samples increased with the number of applications, with over a 179% concentration increase in Low treatments from the Control and nearly a 145% increase in High treatments from the Control (Table 2.2).

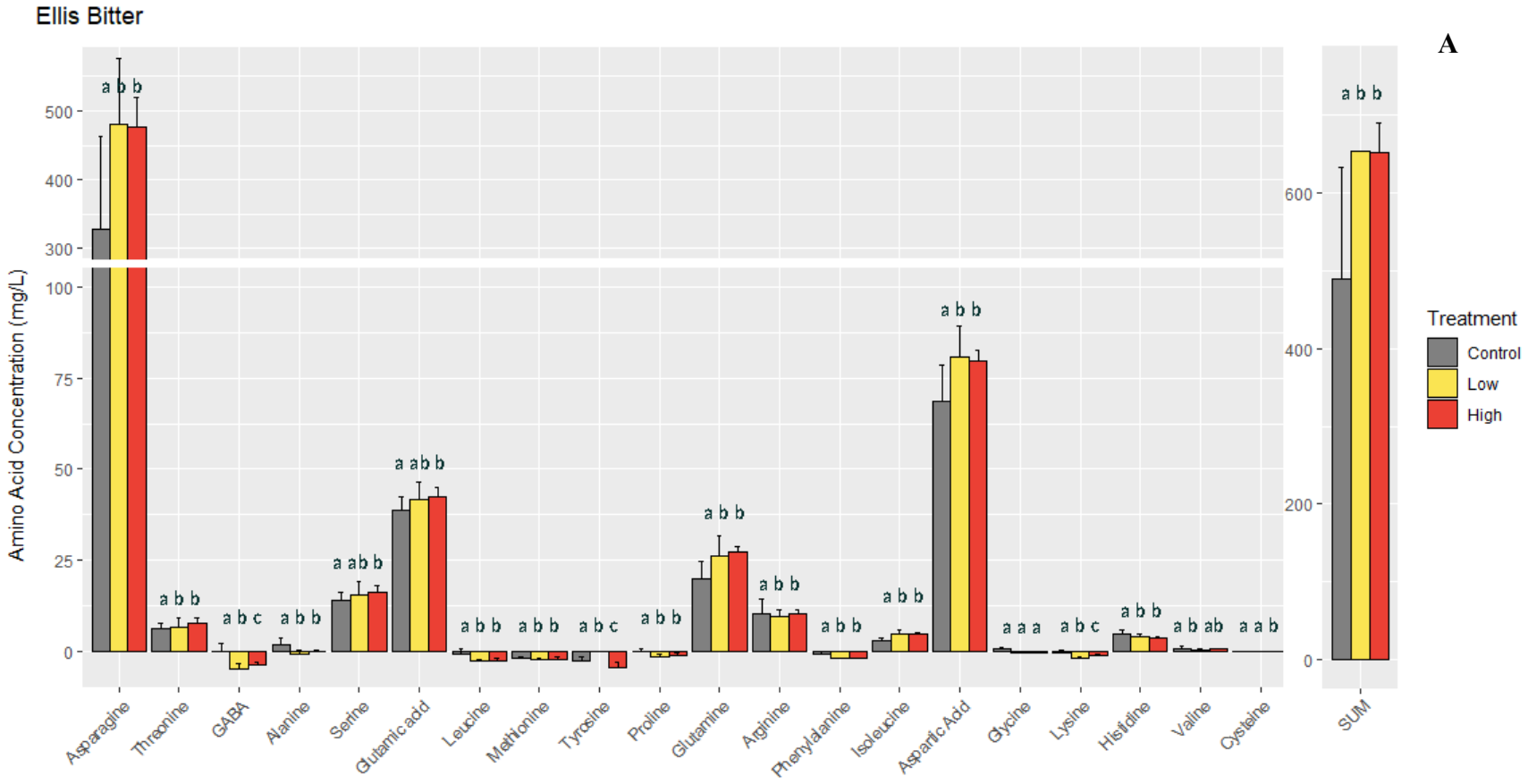
‘Harry Masters Jersey’ demonstrated similar results with a 40% and 130% increase in PAN from the Control for the Low and High treatments, respectively. YAN concentrations from ‘Ellis Bitter’ hard cider samples showed very similar trends to PAN concentrations, with Low treatment ciders 182% and High treatment ciders 149% higher than Control ciders. Ammonia and arginine-urea concentrations did increase with foliar urea fertilization. The PAN-YAN ratio was similar among the treatments, with primary amino nitrogen composing of 94% to 96% of yeast assimilable nitrogen for the ‘Ellis Bitter’ ciders. ‘Harry Masters Jersey’ cider contained ammonia and arginine-urea below the assay’s minimum threshold.

Table 2.2 Juice and cider characteristics of 'Ellis Bitter' and 'Harry Masters Jersey' cultivars after three (Low) and five (High) weekly foliar urea treatments in an experiment conducted in 2021 in Ithaca, NY. Values are mean ± standard error (n=4). Mean separation among treatments at $P \leq 0.05$ indicated by different lower-case letters within each column for each cultivar. PAN = primary amino nitrogen; YAN = yeast assimilable nitrogen; GAE=gallic acid equivalent.

Treatment	Soluble Solids (Juice) (°Brix)	pH (Juice)	Total Polyphenols (Juice) (g GAE/L)	PAN (Juice) (mg N/L)	Total Polyphenols (Cider) (g GAE/L)	PAN (Cider) (mg N/L)	YAN (Cider) (mg N/L)	Residual H_2S (Cider) (mg/L)	Maximum Fermentation Mass Loss Rate (g/L)
Ellis Bitter									
Control	14.0 ± 0.2	3.5 ± 0.0	1.73 ± 0.23 **ab**	54.6 ± 15.3	1.84 ± 0.06	9.0 ± 5.7 **a**	9.4 ± 5.9 **a**	0	12.4 ± 0.0 **a**
Low	14.3 ± 0.1	3.5 ± 0.0	1.52 ± 0.12 **a**	73.7 ± 6.0	1.82 ± 0.05	25.1 ± 7.7 **b**	26.5 ± 7.8 **b**	0	15.2 ± 0.0 **b**
High	14.2 ± 0.3	3.5 ± 0.0	2.11 ± 0.18 **b**	93.0 ± 25.5	1.88 ± 0.08	22.0 ± 7.2 **b**	23.4 ± 7.4 **b**	0	18.0 ± 0.0 **c**
Harry Masters Jersey									
Control	16.7 ± 0.9	3.5 ± 0.0	3.00 ± 0.20	55.4 ± 16.2 **a**	3.06 ± 0.11	13.7 ± 3.9 **a**	13.7 ± 3.9 **a**	0	10.9 ± 0.0 **a**
Low	17.0 ± 0.5	3.5 ± 0.0	3.10 ± 0.14	80.8 ± 16.0 **ab**	3.03 ± 0.10	19.3 ± 7.2 **b**	19.3 ± 7.2 **b**	0	13.2 ± 0.0 **b**
High	16.9 ± 0.7	3.5 ± 0.0	3.03 ± 0.09	108.6 ± 16.2 **b**	2.93 ± 0.09	31.3 ± 3.8 **c**	31.3 ± 3.8 **c**	0	21.8 ± 0.0 **c**

Amino Acid Quantification and Characterization

Asparagine was the most abundant amino acid found in ‘Ellis Bitter’ hard ciders, with GABA and alanine as a distant second and third. For many of the amino acids, the Low treatment ciders had a greater concentration compared to the Control and High, and the amino acid concentration of among all treatments differed with the exception of serine, valine, isoleucine, leucine, and phenylalanine. The Low treatment had the greatest concentration of total amino acids: over 121% greater than the Control and 19% greater than the High treatment ciders. ‘Harry Masters Jersey’ ciders showed similar trends to ‘Ellis Bitter,’ but ciders in the High treatment group had the greatest amino acid concentration total and across a majority of the amino acid groups.


Ellis Bitter
A
Amino Acid Concentration (mg/L)
Treatment
Control
Low
High
Asparagine
Threonine
GABA
Alanine
Serine
Glutamic acid
Leucine
Methionine
Tyrosine
Proline
Glutamine
Arginine
Phenylalanine
Isoleucine
Aspartic Acid
Glycine
Lysine
Histidine
Valine
Cysteine
SUM

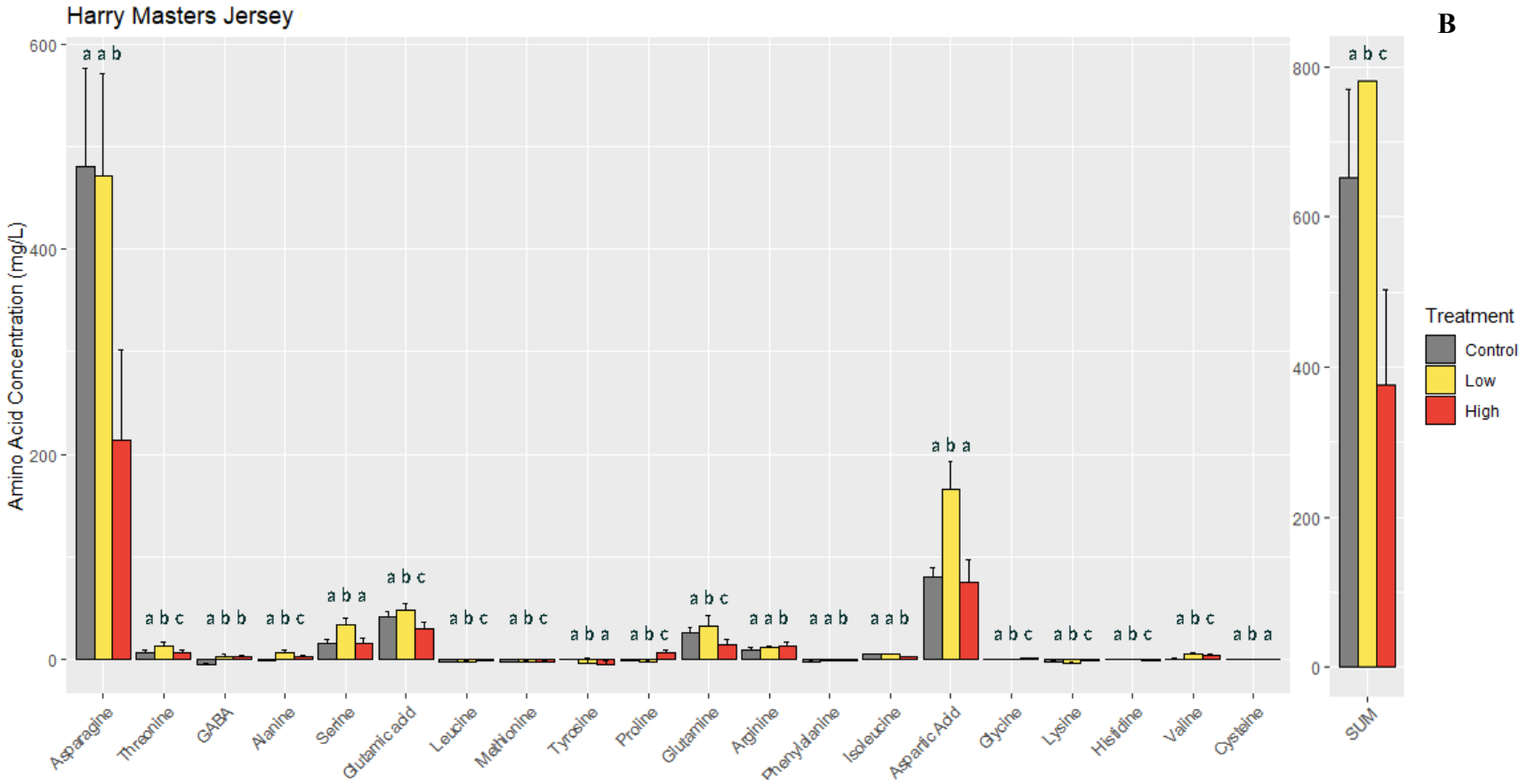


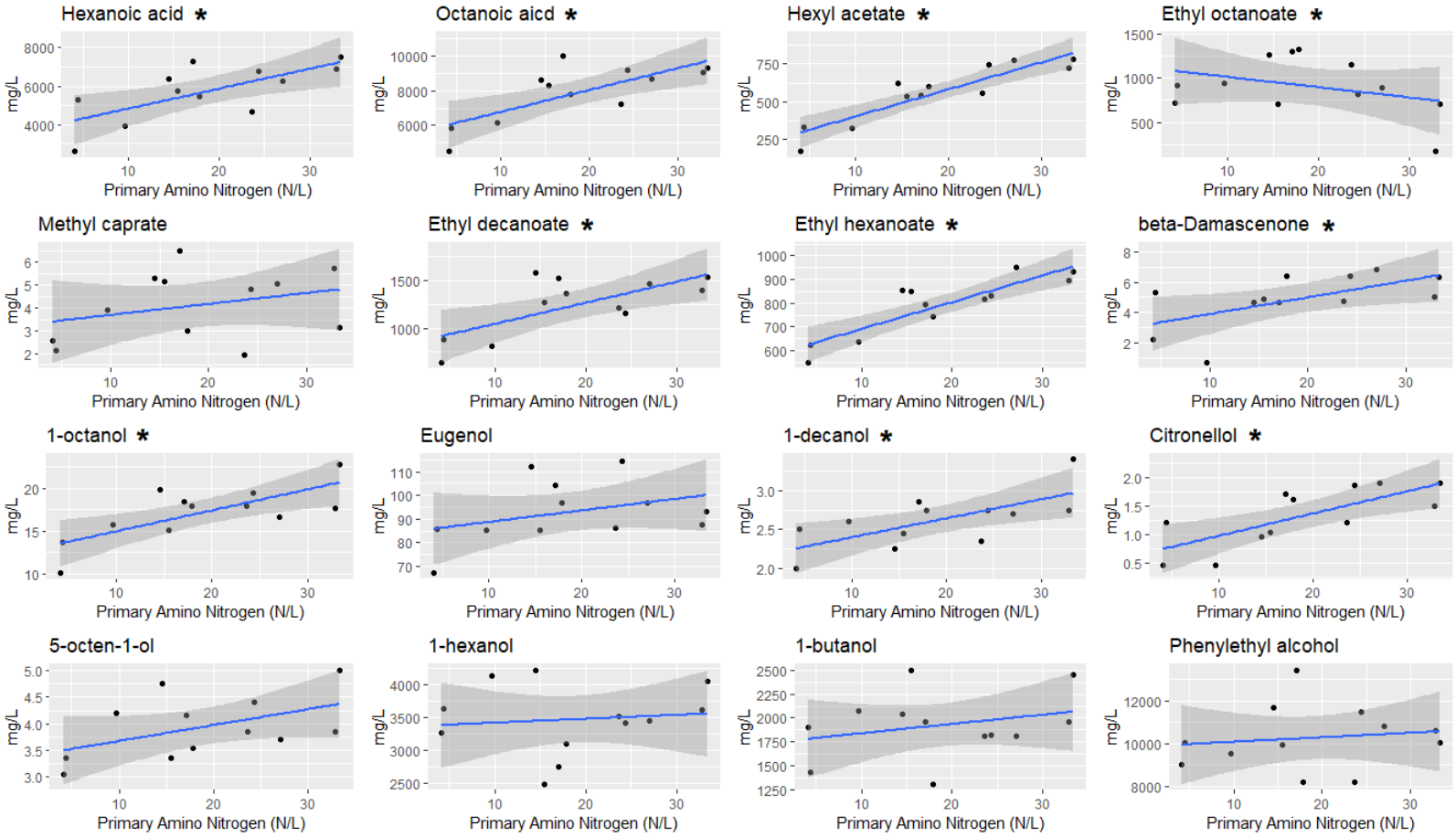
Figure 2.1 The difference in amino acid concentration for 'Ellis Bitter' (A) 'Harry Masters Jersey' (B) from juice to hard cider after three (Low) and five (High) weekly foliar urea treatments in an experiment conducted in 2021 in Ithaca, NY. Values are mean ± standard error (n=4). Mean separation at $P \leq 0.05$ among treatments for each amino acid indicated by lower case letter.

Volatile Organic Compound Quantification and Characterization

A total of 16 volatile organic compounds were identified and quantified from juice and cider samples (Figure 2.2). Of the 16 identified compounds, there were eight higher alcohols, five esters, two acids, and one ketone. Hexyl acetate, ethyl octanoate, and eugenol were identified in 'Ellis Bitter' ciders, but not in 'Harry Masters Jersey'. Median volatile concentrations in cider samples increased with the number of foliar urea applications for 12 of the volatile compounds. Ethyl octanoate, 1-hexanol, 1-butanol, and phenylethyl alcohol concentrations did not change with increased PAN concentration for both cultivars. In juice, volatile concentrations were extremely low in both cultivars, often below the odor detection threshold, and did not differ among treatments. All acids and esters demonstrated a positive correlation with PAN in 'Ellis Bitter' hard ciders with the exception of ethyl octanoate which was negatively correlated. Volatile acid and ester concentrations were also positively correlated in 'Harry Masters Jersey' ciders, but none showed statistical significance. Higher alcohol concentrations across cultivars followed a positive trend but to a weaker degree. Interestingly, 1-butanol and phenylethyl alcohol were not statistically correlated with PAN in 'Ellis Bitter' ciders, but negatively correlated in 'Harry Masters Jersey' samples.

‘Ellis Bitter’

A



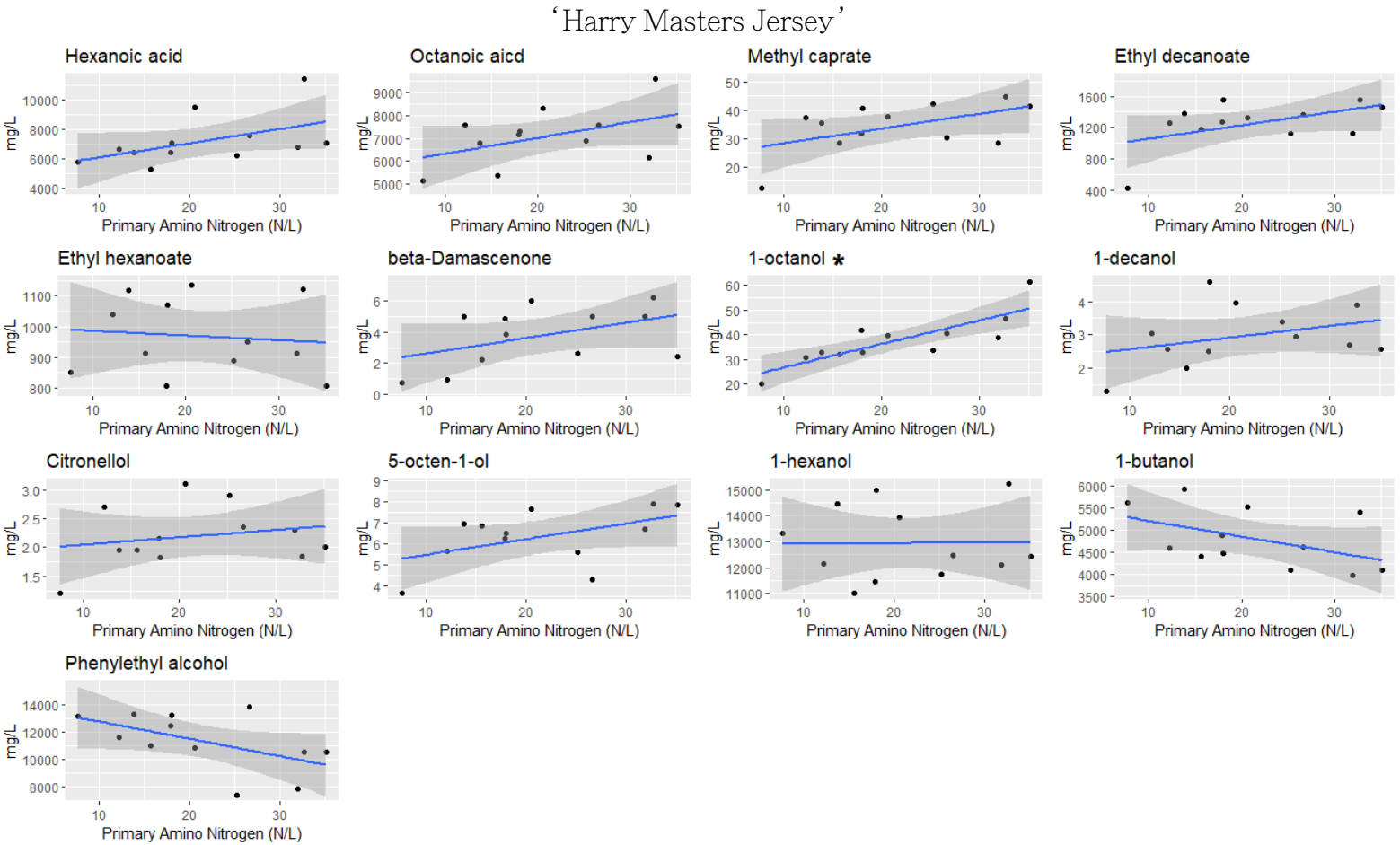


Figure 2.2 Concentration of targeted volatiles with respect to primary amino nitrogen in 'Ellis Bitter' (A) 'Harry Masters Jersey' (B) hard cider samples from an experiment conducted in 2021 in Ithaca, NY. Values are mean ± standard error (n=4). Compounds that had a significant regression ($P \leq 0.05$) are indicated with an asterisk. (*). Shaded band represents 95% confidence interval for the fitted values. Extreme outliers (several hundred magnitudes from the median) were omitted.

Most volatiles, amino acids, PAN concentrations, and leaf nitrogen concentrations were positively correlated with one another (Figure 2.3). The exceptions were norvaline, methionine, tyrosine, cysteine, 1-hexanol, and ethyl octanoate in 'Ellis Bitter' and phenylethyl alcohol and 1-butanol in 'Harry Masters Jersey'. These compounds were either negatively or negligibly correlated with the remaining amino acids and volatiles.

The dimensionality of the amino acid, aromatic volatile, and leaf nitrogen and carbon concentration are reported in Figure 2.4. The first two components of 'Ellis Bitter' represent 89% of the variance, with the Low treatment explaining the greatest set of variables and amino acids making the largest contribution. Histidine, arginine, glycine, aspartic acid, alanine, GABA, proline, and phenylalanine had the strongest correlation to PAN, and the closest relationship with hexanoic acid, octanoic acid, ethyl hexanoate, hexyl acetate, ethyl decanoate, 1-octanol, 1-decanol, citronellol, and beta-damascenone. The first and second components of 'Harry Masters Jersey' represent 76.5% of variance, with the High treatment group explaining the greatest set of variables. One-octanol was strongly correlated to PAN in 'Harry Masters Jersey' but otherwise had similar trends to the 'Ellis Bitter' hard ciders.

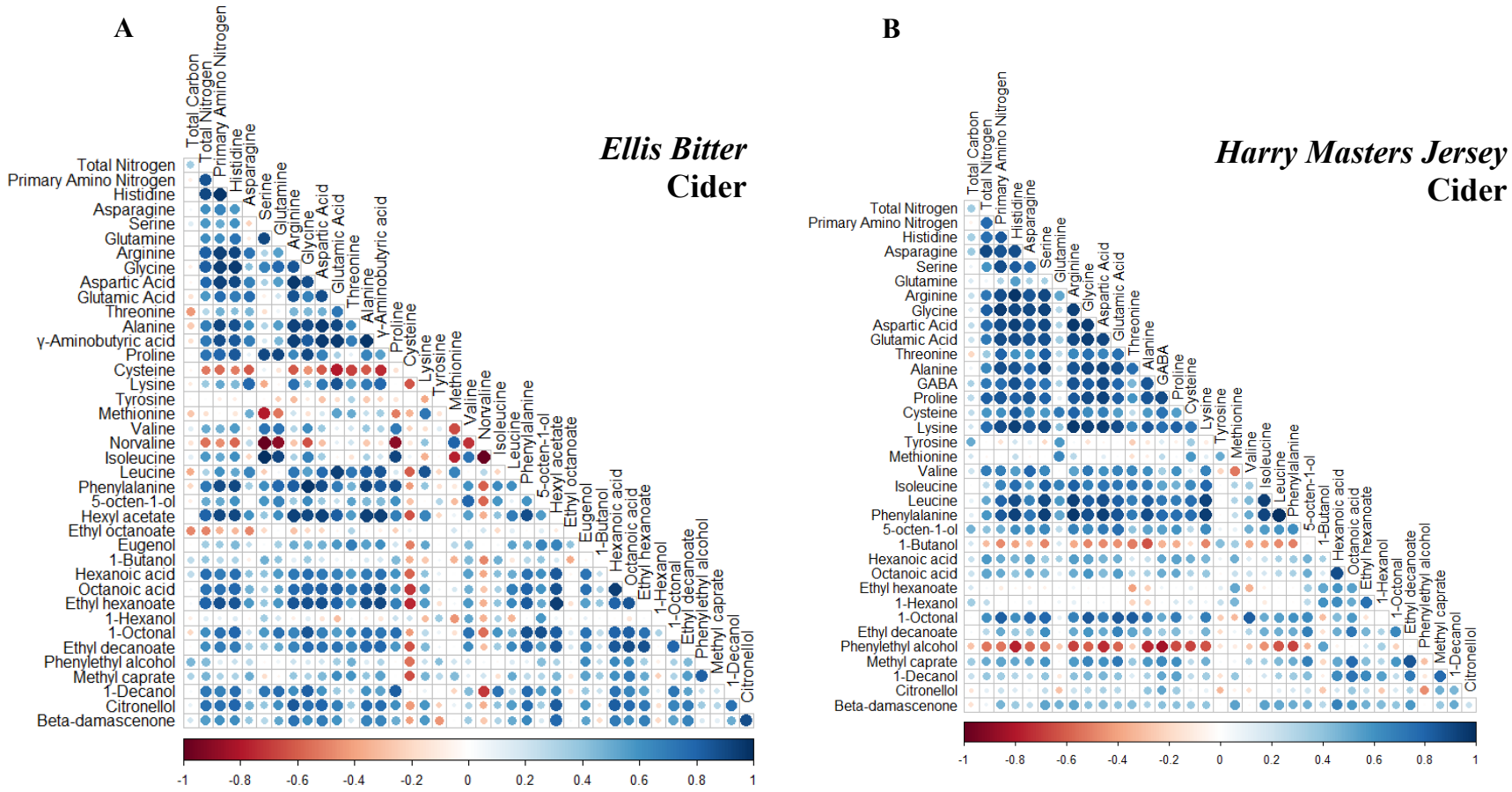
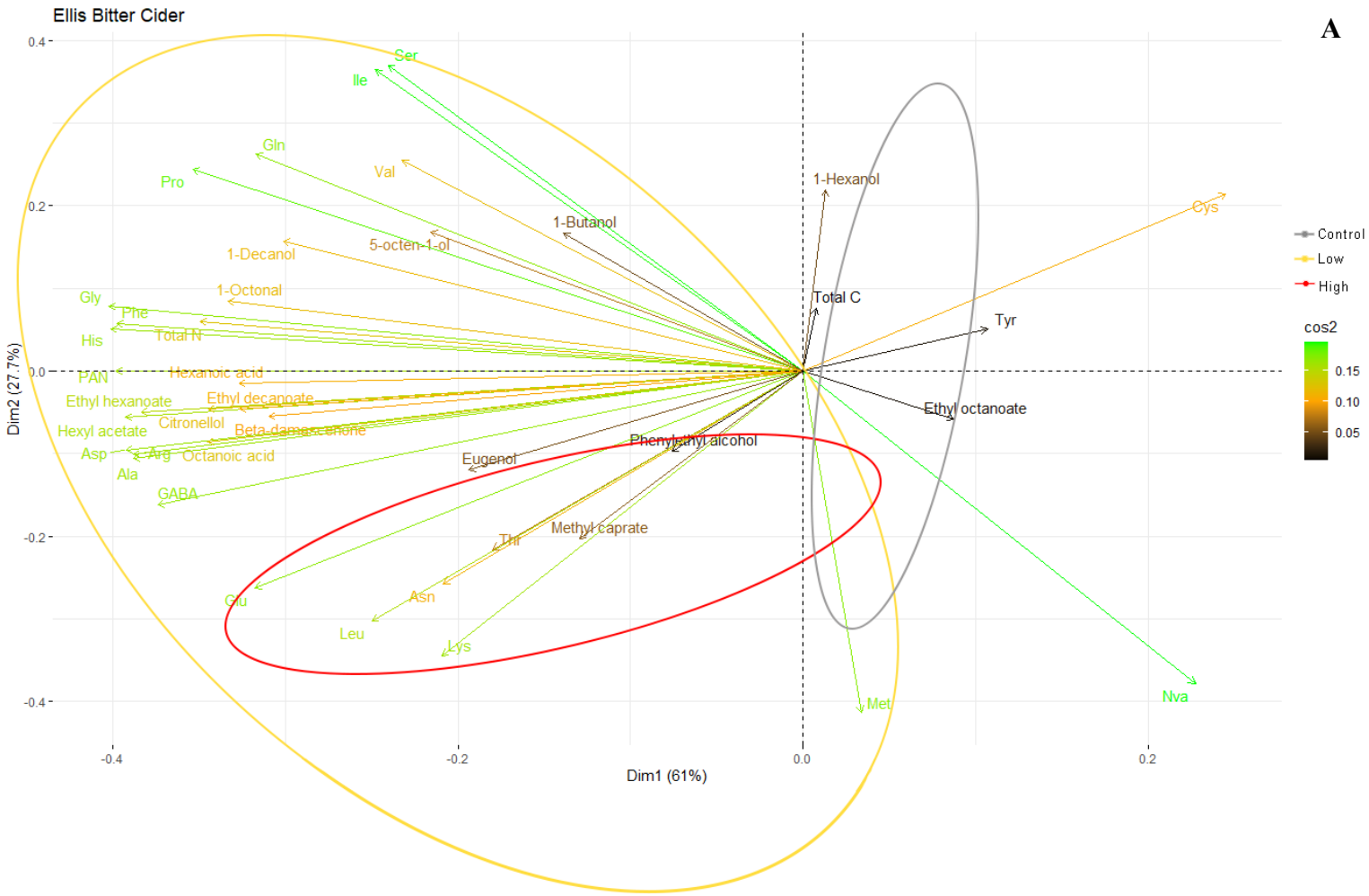


Figure 2.3 Correlation heatmap of aromatic volatile concentration and amino acid concentration for 'Ellis Bitter' (A) 'Harry Masters Jersey' (B) hard cider samples from an experiment conducted in 2021 in Ithaca, NY (n=4). Amino acid data for 'Harry Masters Jersey' hard cider is not shown.


Ellis Bitter Cider
A
Ser
Ile
Gln
Val
Pro
1-Hexanol
Cys
1-Butanol
5-octen-1-ol
1-Decanol
Total C
1-Octonal
Gly
Phe
Tyr
His
Total N
PAN
Hexanoic acid
Ethyl hexanoate
Ethyl decanoate
Ethyl octanoate
Citronellol
Hexyl acetate
Beta-damascenone
Phenylethyl alcohol
Asp
Arg
Octanoic acid
Eugenol
Ala
GABA
Thr
Methyl caprate
Glu
Asn
Leu
Lys
Met
Nva
Control
Low
High
cos2
0.15
0.10
0.05
Dim2 (27.7%)
Dim1 (61%)
0.4
0.2
0.0
-0.2
-0.4

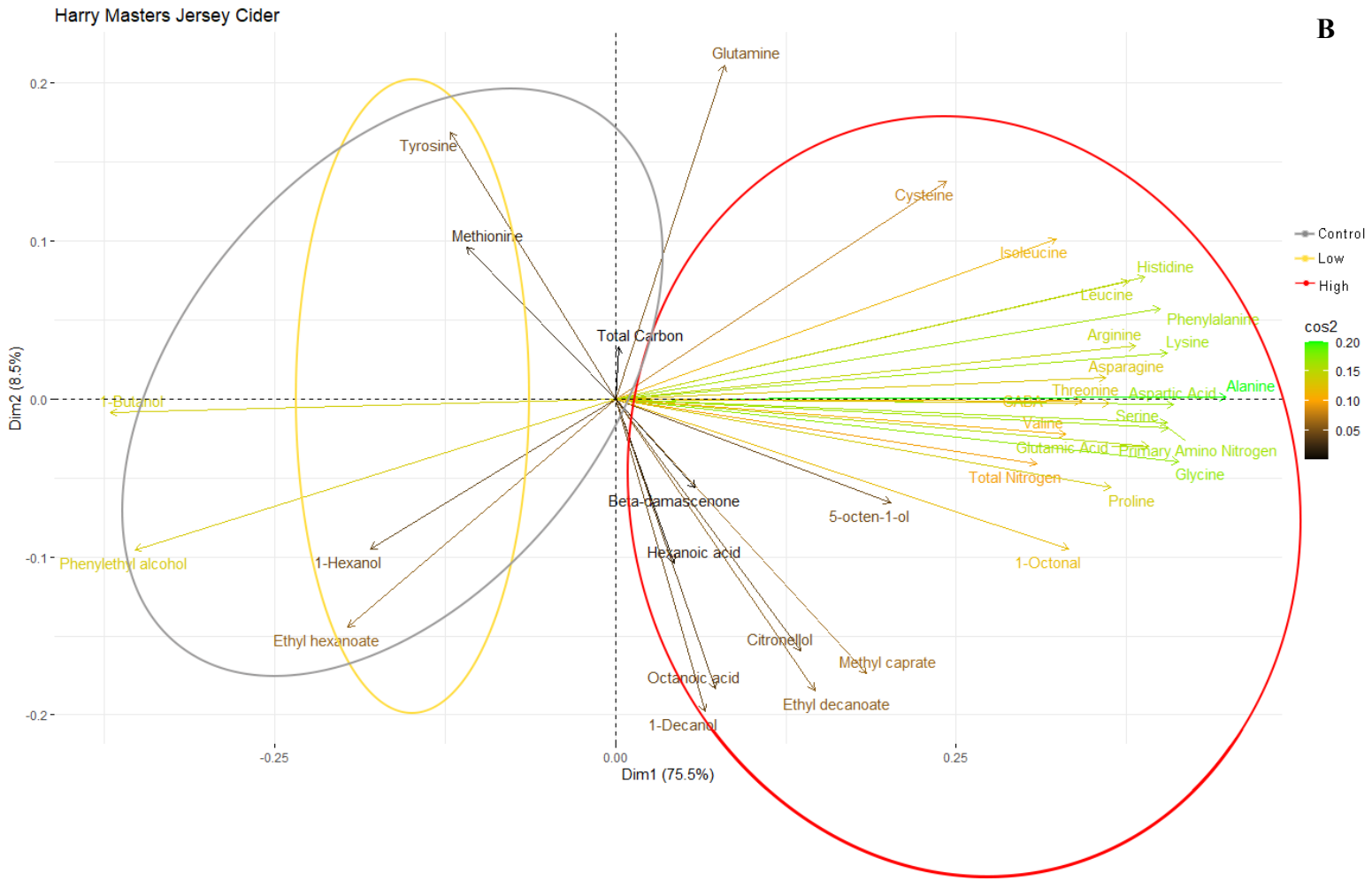


Figure 2.4 Principal component analyses for the concentration of targeted volatiles, leaf carbon and nitrogen concentration, amino acids, and primary amino nitrogen concentration in 'Ellis Bitter' (A) 'Harry Masters Jersey' (B) hard cider samples from an experiment conducted in 2021 in Ithaca, NY (n=4).

Sensory Trials

Within all treatment triangle tests, participants were able to correctly identify the non-matching sample 42% to 56% the time in both cultivars—indicating that treatments were detectable different (Table 2.4). Less than half of 'Ellis Bitter' participants reported a lingering after taste in each treatment, with 26.5% reporting a 'gasoline' lingering retronasal sensations and 14.3% reporting a 'vegetal' lingering retronasal sensations in the Low treatment compared to 12.5% and 4.2% in the Control. Additionally, 'Ellis Bitter' Control treatment ciders scored 50% lower than High treatment cider for rancid aroma intensity, and 16% lower in fruity aroma. 'Harry Masters Jersey' Control ciders were notably different in citrus attributes: Control ciders were 9% less intense than Low ciders for in-mouth citrus aroma and 28% less intense in orthonasal citrus aroma. The 'Harry Masters Jersey' Control ciders also ranked highest for color and appearance liking but ranked the least for overall liking; High treatment ciders were most often ranked most overall preferred.

Table 2.4 Number of sensory study participants (n=100) who correctly identified their hard cider sample triangle discrimination test.

Treatment Triangle	**Percent Correct (%)**	**P-value (One-Tailed)**
Ellis Bitter		
Control + Low	52	0.0049
Control + High	56	0.0008
Low + High	48	0.0222
Harry Masters Jersey		
Control + Low	64	0.0000
Control + High	56	0.0008
Low + High	52	0.0049

Discussion

Increasing the amount of foliar urea applied to the apple trees resulted in an increase in total leaf nitrogen content with more foliar urea applications, but no significant change was observed in several fruit and juice aspects, including total fruit yield, fruit size, fruit ripeness, fruit efficiency, total phenolics, pH, and soluble solids concentration. The study found that as urea applications increased, so did fermentation kinetics, amino acid content, and yeast assimilable nitrogen (YAN) content in the juices. However, the two cultivars responded differently to the two application rates. YAN increased by 130% in 'Ellis Bitter' and 145% in 'Harry Masters Jersey' High treatment juices, compared to the Control juices. The content of ester and fatty acids in both cider cultivars also increased with more urea applications, although the relationship with higher alcohols was inconsistent. Additionally, untrained panelists were able to differentiate aroma and flavor differences from the three treatments. These results indicate that foliar urea applications can serve as a strategy to maintain overall tree health while enhancing YAN and aromatic compounds in hard apple cider.

Increasing leaf nitrogen content with foliar urea applications confirms the efficacy of the treatments, and has been previously reported for hard cider apple trees (Karl et al., 2020a; Plotkowski & Cline, 2021), as well as other apple cultivars (Dong et al., 2005). Foliar urea fertilization is most efficient for optimizing fruit quality during summer months around 6 weeks before harvest since nitrogen uptake during this period is more readily allocated towards fruits as opposed to vegetative tissues or reserves (Amiri et al., 2008; Tan et al., 2021; Wargo et al., 2003). The increasing the number of foliar applications positively impacted ‘Harry Masters Jersey’ yield and

fruit efficiency, but the High treatment resulted in lower fruit yields for ‘Ellis Bitter’. Yield differences were likely an artifact of the crop load thinning process more so than the the urea treatments. Excess nitrogen fertilization has been linked to delayed fruit maturity, decreased red coloration, and reduced flesh firmness (Cheng et al., 2002; Cheng & Fuchigami, 2002). The starch pattern index and chlorophyll a measurements in our study did not indicate significant differences in fruit maturity. Trees that received more urea applications demonstrated a decrease in red peel color, but appearance is not an important attribute for hard cider production. It is uncertain from this study if fruit or tree damage would be evident when reaching higher tree nitrogen content or collecting additional years of data.

Increased foliar urea applications resulted in faster fermentation rates and increased PAN concentrations. The relationship between PAN/YAN and fermentation rate has been well-established in wine and hard cider (Bell & Henschke, 2005; Cairns et al., 2022). Despite urea being an ammonia-based fertilizer, the increases in YAN were almost entirely from PAN rather than inorganic forms. Nitrogen forms are quickly consumed by yeast, leading to deficits later in the fermentation process and potentially increased H_2S production and residual H_2S. Residual H_2S was not found in any of the hard cider treatments. The composition of amino acids was similar to what has been reported in previous research at the stage of fruit development when urea was applied (Karl et al., 2020a; Sugimoto et al., 2011). Asparagine was present in the highest proportion, likely because its metabolism is inactive while many other amino acids, especially aspartic acid, are actively biosynthesized as the fruit approaches maturity (Gomis et al., 1990). Asparagine is used in the transport of nitrogen

molecules, which likely explains the high concentration found during apple growth and maturity (Taiz et al., 2015). The proportion of amino acids was similar across all treatments and cultivars. In a related study on 'CrimsonCrisp®' apple trees, Plotkowski & Cline (2021) examined the effects of early-season urea applications (from petal fall to three to six weeks post petal fall). They found that asparagine and its precursor aspartate were consistently present in the highest concentrations. However, these concentrations varied both yearly and with the number of urea applications. This study, conducted over three consecutive growing seasons, highlighted that the most significant differences arose from the number of urea sprays applied in late summer up to just before harvest. There is evidence from both the present study and others (Karl et al., 2020a, 2020b; Plotkowski & Cline, 2021) that foliar urea nitrogen applications increase total amino acids with proportions favoring higher asparagine with the number of applications. These observations are becoming a repeatable phenomenon, but more research is required to better understand the regulation of amino acid synthesis in apple fruit.

The influence of individual amino acids on the formation of volatile aromatic compounds in hard cider has not been the focus of very many previous studies. Asparagine, aspartate, glutamate, glutamine, and serine are believed to be the greatest contributors to volatile formation since they account for 86 to 95% of the amino acids in juice, and additions of these amino acids have shown to cause changes to both production of volatile compounds and yeast protein synthesis (Eleutério dos Santos et al., 2015). In a study by Santos et al. (2016), individual amino acid additions to apple juices resulted in the increased production of esters with aspartate and asparagine

additions while glutamate produced fewer esters. Serine produced the highest concentration of acetaldehyde, which imparts a pleasantly fruity aroma and is a crucial intermediate for fatty acids, higher alcohols, and esters, but in high concentrations produces typically undesirable aromas and acetic acid (Liu & Pilone, 2000). A mixture of 43% aspartate and 56% glutamate was recommended to maximize the production of esters for hard ciders by Santos et al. (2016). Foliar urea additions did not significantly impact the concentration of serine, but increased glutamate and decreased aspartate concentration in 'Ellis Bitter' hard ciders. It is possible aspartate was utilized for other compounds when YAN was increased since aspartate is a precursor to several other amino acids (threonine, lysine, methionine, isoleucine, and asparagine) and is a metabolically reactive nitrogen donor to numerous aminotransferase reactions (Buchanan et al., 2012).

The production of amino acids within an apple tree is governed by numerous metabolic pathways, each influenced by the availability of nitrogen, the complexity of the synthesis pathways, the availability of precursor molecules and ATP, and the activity of specific enzymes. For instance, glutamine and asparagine are directly synthesized from glutamate and aspartate, respectively, through the addition of an amide group. These amino acids can act as a storage form of nitrogen within the plant and are often the first to increase when nitrogen is abundant. An increase in both glutamine and asparagine was measured with increases to PAN concentration in this study. The added complexity of the plant system versus bacterial and fungal systems comes from the presence of multiple plant-specific isoenzymes that can catalyze the same synthesis reactions, and these isoenzymes are often localized to distinct

subcellular compartments or exist at different developmental stages. Amino acid biosynthesis regulation is not just determining the context of the respective pathway, but also the context of the metabolic network.

The relationship between YAN additions to juice and volatile concentrations has been previously reported in hard cider (Cairns et al., 2022; Carrau et al., 2008; Xu et al., 2022), but urea applications to increase YAN concentrations has not been comprehensively studied. Inorganic YAN additions to juice/must predominately contribute to increases in volatile compounds, most notably ethyl and acetate esters which can been reported to influence sensory perception in wine (Espino-Díaz et al., 2016; Satora et al., 2008; Xu et al., 2022). The relationship between the addition of YAN and the production of higher alcohols has shown to be more complex. Generally, a direct relationship between initial nitrogen content and higher alcohol concentration has been observed when the nitrogen content in a fermentation remained low; however, at moderate to high nitrogen concentrations during fermentation, there is an inverse relationship (Carrau et al., 2008; Jiménez-Martí et al., 2007; Mouret et al., 2014; Rollero et al., 2015; Vilanova et al., 2007, 2012). Rollero (2017) proposed a model suggesting that when nitrogen resources are limited, most free amino acids are catabolized to provide intracellular nitrogen for yeast development. This model was based on a quantitative exploration of changes in valine, leucine, and fermentation aromas in wine samples at 70 mg/L, 250 mg/L, and 425 mg/L nitrogen concentrations, derived from an ammonium-amino acid mixture composed of valine and leucine only. In yeast, α-ketoacids are a key intermediary between central carbon metabolism and nitrogen metabolism. They have been reported as the main precursor acids and

alcohols synthesized through the Ehrlich pathway and to amino acid anabolism (Albers et al., 1996; Hazelwood et al., 2008). α-ketoacids are released during this process, resulting in the moderate production of higher alcohols and removal of surplus α-ketoacids by central carbon metabolism. At higher nitrogen concentrations, there is a greater anabolic demand for amino acids to supplement cell growth and so α-ketoacids are instead directed towards the synthesis of amino acids at the expense of higher alcohol formation (Rollero et al., 2017). This mixed relationship of YAN and higher alcohols has also been previously reported in hard cider (Santos et al., 2016; Xu et al., 2022), but further research on the changes to intracellular α-ketoacid content in response to assimilable nitrogen availability will help to support and strengthen the hypothesis.

Seguinot et al. (2018) observed a decrease in ethyl octanoate with the addition of nitrogen to a synthetic grape juice medium, similar to the observations in our study. The less understood production of ethyl esters in cider fermentations depends on the concentration of acetyl-CoA—a precursor to ethyl esters—within yeast cells. Changes in juice nitrogen availability affect the redox balance in yeast cells which can potentially impact the availability of acetyl-CoA (Bloem et al., 2016).

The discrimination triangle tests indicated strong evidence that the number of foliar urea applications a tree received significantly influenced sensory profile; however, the differences among treatment cannot be fully quantified with untrained panelists. Other studies have found inorganic nitrogen additions to grape an apple juices to significantly impact the perception of fruity aromatics because of increased ester production (Christofi et al., 2022; Ugliano et al., 2010; Xu et al., 2022), but other

changes in sensory perception are cultivar-dependent. The sensory profile of hard cider is the composite of hundreds of volatile and nonvolatile compounds within a matrix, which can impact through effects such as enhancement or masking (Polášková et al., 2008). In the case of 'Harry Masters Jersey' ciders, increased urea application rates significantly enhanced overall liking among participants, but this was not the case for 'Ellis Bitter'. This suggests that urea fertilization impacts sensory characteristics in a way that may be contingent on each cultivar's unique aromatic profile and matrix.

Conclusion

Our study found that applying foliar urea to apple trees increased juice YAN concentration, particularly in the form of PAN, with asparagine as the predominant amino acid. Greater YAN concentration resulted in faster fermentation rates and increased production of most measured esters, fatty acids, and higher alcohols. These compounds are beneficial to hard cider producers hoping to increase fruity aromas. The sensory perception of these changes varied by cultivar, but either improved or did not influence the overall likeability of the cider as YAN increased. Foliar urea applications can benefit hard cider orchards by providing fruit with improved nitrogen content, as well as general tree health. Predicting the composition and concentration of YAN and the resulting aromatic flavor volatile compounds is a difficult but worthwhile area for future research. Commercial cider apple growers would likely benefit from foliar urea applications as a cost-effective method to increase the nitrogen content and aromatic flavor volatile precursors in juices so long as it fits into preexisting management practices.

REFERENCES

Agnello, A., Brown, B., Carroll, J., Cheng, L., Cox, K., Curtis, P., Dunn, A., Helms, M., Robinson, T., & Sosnoskie, L. (2021). 2022 Cornell pest management guidelines for commercial tree fruit production. *Cornell Cooperative Extension.*

Albers, E., Larsson, C., Lid n, G., Niklasson, C., & Gustafsson, L. (1996). Influence of the nitrogen source on *Saccharomyces cerevisiae* anaerobic growth and product formation. *Applied and Environmental Microbiology*, *62*(9), 3187–3195. https://doi.org/10.1128/aem.62.9.3187-3195.1996

Amiri, M. E., Fallahi, E., & Golchin, A. (2008). Influence of foliar and ground fertilization on yield, fruit quality, and soil, leaf, and fruit mineral nutrients in apple. *Journal of Plant Nutrition*, *31*(3), 515–525. https://doi.org/10.1080/01904160801895035

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

Blanpied, G. D., & Silsby, K. J. (1992). *Predicting Harvest Date Windows for Apples*. https://ecommons.cornell.edu/handle/1813/3299

Bloem, A., Sanchez, I., Dequin, S., & Camarasa, C. (2016). Metabolic impact of redox cofactor perturbations on the formation of aroma compounds in Saccharomyces cerevisiae. *Applied and Environmental Microbiology*, *82*(1), 174–183. https://doi.org/10.1128/AEM.02429-15

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

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

Buchanan, B. B., Gruissem, W., & Jones, R. L. (2012). *Biochemistry and Molecular Biology of Plants* (Second edition). Wiley.

Burdock, G. A. (2009). *Fenaroli's Handbook of Flavor Ingredients* (6th ed.). CRC Press. https://doi.org/10.1201/9781439847503

Cairns, P., Hamilton, L., Racine, K., Phetxumphou, K., Ma, S., Lahne, J., Gallagher, D., Huang, H., Moore, A. N., & Stewart, A. C. (2022). Effects of hydroxycinnamates and exogenous yeast assimilable nitrogen on cider aroma

and fermentation performance. *Journal of the American Society of Brewing Chemists*, *80*(3), 236–247. https://doi.org/10.1080/03610470.2021.1968171

Canuti, V., Conversano, M., Calzi, M. L., Heymann, H., Matthews, M. A., & Ebeler, S. E. (2009). Headspace solid-phase microextraction–gas chromatography–mass spectrometry for profiling free volatile compounds in *Cabernet Sauvignon* grapes and wines. *Journal of Chromatography A*, *1216*(15), 3012–3022. https://doi.org/10.1016/j.chroma.2009.01.104

Carrau, F. M., Medina, K., Farina, L., Boido, E., Henschke, P. A., & Dellacassa, E. (2008). Production of fermentation aroma compounds by *Saccharomyces cerevisiae* wine yeasts: Effects of yeast assimilable nitrogen on two model strains. *FEMS Yeast Research*, *8*(7), 1196–1207. https://doi.org/10.1111/j.1567-1364.2008.00412.x

Cheng, L., Dong, S., & Fuchigami, L. H. (2002). Urea uptake and nitrogen mobilization by apple leaves in relation to tree nitrogen status in autumn. *The Journal of Horticultural Science and Biotechnology*, *77*(1), 13–18. https://doi.org/10.1080/14620316.2002.11511449

Cheng, L., & Fuchigami, L. H. (2002). Growth of young apple trees in relation to reserve nitrogen and carbohydrates. *Tree Physiology*, *22*(18), 1297–1303. https://doi.org/10.1093/treephys/22.18.1297

Cheng, L., & Raba, R. (2009). Accumulation of macro- and micronutrients and nitrogen demand-supply relationship of 'Gala'/'Malling 26' apple trees grown in sand culture. *Journal of the American Society for Horticultural Science*, *134*(1), 3–13. https://doi.org/10.21273/JASHS.134.1.3

Cheng, L., & Schupp, J. (2004). *Nitrogen fertilization of apple orchards*.

Christofi, S., Papanikolaou, S., Dimopoulou, M., Terpou, A., Cioroiu, I. B., Cotea, V., & Kallithraka, S. (2022). Effect of yeast assimilable nitrogen content on fermentation kinetics, wine chemical composition and sensory character in the production of *Assyrtiko* wines. *Applied Sciences*, *12*(3), Article 3. https://doi.org/10.3390/app12031405

Cristea, G., Voica, C., Feher, I., Radu, S., & Magdas, D. A. (2019). Isotopic and elemental characterization of cider commercialized on Romanian market. *Analytical Letters*, *52*(1), 139–149. https://doi.org/10.1080/00032719.2018.1434189

Dong, S., Cheng, L., Scagel, C. F., & Fuchigami, L. H. (2005). Method of nitrogen application in summer affects plant growth and nitrogen uptake in autumn in young *Fuji*/M.26 apple trees. *Communications in Soil Science and Plant Analysis*, *36*(11–12), 1465–1477. https://doi.org/10.1081/CSS-200058491

Doty, R. L. (2002). Olfaction. In V. S. Ramachandran (Ed.), *Encyclopedia of the Human Brain* (pp. 717–727). Academic Press. https://doi.org/10.1016/B0-12-227210-2/00259-4

Eleutério dos Santos, C. M., Pietrowski, G. de A. M., Braga, C. M., Rossi, M. J., Ninow, J., Machado dos Santos, T. P., Wosiacki, G., Jorge, R. M. M., &

Nogueira, A. (2015). Apple aminoacid profile and yeast strains in the formation of fusel alcohols and esters in cider production. *Journal of Food Science*, *80*(6), C1170–C1177. https://doi.org/10.1111/1750-3841.12879

Espino-Díaz, M., Sepúlveda, D. R., González-Aguilar, G., & Olivas, G. I. (2016). Biochemistry of apple aroma: A review. *Food Technology and Biotechnology*, *54*(4), 375–397. https://doi.org/10.17113/ftb.54.04.16.4248

Fageria, N. K., Filho, M. P. B., Moreira, A., & Guimarães, C. M. (2009). Foliar fertilization of crop plants. *Journal of Plant Nutrition*, *32*(6), 1044–1064. https://doi.org/10.1080/01904160902872826

Fallahi, E. (1997). Preharvest nitrogen optimization for maximizing yield and postharvest fruit quality of apples. *Acta Horticulturae*, *448*, 415–420. https://doi.org/10.17660/ActaHortic.1997.448.77

Fallahi, E., Conway, W. S., Hickey, K. D., & Sams, C. E. (1997). The role of calcium and nitrogen in postharvest quality and disease resistance of apples. *HortScience*, *32*(5), 831–835. https://doi.org/10.21273/HORTSCI.32.5.831

Francis, L. l., & Williamson, P. O. (2015). Application of consumer sensory science in wine research. *Australian Journal of Grape and Wine Research*, *21*(S1), 554–567. https://doi.org/10.1111/ajgw.12169

Garde-Cerdán, T., & Ancín-Azpilicueta, C. (2008). Effect of the addition of different quantities of amino acids to nitrogen-deficient must on the formation of esters, alcohols, and acids during wine alcoholic fermentation. *LWT - Food Science and Technology*, *41*(3), 501–510. https://doi.org/10.1016/j.lwt.2007.03.018

Gerhardt, K. O. (1990). Gas chromatography—Mass spectrometry. In M. H. Gordon (Ed.), *Principles and Applications of Gas Chromatography in Food Analysis* (pp. 59–85). Springer US. https://doi.org/10.1007/978-1-4613-0681-8_2

Gomis, D. B., Lobo, A. M. P., Alvarez, M. D. G., & Alonso, J. J. M. (1990). Determination of amino acids in apple extracts by high performance liquid chromatography. *Chromatographia*, *29*(3), 155–160. https://doi.org/10.1007/BF02268703

Hampel, D., Robinson, A. L., Johnson, A. J., & Ebeler, S. E. (2014). Direct hydrolysis and analysis of glycosidically bound aroma compounds in grapes and wines: Comparison of hydrolysis conditions and sample preparation methods. *Australian Journal of Grape and Wine Research*, *20*(3), 361–377. https://doi.org/10.1111/ajgw.12087

Hazelwood, L. A., Daran, J. M., Van Maris, A. J. A., Pronk, J. T., & Dickinson, J. R. (2008). The Ehrlich pathway for fusel alcohol production: A century of research on *Saccharomyces cerevisiae* metabolism. *Applied and Environmental Microbiology*, *74*(8), 2259–2266. Scopus. https://doi.org/10.1128/AEM.02625-07

Hein, K., Ebeler, S. E., & Heymann, H. (2009). Perception of fruity and vegetative aromas in red wine. *Journal of Sensory Studies*, *24*(3), 441–455. https://doi.org/10.1111/j.1745-459X.2009.00220.x

Hernandez-Orte, P., Bely, M., Cacho, J., & Ferreira, V. (2006). Impact of ammonium additions on volatile acidity, ethanol, and aromatic compound production by different *Saccharomyces cerevisiae* strains during fermentation in controlled synthetic media. *Australian Journal of Grape and Wine Research*, *12*(2), 150–160. https://doi.org/10.1111/j.1755-0238.2006.tb00055.x

Herraiz, T., & Ough, C. S. (1993). Formation of ethyl esters of amino acids by yeasts during the alcoholic fermentation of grape juice. *American Journal of Enology and Viticulture*, *44*(1), 41–48. https://doi.org/10.5344/ajev.1993.44.1.41

Jiménez-Martí, E., Aranda, A., Mendes-Ferreira, A., Mendes-Faia, A., & del Olmo, M. lí. (2007). The nature of the nitrogen source added to nitrogen depleted vinifications conducted by a *Saccharomyces cerevisiae* strain in synthetic must affects gene expression and the levels of several volatile compounds. *Antonie Van Leeuwenhoek*, *92*, 61–75. https://doi.org/10.1007/s10482-006-9135-1

Karl, A. D., Brown, M. G., Ma, S., Sandbrook, A., Stewart, A. C., Cheng, L., Mansfield, A. K., & Peck, G. M. (2020a). Foliar urea applications increase yeast assimilable nitrogen concentration and alcoholic fermentation rate in 'Red Spy' apples used for cider production. *HortScience*, *55*(8), 1356–1364. https://doi.org/10.21273/HORTSCI15029-20

Karl, A. D., Brown, M. G., Ma, S., Sandbrook, A., Stewart, A. C., Cheng, L., Mansfield, A. K., & Peck, G. M. (2020b). Soil nitrogen fertilization increases yeast assimilable nitrogen concentrations in 'Golden Russet' and 'Medaille D'or' apples used for cider production. *HortScience*, *55*(8), 1345–1355. https://doi.org/10.21273/HORTSCI15028-20

King, E. S., Kievit, R. L., Curtin, C., Swiegers, J. H., Pretorius, I. S., Bastian, S. E. P., & Leigh Francis, I. (2010). The effect of multiple yeasts co-inoculations on *Sauvignon Blanc* wine aroma composition, sensory properties and consumer preference. *Food Chemistry*, *122*(3), 618–626. https://doi.org/10.1016/j.foodchem.2010.03.021

Liu, S.-Q., & Pilone, G. J. (2000). An overview of formation and roles of acetaldehyde in winemaking with emphasis on microbiological implications. *International Journal of Food Science & Technology*, *35*(1), 49–61. https://doi.org/10.1046/j.1365-2621.2000.00341.x

Lorenzini, M., Simonato, B., Slaghenaufi, D., Ugliano, M., & Zapparoli, G. (2019). Assessment of yeasts for apple juice fermentation and production of cider volatile compounds. *LWT*, *99*, 224–230. https://doi.org/10.1016/j.lwt.2018.09.075

Ma, S., Neilson, A. P., Lahne, J., Peck, G. M., O'Keefe, S. F., & Stewart, A. C. (2018). Free amino acid composition of apple juices with potential for cider making as determined by UPLC-PDA. *Journal of the Institute of Brewing*, *124*(4), 467–476. https://doi.org/10.1002/jib.519

Merwin, I. A., & Stiles, W. C. (1994). Orchard groundcover management impacts on apple tree growth and yield, and nutrient availability and uptake. *Journal of the American Society for Horticultural Science*, *119*(2), 209–215.

Moss, J. R. (2016). *Evaluation of nitrogen management schemes in cover cropped vineyards* [Thesis, Virginia Tech]. https://vtechworks.lib.vt.edu/handle/10919/80510

Mouret, J. R., Camarasa, C., Angenieux, M., Aguera, E., Perez, M., Farines, V., & Sablayrolles, J.-M. (2014). Kinetic analysis and gas–liquid balances of the production of fermentative aromas during winemaking fermentations: Effect of assimilable nitrogen and temperature. *Food Research International*, *62*, 1–10. https://doi.org/10.1016/j.foodres.2014.02.044

Neilsen, G. H., Neilsen, D., Bowen, P., Bogdanoff, C., & Usher, K. (2010). Effect of timing, rate, and form of N fertilization on nutrition, vigor, yield, and berry yeast-assimilable N of grape. *American Journal of Enology and Viticulture*, *61*(3), 327–336. https://doi.org/10.5344/ajev.2010.61.3.327

Niu, J., Liu, C., Huang, M., Liu, K., & Yan, D. (2021). Effects of foliar fertilization: A review of current status and future perspectives. *Journal of Soil Science and Plant Nutrition*, *21*(1), 104–118. https://doi.org/10.1007/s42729-020-00346-3

Nordström, K. (1965). Possible control of volatile ester formation in brewing. *Proceedings of the 10th Congress of the European Brewing Convention*, *10*, 195–208. https://doi.org/10.1111/j.1751-7915.2009.00106.x

Peck, G., & Knickerbocker, W. (2018). Economic Case Studies of Cider Apple Orchards in New York State. . . *NUMBER*, *26*(3), 6.

Peck, G., McGuire, M., Boudreau, T., & Stewart, A. (2016). Crop load density affects 'York' apple juice and hard cider quality. *HortScience*, *51*(9), 1098–1102. https://doi.org/10.21273/HORTSCI10962-16

Pino, J. A., & Queris, O. (2011). Analysis of volatile compounds of mango wine. *Food Chemistry*, *125*(4), 1141–1146. https://doi.org/10.1016/j.foodchem.2010.09.056

Plotkowski, D. J., & Cline, J. A. (2021). Seasonal and postharvest changes in amino acid composition in 'Crimson Crisp' apple (*Malus Domestica* Borkh.) in response to summer foliar urea applications. *HortScience*, *56*(9), 1041–1052. https://doi.org/10.21273/HORTSCI15982-21

Polášková, P., Herszage, J., & E. Ebeler, S. (2008). Wine flavor: Chemistry in a glass. *Chemical Society Reviews*, *37*(11), 2478–2489. https://doi.org/10.1039/B714455P

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

Raese, J. T., Drake, S. R., & Curry, E. A. (2007). Nitrogen fertilizer influences fruit quality, soil nutrients and cover crops, leaf color and nitrogen content, biennial bearing and cold hardiness of 'Golden Delicious.' *Journal of Plant Nutrition*, *30*(10), 1585–1604. https://doi.org/10.1080/01904160701615483

Rita, R.-D., Zanda, K., Daina, K., & Dalija, S. (2011). Composition of aroma compounds in fermented apple juice: Effect of apple variety, fermentation temperature and inoculated yeast concentration. *Procedia Food Science*, *1*, 1709–1716. https://doi.org/10.1016/j.profoo.2011.09.252

Roberto, R. M., García, N. P., Hevia, A. G., & Valles, B. S. (2005). Application of purge and trap extraction and gas chromatography for determination of minor esters in cider. *Journal of Chromatography A*, *1069*(2), 245–251. https://doi.org/10.1016/j.chroma.2005.02.019

Rollero, S., Bloem, A., Camarasa, C., Sanchez, I., Ortiz-Julien, A., Sablayrolles, J.-M., Dequin, S., & Mouret, J.-R. (2015). Combined effects of nutrients and temperature on the production of fermentative aromas by *Saccharomyces cerevisiae* during wine fermentation. *Applied Microbiology and Biotechnology*, *99*, 2291–2304. https://doi.org/10.1007/s00253-014-6210-9

Rollero, S., Mouret, J.-R., Bloem, A., Sanchez, I., Ortiz-Julien, A., Sablayrolles, J.-M., Dequin, S., & Camarasa, C. (2017). Quantitative 13C-isotope labelling-based analysis to elucidate the influence of environmental parameters on the production of fermentative aromas during wine fermentation. *Microbial Biotechnology*, *10*(6), 1649–1662. https://doi.org/10.1111/1751-7915.12749

Romano, P., Suzzi, G., Comi, G., & Zironi, R. (1992). Higher alcohol and acetic acid production by apiculate wine yeasts. *Journal of Applied Bacteriology*, *73*(2), 126–130. https://doi.org/10.1111/j.1365-2672.1992.tb01698.x

Rühmann, S., Leser, C., Bannert, M., & Treutter, D. (2002). Relationship between growth, secondary metabolism, and resistance of apple. *Plant Biology*, *4*(2), 137–143. https://doi.org/10.1055/s-2002-25727

Saerens, S. M. G., Delvaux, F. R., Verstrepen, K. J., & Thevelein, J. M. (2010). Production and biological function of volatile esters in *Saccharomyces cerevisiae*. *Microbial Biotechnology*, *3*(2), 165–177. https://doi.org/10.1111/j.1751-7915.2009.00106.x

Santos, C. M. E. dos, Alberti, A., Pietrowski, G. de A. M., Zielinski, A. A. F., Wosiacki, G., Nogueira, A., & Jorge, R. M. M. (2016). Supplementation of amino acids in apple must for the standardization of volatile compounds in ciders. *Journal of the Institute of Brewing*, *122*(2), 334–341. https://doi.org/10.1002/jib.318

Satora, P., Sroka, P., Duda-Chodak, A., Tarko, T., & Tuszyński, T. (2008). The profile of volatile compounds and polyphenols in wines produced from dessert varieties of apples. *Food Chemistry*, *111*(2), 513–519. https://doi.org/10.1016/j.foodchem.2008.04.007

Seguinot, P., Rollero, S., Sanchez, I., Sablayrolles, J.-M., Ortiz-Julien, A., Camarasa, C., & Mouret, J.-R. (2018). Impact of the timing and the nature of nitrogen additions on the production kinetics of fermentative aromas by *Saccharomyces cerevisiae* during winemaking fermentation in synthetic media. *Food Microbiology*, *76*, 29–39. https://doi.org/10.1016/j.fm.2018.04.005

Soil Survey Staff. (2022). *Keys to Soil Taxonomy, 13th ed*. USDA-Natural Resources Conservation Service.

Stiles, W. C., & Reid, W. S. (1991). Orchard nutrition management. *Cornell Cooperative Extension*, *Information Bulletin 219. Ithaca, NY*. https://ecommons.cornell.edu/handle/1813/3305

Sugimoto, N., Jones, A. D., & Beaudry, R. (2011). Changes in free amino acid content in 'Jonagold' apple fruit as related to branched-chain ester production, ripening, and senescence. *Journal of the American Society for Horticultural Science*, *136*(6), 429–440. https://doi.org/10.21273/JASHS.136.6.429

Tahim, C. M., & Mansfield, A. K. (2019). Yeast assimilable nitrogen optimization for cool-climate *Riesling*. *American Journal of Enology and Viticulture*, *70*(2), 127–138. https://doi.org/10.5344/ajev.2018.17087

Taiz, L., Zeiger, E., Møller, I. M., & Murphy, A. (2015). Plant physiology and development. *Plant Physiology and Development.*, *Ed. 6*. https://www.cabdirect.org/cabdirect/abstract/20173165866

Tan, B. Z., Close, D. C., Quin, P. R., & Swarts, N. D. (2021). Nitrogen use efficiency, allocation, and remobilization in apple trees: Uptake is optimized with pre-harvest N supply. *Frontiers in Plant Science*, *12*. https://www.frontiersin.org/articles/10.3389/fpls.2021.657070

Ugliano, M., Travis, B., Francis, I. L., & Henschke, P. A. (2010). Volatile composition and sensory properties of *Shiraz* wines as affected by nitrogen supplementation and yeast species: Rationalizing nitrogen modulation of wine aroma. *Journal of Agricultural and Food Chemistry*, *58*(23), 12417–12425. https://doi.org/10.1021/jf1027137

Vidrih, R., & Hribar, J. (1999). Synthesis of higher alcohols during cider processing. *Food Chemistry*, *67*(3), 287–294. https://doi.org/10.1016/S0308-8146(99)00136-3

Vilanova, M., Siebert, T. E., Varela, C., Pretorius, I. S., & Henschke, P. A. (2012). Effect of ammonium nitrogen supplementation of grape juice on wine volatiles and non-volatiles composition of the aromatic grape variety *Albariño*. *Food Chemistry*, *133*(1), 124–131. https://doi.org/10.1016/j.foodchem.2011.12.082

Vilanova, M., Ugliano, M., Varela, C., Siebert, T., Pretorius, I. S., & Henschke, P. A. (2007). Assimilable nitrogen utilisation and production of volatile and non-volatile compounds in chemically defined medium by <i>Saccharomyces cerevisiae<i> wine yeasts. *Applied Microbiology and Biotechnology*, *77*(1), 145–157. https://doi.org/10.1007/s00253-007-1145-z

Villière, A., Arvisenet, G., Bauduin, R., Quéré, J.-M. L., & Sérot, T. (2015). Influence of cider-making process parameters on the odourant volatile composition of hard ciders. *Journal of the Institute of Brewing*, *121*(1), 95–105. https://doi.org/10.1002/jib.197

Wargo, J. M., Merwin, I. A., & Watkins, C. B. (2003). Fruit size, yield, and market value of `Goldrush' apple are affected by amount, timing and method of

nitrogen fertilization. *HortTechnology*, *13*(1), 153–161. https://doi.org/10.21273/HORTTECH.13.1.0153

Wargo, J. M., Merwin, I. A., & Watkins, C. B. (2004). Nitrogen fertilization, midsummer trunk girdling, and avg treatments affect maturity and quality of `Jonagold' apples. *HortScience*, *39*(3), 493–500. https://doi.org/10.21273/HORTSCI.39.3.493

Webb, A. D., & Ingraham, J. L. (1963). Fusel oil. In W. W. Umbreit (Ed.), *Advances in Applied Microbiology* (Vol. 5, pp. 317–353). Academic Press. https://doi.org/10.1016/S0065-2164(08)70014-5

Xia, G., Cheng, L., Lakso, A., & Goffinet, M. (2009). Effects of nitrogen supply on source-sink balance and fruit size of 'Gala' apple trees. *Journal of the American Society for Horticultural Science*, *134*(1), 126–133. https://doi.org/10.21273/JASHS.134.1.126

Xu, J., Guo, L., Wang, T., Ma, M., Wang, B., Wei, X., & Fan, M. (2022). Effect of inorganic and organic nitrogen supplementation on volatile components and aroma profile of cider. *Food Research International*, *161*, 111765. https://doi.org/10.1016/j.foodres.2022.111765

Yair, M. (1997). *Concepts in wine chemistry* (pp. 23–24). The wine appreciation guild.

Ye, M., Yue, T., & Yuan, Y. (2014). Changes in the profile of volatile compounds and amino acids during cider fermentation using dessert variety of apples. *European Food Research and Technology*, *239*(1), 67–77. https://doi.org/10.1007/s00217-014-2204-1

Yegge, J. M. (2001). *Influence of sensory and non-sensory attributes of* Chardonnay *wine on acceptance and purchase intent* [Ph.D., University of California, Davis]. https://www.proquest.com/docview/304684825/abstract/6FD5B870A74C4E4APQ/1

Zakalik D. and Peck, G.M.. 2023. High-tannin apple supply and demand in North America: results from a 2021 cider industry survey. *Fruit Quarterly*. 29(1):30–34. https://nyshs.org/fruit-quarterly/

CHAPTER 3

CONCLUDING REMARKS AND REFLECTIONS

The objective for nitrogen management in fresh-market and processing apple orchards is to provide sufficient nitrogen for tree metabolism while avoiding excessive vegetative grown and reductions in fruit quality (Cheng & Raba, 2009; Fallahi, 1997; Raese et al., 2007; Wargo et al., 2003; Xia et al., 2009). Some of the negative fruit quality attributes that are affected by nitrogen fertilization, such as decreased flesh firmness and red coloration, are not as important qualities for hard cider apples, and so larger amounts of nitrogen applied to trees intended for hard cider may offer the benefits of high juice yeast assimilable nitrogen (YAN) concentration without detrimental impacts to the finished cider product. The experiments described in this thesis investigated the impact of foliar urea applications on hard cider volatiles and aromatic volatiles, specifically, the potential impact of foliar urea fertilization on tree and fruit physiology, fermentation kinetics, aromatic volatile and amino acid composition and concentration, and YAN content; and if different levels of foliar urea fertilization produce ciders that are sensorily distinct and discernable by consumers.

Foliar urea applications were effective at increasing juice YAN content with the highest rate of foliar fertilization increasing juice YAN by 130% to 145% compared to the Control without negatively impacting important for hard cider parameters such as total polyphenols, sugar content and composition, or fruit yield. Changes to leaf nitrogen content were nominal compared to the increases seen in juice YAN, indicating that nitrogen fertilization within five weeks before harvest has a greater impact on fruit YAN than tree growth and vigor. Based on the findings in this study, foliar urea applied to hard cider trees close to their projected harvest date may be an effective management technique to increase hard cider juice quality. Further research to help understand the range of which management can enhance hard cider

production via orchard-level nitrogen supplementation without loss or diminishing return to fruit quality.

Aromatic volatiles were differentially impacted by increases in juice YAN content, but ester concentration—which has some of the greatest aromatic influence on hard cider aroma (Lorenzini et al., 2019)—demonstrated the strongest positive correlation with the increase in YAN. A negative effect, or lack thereof, on higher alcohols with the increase in YAN is a phenomenon that requires a further study into amino acid biosynthesis and catabolism to fully predict and explain the mechanisms behind nitrogen-related changes in these aromatic volatile compounds. Amino acid composition will also greatly affect higher alcohol synthesis since they are precursors to α-ketoacids which serve as intermediaries in higher alcohol synthesis. The proportion of amino acids in juices and ciders did vary among treatments. Extrapolating the relationship among these compositional changes and changes to higher alcohol concentration requires additional research. It would be beneficial to identify and quantify additional higher alcohols from this dataset, such as isoamyl alcohol, that are known to have sensory effects in cider since a large number of aromatic higher alcohols do not present a sensory impact due to their high odor threshold (Romano et al., 1992; Vidrih & Hribar, 1999; Yair, 1997).

The intricate relationship between primary amino nitrogen (PAN) and volatile concentration, as well as the surprising influence of apple cultivars on aromatic composition, highlights the complexities of hard cider production and orchard management and immense potential to these industries. Reflecting on the project, I'd recommend a more aggressive approach to nitrogen fertilization in future research. A broader investigation, encompassing YAN concentrations that match and surpass those typically found in juices for commercial wines and hard ciders, could yield findings directly applicable to commercial cidery conditions.

The differences among treatments were sensorily distinguishable by untrained panelists, which adds significantly to the current breadth of knowledge on the impact of management practices on cider aroma. The results of this study may be most beneficial for vertically integrated operations where the cider producer is also the orchard manager. Such a business model grants the cidermaker the greatest control over nitrogen management to craft their ideal aromatic profile. The evident malleability and scalability of cider production underscores hard cider as a veritable gold mine waiting to be struck that accommodates both small startups and growing businesses. This dynamic, coupled with the discoveries from this study, leaves me convinced that the interplay between science and the art of cider production is a rich field for exploration.

REFERENCES

Cheng, L., & Raba, R. (2009). Accumulation of macro- and micronutrients and nitrogen demand-supply relationship of 'Gala'/'Malling 26' apple trees grown in sand culture. *Journal of the American Society for Horticultural Science*, *134*(1), 3–13. https://doi.org/10.21273/JASHS.134.1.3

Fallahi, E. (1997). Preharvest nitrogen optimization for maximizing yield and postharvest fruit quality of apples. *Acta Horticulturae*, *448*, 415–420. https://doi.org/10.17660/ActaHortic.1997.448.77

Lorenzini, M., Simonato, B., Slaghenaufi, D., Ugliano, M., & Zapparoli, G. (2019). Assessment of yeasts for apple juice fermentation and production of cider volatile compounds. *LWT*, *99*, 224–230. https://doi.org/10.1016/j.lwt.2018.09.075

Raese, J. T., Drake, S. R., & Curry, E. A. (2007). Nitrogen fertilizer influences fruit quality, soil nutrients and cover crops, leaf color and nitrogen content, biennial bearing and cold hardiness of 'Golden Delicious.' *Journal of Plant Nutrition*, *30*(10), 1585–1604. https://doi.org/10.1080/01904160701615483

Romano, P., Suzzi, G., Comi, G., & Zironi, R. (1992). Higher alcohol and acetic acid production by apiculate wine yeasts. *Journal of Applied Bacteriology*, *73*(2), 126–130. https://doi.org/10.1111/j.1365-2672.1992.tb01698.x

Vidrih, R., & Hribar, J. (1999). Synthesis of higher alcohols during cider processing. *Food Chemistry*, *67*(3), 287–294. https://doi.org/10.1016/S0308-8146(99)00136-3

Wargo, J. M., Merwin, I. A., & Watkins, C. B. (2003). Fruit size, yield, and market value of `Goldrush' apple are affected by amount, timing and method of nitrogen fertilization. *HortTechnology*, *13*(1), 153–161. https://doi.org/10.21273/HORTTECH.13.1.0153

Xia, G., Cheng, L., Lakso, A., & Goffinet, M. (2009). Effects of nitrogen supply on source-sink balance and fruit size of 'Gala' apple trees. *Journal of the American Society for Horticultural Science*, *134*(1), 126–133. https://doi.org/10.21273/JASHS.134.1.126

Yair, M. (1997). *Concepts in wine chemistry* (pp. 23–24). The wine appreciation guild.

Appendix

Table A2-1. Sugar contents of 'Ellis Bitter' and 'Harry Masters Jersey' cider before and after fermentation. Values are means ± standard error across blocks within treatment groups. a, b, and c, indicate significant ($p < 0.05$) similarity and dissimilarity among treatments within the same juice/cider group.

	Juice (g/L)			**Cider (g/L)**		
	Control	Low	High	Control	Low	High
Ellis Bitter						
Sorbitol	9.0±0.9 **a**	8.5±1.3 **a**	11.0±0.9 **a**	9.9±0.6	10.6±1.0	10.8±0.9
Glucose	24.7±3.0 **ab**	22.1±1.0 **a**	29.6±4.8 **b**	0.4±0.0	0.4±0.2	0.5±0.0
Fructose	60.3±4.5 **ab**	53.3±4.5 **a**	71.2±8.6 **b**	0.2±0.1	0.1±0.0	0.1±0.0
Sucrose	28.7±2.3 **a**	25.5±4.5 **a**	34.4±3.4 **a**	0.1±0.0	0.1±0.0	0.1±0.0
Harry Masters Jersey						
Sorbitol	15.0±3.4	14.7±1.5	13.8±2.4	15.2±3.5	15.2±1.4	14.4±2.4
Glucose	25.2±3.0	26.9±0.6	27.6±2.3	0.9±0.1	1.0±0.1	0.9±0.4
Fructose	75.2±2.5	75.6±1.1	74.9±3.1	0.1±0.0	0.1±0.1	0.1±0.1
Sucrose	31.8±1.3	31.0±1.9	28.4±1.7	0.2±0.0	0.2±0.0	0.1±0.1

Table A2-2. Amino acids of 'Ellis Bitter' cider after three (Low) and five (High) weekly foliar urea treatments. Values are means and standard error of the means across blocks within treatments. a, b, and c, indicate significant ($p < 0.05$) similarity and dissimilarity using post-hoc Tukey HSD and n=4.

	Histidine	Asparagine	Serine	Glutamine	Arginine	Glycine	Aspartate	Glutamate	Threonine	Alanine	GABA	Proline	Cysteine	Lysine	Tyrosine	Methionine	Valine	Norvaline	Isoleucine	Leucine	Phenylalanine	SUM
Ellis Bitter																						
Control (mg/L)	0.32 **a**	5.43 **a**	2.69 **a**	1.90 **a**	1.07 **a**	0.88 **a**	0.91 **a**	1.56 **a**	5.06 **a**	2.39 **a**	2.47 **a**	1.60 **a**	6.88 **a**	0.62 **a**	2.85 **a**	2.64 **a**	0.14 **a**	29.74 **a**	1.32 **a**	2.24 **a**	1.22 **a**	**73.50 a**
Low (mg/L)	1.23 **b**	73.16 **b**	4.86 **b**	2.70 **b**	2.94 **b**	2.06 **b**	2.34 **b**	4.69 **b**	6.28 **b**	6.38 **b**	8.20 **b**	3.24 **b**	0.00 **b**	2.37 **b**	0.49 **b**	3.48 **b**	0.57 **a**	28.78 **a**	2.01 **b**	4.11 **b**	2.35 **b**	**162.72 b**
High (mg/L)	1.02 **c**	49.12 **c**	4.62 **b**	2.28 **c**	2.11 **c**	1.84 **c**	1.85 **c**	4.07 **c**	7.06 **c**	5.49 **c**	6.88 **c**	2.75 **c**	0.02 **b**	1.46 **c**	4.63 **c**	2.94 **c**	1.24 **c**	29.56 **a**	2.01 **b**	3.95 **b**	2.38 **b**	**137.00 c**

Table A2-3. Volatile aromatics of 'Ellis Bitter' cider after foliar urea treatments. Values are means ± standard error across blocks within treatment groups. a, b, and c, indicate significant ($p < 0.05$) similarity and dissimilarity.

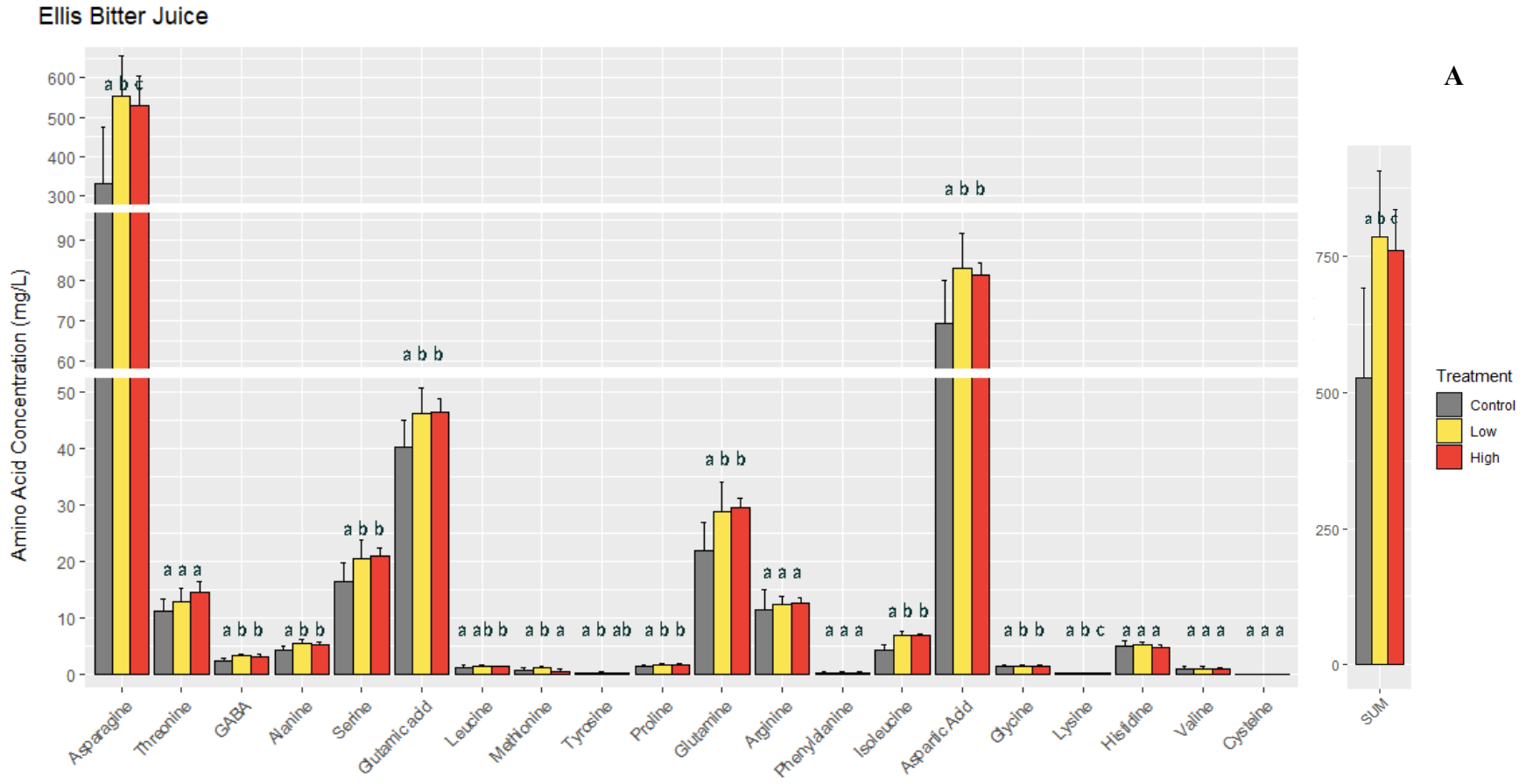
	'Ellis Bitter'					
	Juice			Cider		
	Control	Low	High	Control	Low	High
Higher Alcohols						
5-octen-1-ol	3.3±0.6	3.1±1.5	3.1±1.8	3.2±1.0	3.7±1.3	6.4±6.4
1-hexanol	5187.0±567.6	8975.0±7074.2	10813.1±5307.2	3501.3±762.2	3432.9±1193.4	3251.2±1142.0
Phenylethyl Alcohol	29.9±0.9	44.5±26.3	63.7±35.9	8386.3±2521.1	9947.9±3259.9	9448.8±3512.5
1-octonal	3.3±0.5	2.5±1.3	6.9±4.2	12.7±5.4	16.5±6.5	16.4±5.6
Eugenol	40.2±0.1	41.6±2.8	46.5±6.6	81.9±14.8	126.1±12.8	96.1±81.5
1-butanol	2317.0±424.0	4465.9±4006.7	3972.7±1412.2	1703.2 ± 437.7	2480.2±679.2	2187.9±1530.8
Citronellol	0.1±0.0	0.4±0.7	2.2±2.8	0.9±0.7	1.8±0.5	1.6±1.3
1-decanol	0.1±0.0	0.2±0.3	4.7±5.2	2.4±0.4	4.8±0.9	2.6±6.4
Esters						
Ethyl hexanoate	0.0±0.0	0.0±0.0	0.4±0.8	571.3±204.4	795.8±293.9	791.2±301.8
Hexyl acetate	2.7±3.4	0.1±0.1	0.5±0.8	334.8±169.7 **A**	604.0±133.51 **B**	688.6±161.4 **B**
Ethyl decanoate	0.0±0.0	0.0±0.0	0.0±0.0	827.8±381.7	1285.1±460.1	1204.7±469.0
Ethyl Octanoate	0.0±0.0	0.0±0.0	0.0±0.0	947.5± 247.2	1009.5± 644.2	774.0±672.9
Methyl Caprate	0.3±0.0	0.6±0.5	0.4±0.0	2.9±1.3	5.1±1.9	4.6±2.1
Aldehydes and ketones						

Beta-damascenone	1.9±0.3	1.7±0.5	1.6±0.7	3.5±2.6	4.8±1.9	5.5±0.4
Acids						
Octanoic Acid	180.4±7.5	186.6±13.3	247.4±71.6	5887.6±1406.0	34980.8±542.1	8778.5±74339.6
Hexanoic Acid	0.0±0.0	0.0±0.0	0.0±0.0	5049.5±4263.0	34839.1±3223.3	6501.1±82423.6
Total VOCs	**3106±3756**	**59389±3267**	**60653±153991**	**245849±989**	**303810±12158**	**805865±6832**

Table A2-4. Volatile aromatics of 'Harry Masters Jersey' cider after foliar urea treatments. Values are means ± standard error across blocks within treatment groups. a, b, and c, indicate significant ($p < 0.05$) similarity and dissimilarity. n.d. indicates compounds that were not identified in the samples.

	'Harry Masters Jersey'					
	Juice			Cider		
	Ctrl.	Low	High	Ctrl.	Low	High
Alcohols						
5-octen-1-ol	8.2±1.5	5.8±3.5	5.3±1.8	7.5±5.0	6.1±1.6	7.2±2.5
1-hexanol	18068.4±1093.9 **A**	17728.2±1835.5 **AB**	10629.8±5678.1 **B**	15826.1±9364.4	13569.2±2280.8	11758.3±4171.1
Phenylethyl Alcohol	84.6±2.4	87.6±13.8	57.0±29.2	11147.5±3988.5	11061.6±4247.5	8328.6±2992.3
1-octonal	16.2±3.3	14.7±3.6	9.1±6.6	30.4±9.3	32.2±11.8	40.9±17.4
Eugenol	n.d.	n.d.	n.d.	n.d.	n.d.	191.9±0.0
1-butanol	7066.2±704.8**A**	6538.5±932.8 **AB**	3912.4±1509.1 **B**	8471.1±9263.7	5835.4±2974.3	4168.4±1004.6
Citronellol	6.1±1.7	5.5±2.1	3.4±3.3	1.7±0.4	2.4±0.6	2.2±1.0
1-decanol	7.9±3.2	5.9±3.9	4.2±4.4	2.0±0.6 **A**	3.7±1.7 **B**	3.1±0.9 **AB**
Esters						
Ethyl hexanoate	14.0±8.3 **A**	7.6±6.7 **AB**	0.1±0.2 **B**	820.8±320.4	932.8±353.5	856.8±326.0
Hexyl acetate	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Ethyl decanoate	0.0±0.0	0.0±0.0	0.0±0.0	990.4±450.6	1222.5±469.2	1208.9±443.6
Ethyl Octanoate	n.d.	n.d.	n.d.	n.d.	n.d.	1692.1±0.0

Methyl Caprate	0.4±0.1	0.4±0.1	0.4±0.0	25.5±9.8	32.8±11.8	35.9±13.4
Aldehydes and ketones						
Beta-damascenone	1.6±2.2	1.8±0.8	1.4±0.4	3.0±1.3	3.9±2.2	3.9±0.5
Acids						
Octanoic Acid	235.6±50.0	308.6±69.2	256.3±109.5	5479.2±2030.7	6849.8±2584.2	11580.4±11205.3
Hexanoic Acid	75.1±130.1	19.6±33.9	0.0±0.0	4613.5±2587.2	6866.6±2751.1	18169.4±35301.9
Total VOCs	**102337±11473**	**98896±6704**	**59518±39114**	**426769±1698**	**417771±2711**	**507455±7294**


Ellis Bitter Juice
A
Amino Acid Concentration (mg/L)
Treatment
Control
Low
High
Asparagine
Threonine
GABA
Alanine
Serine
Glutamic acid
Leucine
Methionine
Tyrosine
Proline
Glutamine
Arginine
Phenylalanine
Isoleucine
Aspartic Acid
Glycine
Lysine
Histidine
Valine
Cysteine
SUM

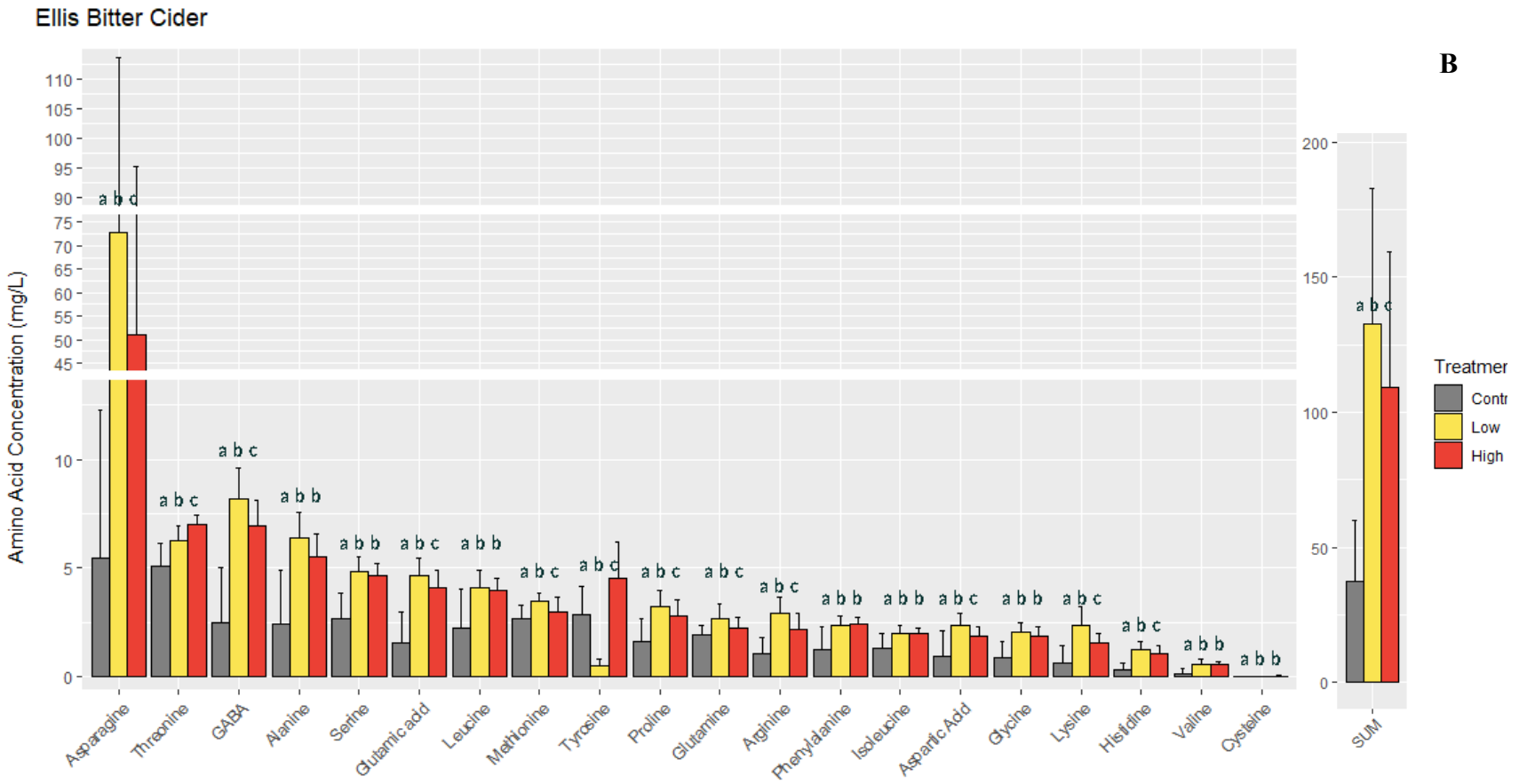
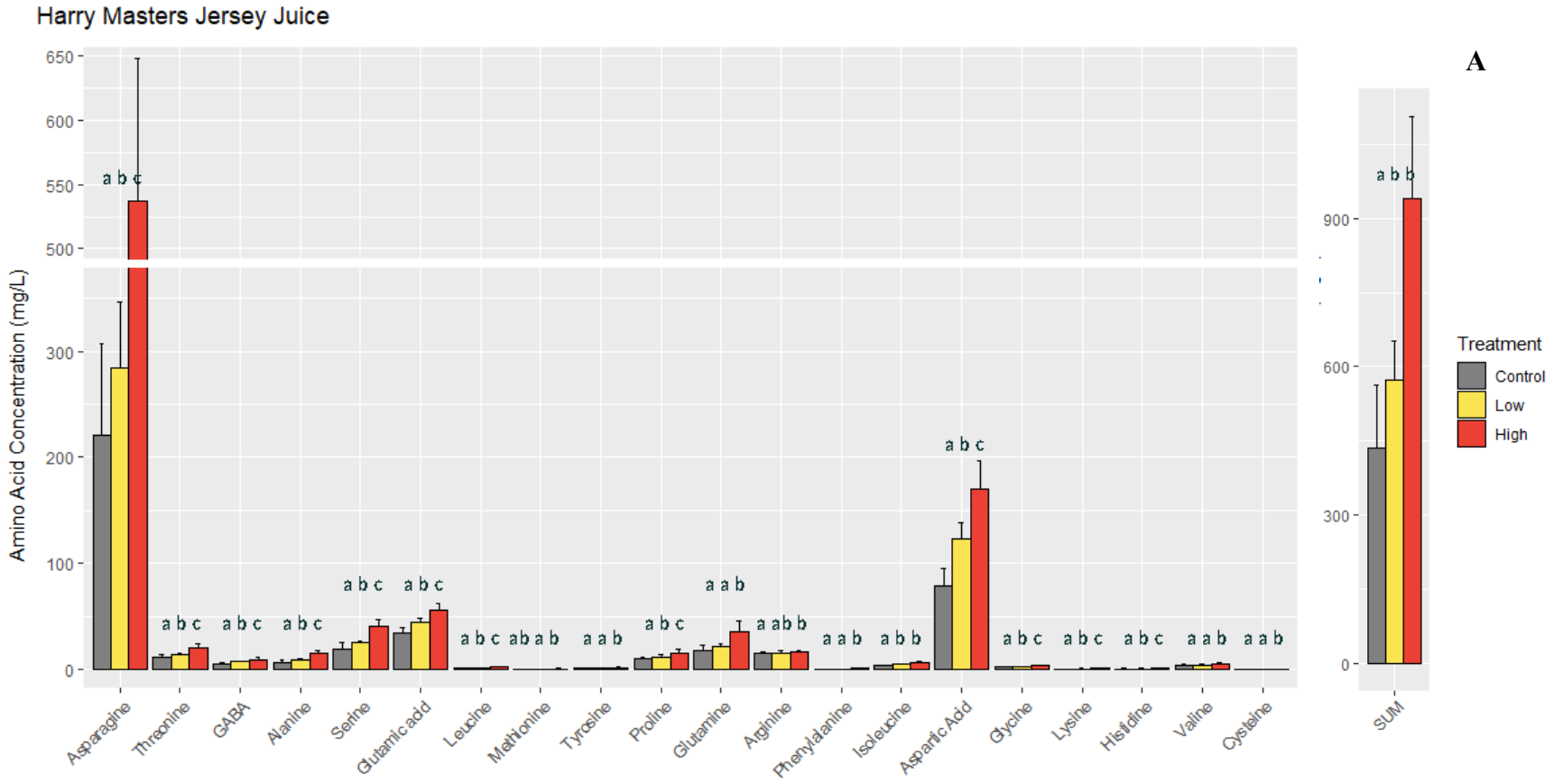


Figure A2-1. Amino acid concentration for 'Ellis Bitter' juice (A) hard cider (B) after three (Low) and five (High) weekly foliar urea treatments in an experiment conducted in 2021 in Ithaca, NY. Values are mean ± standard error (n=4). Mean separation indicated by a, b, and c.


Harry Masters Jersey Juice
A
Amino Acid Concentration (mg/L)
650
600
550
500
300
200
100
0
900
600
300
0
a b c
a b b
a b c
a b c
a b c
a b c
a b c
a b c
a b c
a b c
a a b
a b c
a a b
a b b
a b c
a b c
a b c
a b c
a a b
a a b
Asparagine
Threonine
GABA
Alanine
Serine
Glutamic acid
Leucine
Methionine
Tyrosine
Proline
Glutamine
Arginine
Phenylalanine
Isoleucine
Aspartic Acid
Glycine
Lysine
Histidine
Valine
Cysteine
SUM
Treatment
Control
Low
High

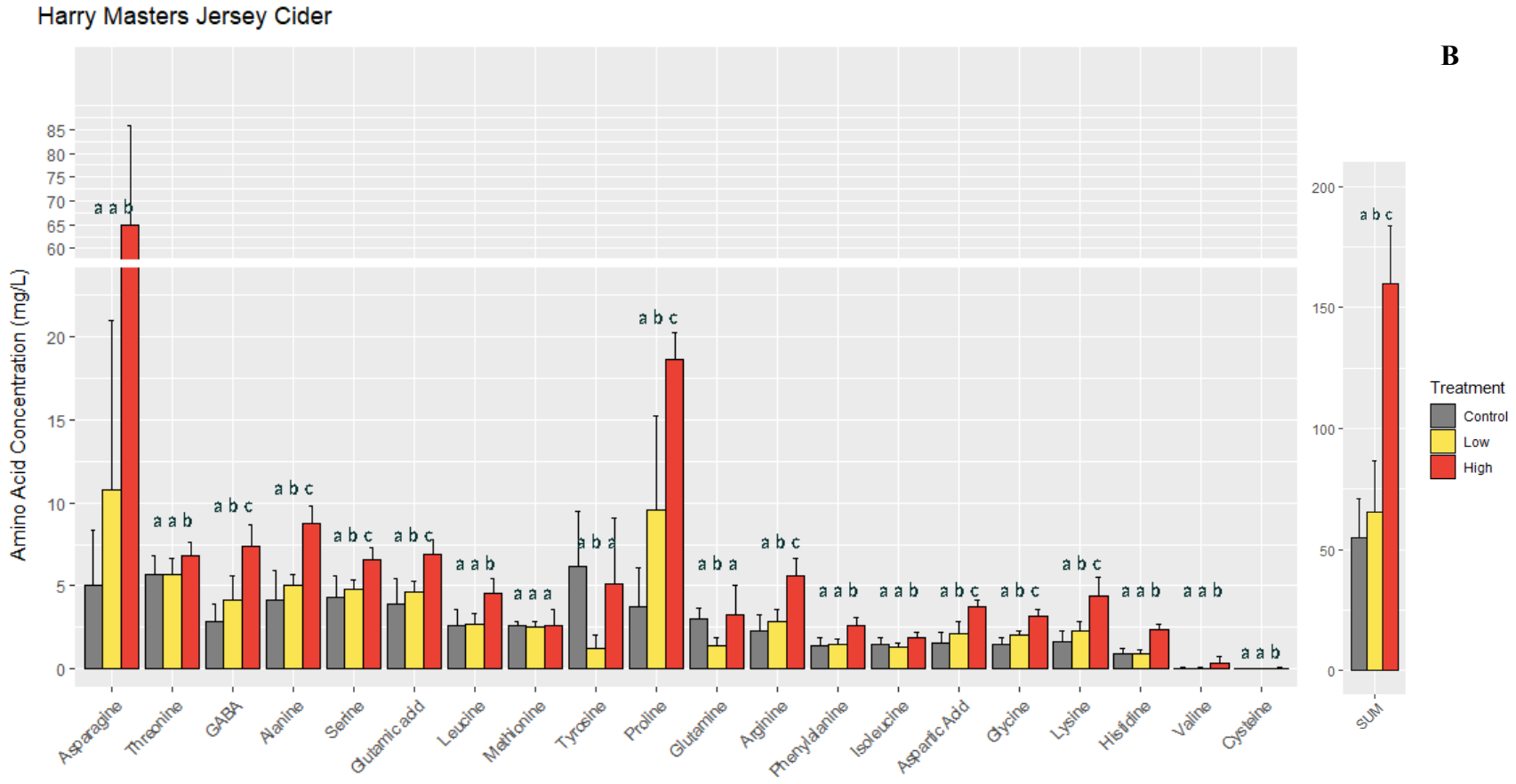


Figure A2-2. Amino acid concentration for 'Harry Masters Jersey' juice (top) hard cider (bottom) after three (Low) and five (High) weekly foliar urea treatments in an experiment conducted in 2021 in Ithaca, NY. Values are mean ± standard error (n=4). Mean separation indicated by a, b, and c.

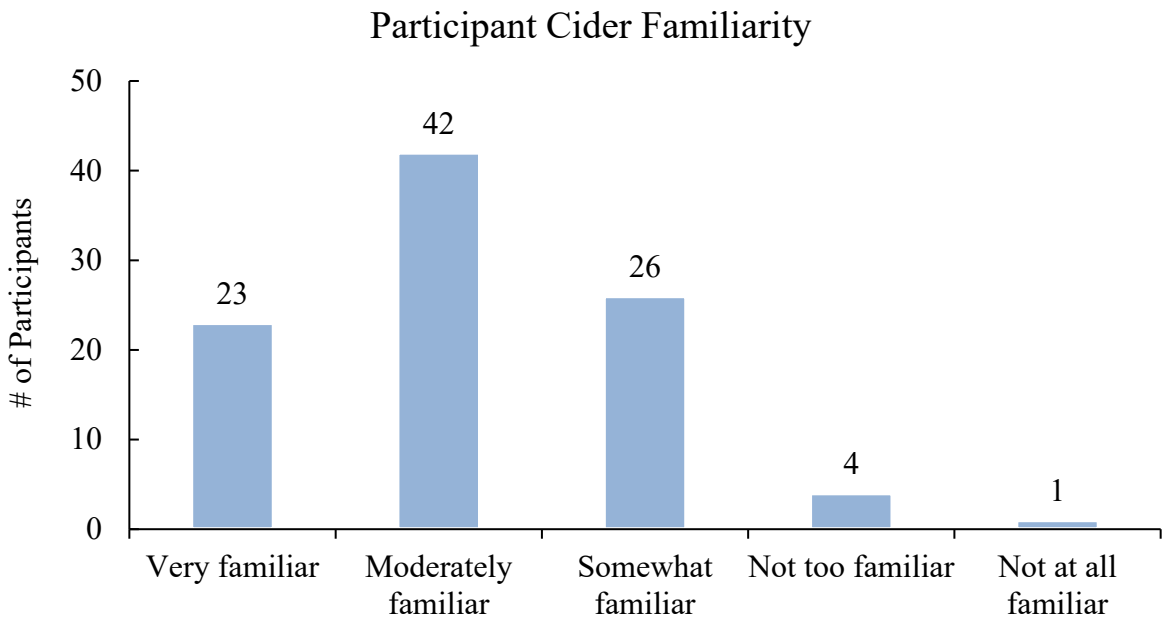


Figure A2-3. Number of sensory study participants (n=100) and their self-reported familiarity with hard apple cider.

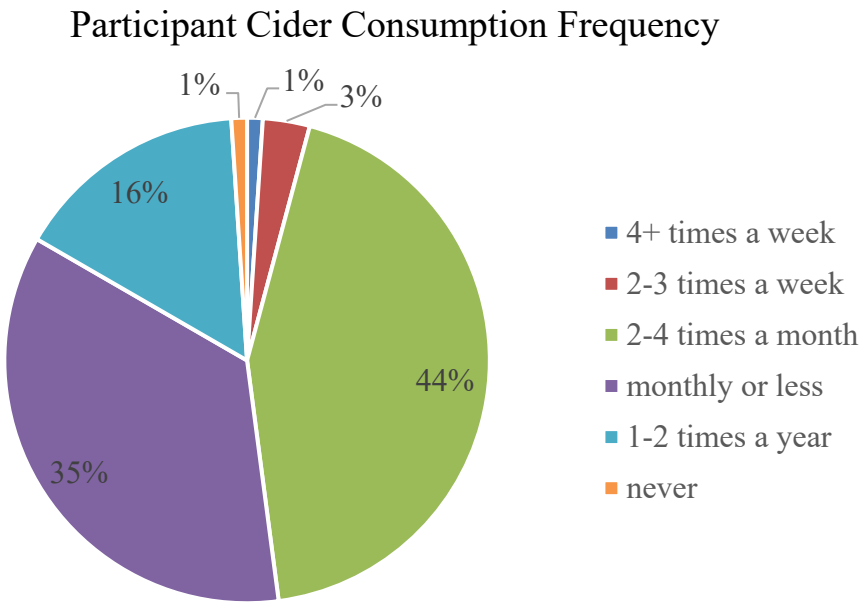


Figure A2-4. Percentage of sensory study participants (n=100) and their self-reported consumption frequency with hard apple cider.

Table A2-5. Percentage of sensory study participants who reported lingering in-mouth sensation by descriptor. Participants were allowed to report more than one lingering sensation.

	Ellis Bitter: Control	**Ellis Bitter: Low**	**Ellis Bitter: High**	**Harry Masters: Control**	**Harry Masters: Low**	**Harry Masters: High**
Aftertaste Reported	**46%**	**47%**	**42%**	**45%**	**54%**	**44%**
Other	6%	4%	5%	3%	2%	0%
Bitterness	46%	61%	61%	24%	32%	22%
Acidity/Sourness	54%	51%	59%	30%	34%	25%
Gasoline/Petrol	4% **a**	14% **ab**	20% **b**	8%	11%	8%
Green/Vegetal	13% **a**	27% **b**	16% **ab**	5%	7%	8%
Rancid	2%	6%	5%	5%	5%	11%
Fatty/Cheese	6%	16%	7%	11%	5%	6%
Floral	19%	10%	14%	3%	2%	3%
Citrus	17%	29%	16%	0%	0%	3%
Cooked Fruit	21%	14%	16%	5%	9%	11%
Green Apple	25%	29%	34%	8%	9%	11%
Fruitiness	23%	24%	23%	5%	5%	8%

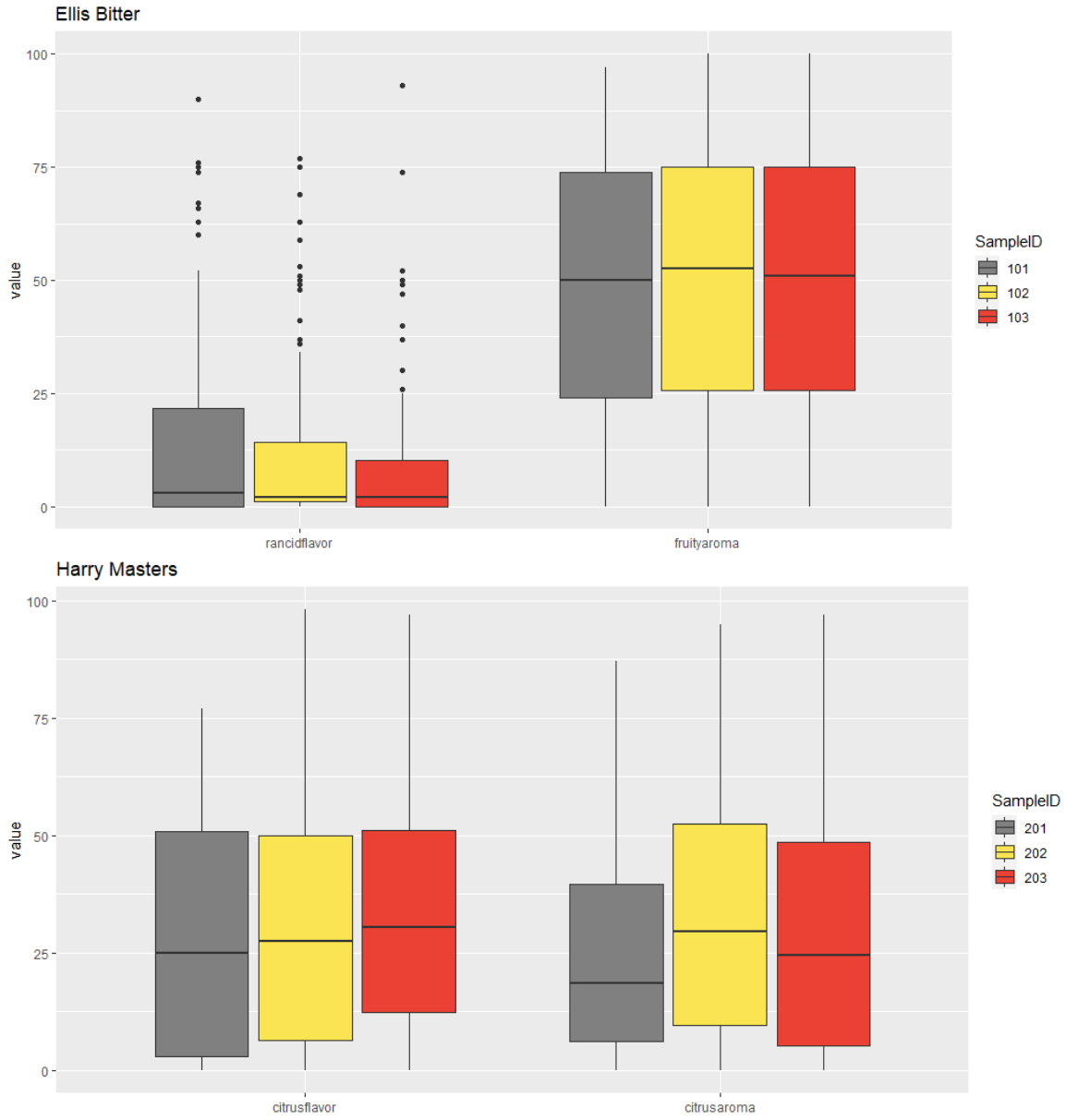


Figure A2-5. Sensory attributes with statistical significance. a, b, and c, indicate significant ($p < 0.05$) similarity and dissimilarity among treatments.

	Control	**Low**	**High**
Ellis Bitter			
Fruity aroma	45.9±28.9 **a**	51.5±28.3 **ab**	52.3±27.4 **b**
Rancid flavor	15.2±23.2 **a**	12.3±19.4 **ab**	10.1±17.5 **b**
Harry Masters			
Citrus aroma	25.8±23.5 **a**	33.0±25.1 **b**	28.2±25.0 **ab**
Citrus flavor	29.0±25.1 **a**	31.5±26.3 **b**	34.7±25.1 **ab**